Sensitive electrochemical detection of bisphenol A at screen-printed graphite electrode modified with nitrogen-doped graphene sheets

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Abstract

A novel voltammetric sensor was developed by modifying screen-printed graphite electrode (SPGE) with nitrogen doped graphene sheets (N-GSs) to detect bisphenol A. The electrochemical results exhibited that N-GSs / modified SPGE has high sensing performance towards the oxidation of bisphenol A. Excellent results were obtained for bisphenol A detection in the linear range from 0.08 to 300.0 µM with a sensitivity of 0.1626 µA µM⁻¹ and limit of detection of 0.02 µM. Also, the fabricated N-GSs/SPGE sensor showed good stability. The as-prepared sensor was tested towards the detection of bisphenol A in real samples. The measured results established the great sensing ability of N-GSs/SPGE for bisphenol A with high selectivity and good stability in real samples.

Keywords
voltammetric sensor; high sensitivity; lengthy stability; reproducibility.

Introduction

Bisphenol A is a compound widely used in the synthesis of polycarbonate plastics and epoxy resins. It can be used in medical materials, thermal receipts, and packagings such as canned beverage containers, nursing bottles, and children’s toys. Thus, trace amounts of bisphenol A can migrate into food and beverage packing of products and consequently are absorbed by humans [1–4]. Besides, bisphenol A is also one of the endocrine-disrupting compounds, which can affect the reproduction of aquatic organisms and cause a different of unfavorable health problems to human beings [5]. Because of its severe threat to the environment and human health, developing a more convenient and specific method of higher sensitivity is crucial to detect bisphenol A rapidly. At present, the detection of bisphenol A relies on spectrophotometry [6], chromatography [7], and immunoassay [8,9], which are expensive and time-consuming techniques. By contrast, electrochemical methods are considered an effective method for quantifying analytes due to their high sensitivity, simplicity, and low cost [10-16].
Electrochemical sensors based on screen-printed electrodes (SPEs) have gained increasing interest as analytical tools for electro-analysis. SPEs provide great advantages that make these kinds of sensors have important characteristics of ideal sensors: ease of use, low-cost, and portability [17,18]. So, the screen-printed technology has significantly contributed to the transition from traditional unwieldy electrochemical cells to miniaturized and portable electrodes that meet the needs for on-site analysis [19,20]. Different materials and methods can be used to modify the surface of the working electrode to enhance the electro-activity and sensitivity of the modified electrode [21-26]. The sensing materials based on nano-materials are usually used due to their high electrical conductivity, high thermal conductivity, and unique chemical and mechanical properties [27-33].

Graphene, a carbon monolayer packed into a 2D honeycomb lattice, has spurred extensive interest in the area of electrochemical sensors because of its superior electronic conductivity, low cost, and chemical and mechanical stability [34]. The capacity of graphene is greatly dependent on its specific surface area, pore structure, doping, and interlayer distance. Various strategies have been developed, such as the design of graphene foam [35], assembly with the assistance of templates [36], the recombination of graphene and carbon nanotube [37], and introduction of heteroatoms into graphene lattice [38]. Among them, nitrogen doping has been an effective way to improve the sensing properties of graphene for electrochemical sensors [39-41]. As is well known, N-doping would further promote the electrochemical performance of graphene electrodes, which could be attributed to more active sites induced by doping, and the increased specific surface area.

In this study, the electrochemical detection of bisphenol A using N-GSs modified SPGE is reported. The electrochemical and electrocatalytic properties of N-GSs modified SPGE toward bisphenol A are studied. Finally, accurate detection of bisphenol A in real samples is demonstrated using N-GSs/SPGE as an electrochemical sensor.

**Experimental**

**Chemicals and instruments**

The chemicals and reagents were of analytical grade without any further purification. All chemicals were obtained from Sigma-Aldrich. In addition, ortho-phosphoric acid and the respective salts (KH$_2$PO$_4$, K$_2$HPO$_4$, K$_3$PO$_4$) with a pH ranging between 2.0 and 9.0 have been utilized to procure buffer solution. Electrochemical measurements were carried out on a PGSTAT-302N Autolab potentiostat/galvanostat (Eco Chemie, The Netherlands). All the measurements were done using SPEs system (Dropsens, DRP-110, Spain) with a three-electrode cell system consisting of graphite as a working electrode, a silver pseudo-reference electrode and graphite used as an auxiliary electrode. A digital pH-meter (Metrohm 710) was employed for measuring pH values.

**Synthesis of N-GSs**

N-GSs were synthesized via hydrothermal treatment method with graphene oxide (GO) as the base material and urea as the reducing and doping agent. Briefly, exfoliated GO (50 mg) was dispersed through ultrasonication in 50 mL of redistilled water, and the pH of the solution was adjusted to 10 by adding NH$_3$·H$_2$O (30 %). Next, urea (3.0 g) was added and ultrasonicated for three hours. Then, the mixture was sealed in a polytetrafluoroethylene (Teflon)-lined autoclave and maintained at 180 °C for 12 h. After being cooled down to room temperature, the finally obtained hydrothermal product of N-GSs was collected and washed several times with double distilled water and its pH was adjusted to neutral. Finally, the N-GSs were freeze-dried. A typical SEM image of the synthesized N-GSs is shown in Figure 1.
Electrode preparation

Firstly, 1 mg of N-GSs was put into 1.0 mL of redistilled water to form a homogeneous solution through ultrasonic treatment. The N-GSs/SPGE was obtained by adding 4 µL of the dispersed solution on the SPGE surface directly and finally dried at the ambient temperature. The surface areas of N-GSs/SPGE and bare SPGE were obtained by cyclic voltammetry (CV) using 1 mM K₃Fe(CN)₆ at different scan rates. Using the Randles-Sevcik formula [42], the electrode surface area of N-GSs/SPGE was calculated as 0.116 cm², which was about 3.7 times greater than the surface area of bare SPGE.

Results and discussion

Electrocatalytic oxidation of bisphenol A at N-GSs/SPGE

The electrochemical behavior of bisphenol A is strongly determined by the pH value of the aqueous electrolyte solution (Scheme 1). Hence, to ensure the optimal performance of N-GSs/SPGE in the determination of bisphenol A, the influence of pH was investigated through the cyclic voltammetry behavior of N-GSs/SPGE toward 100.0 μM bisphenol A in the pH range between 2.0 and 9.0 at the scan rate of 50 mV/s.

Scheme 1. Electrochemical oxidation mechanism of bisphenol A at the surface of the modified electrode

The oxidation peak current value of bisphenol A showed a tendency to rise to pH 7 and fall beyond it (Figure 2). Since the maximum oxidation peak current value of bisphenol A was achieved at pH value 7.0, it was chosen as the optimal pH value for subsequent measurements in 0.1 M PBS.
DETECTION OF BISPHENOL A AT SPG ELECTRODE

Figure 2. Plot of $I_p$ vs. pH obtained from CVs of N-GSS/SPGE in a solution containing 100.0 μM of bisphenol A in 0.1 PBS of different pH (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0).

The electrochemical properties of the SPGE before and after modification with N-GSSs were investigated by CV. CV curves recorded at the scan rate of 50 mV s$^{-1}$ are shown in Figure 3. It can be seen in Figure 3 that the oxidation current response of bisphenol A at N-GSSs/SPGE was significantly higher than at SPGE. In addition, the anodic peak potential of bisphenol A at the surface of the N-GSSs/SPGE is shifted to a negative value relative to the bare electrode. The reason could be that the N-GSSs provided a large specific surface area of high electrical conductivity, which accelerated the electron transfer rate of electroactive compounds at the interface between the electrode and solution.

Figure 3. CV response (50 mV s$^{-1}$) of 100.0 μM of bisphenol A in 0.1 M PBS of pH 7.0 at: a - bare SPGE and b - N-GSSs/SPGE

Effect of scan rate on the response of bisphenol A at N-GSSs/SPGE

The effect of the scan rate on the oxidation peak current values of 70.0 μM bisphenol A was investigated using linear sweep voltammetry (LSV) (Figure 4A). Various scanning rates have been tested (10, 25, 50, 75, 100, 200 and 400 mV s$^{-1}$). The results show that the oxidation peak current increased upon increasing the scan rate. Moreover, the peak potential is slightly shifted to more positive potentials. The results show that at higher scan rates, a kinetic limitation between bisphenol A and modified electrode surface can occur. According to Figure 4B, the anodic peak current ($I_{pa}$) of bisphenol A is proportional to the square root of scan rate ($\sqrt{\nu}$), suggesting that the reaction of bisphenol A at N-GSSs/SPGE is controlled by diffusion.
To define the electron transfer coefficient ($\alpha$) between bisphenol A and N-GSs/SPGE electrode, a Tafel diagram ($E$ vs. $\log I$) was plotted (Figure 5B), using the activation region (rising part) of the voltammogram recorded at 10 mV s$^{-1}$ for 70.0 μM bisphenol A (Figure 5A). The slope from the linear plot was estimated to be 0.0898 V$^{-1}$. The slope value is equal to $n_\alpha (1-\alpha)F/2.3RT$. From this equation, the $\alpha$ value can be estimated to be 0.34 (assuming $n_\alpha = 1$).

**CHA studies**

According to the previous section, a diffusion-controlled process dominated during the electro-oxidation of bisphenol A at the surface of N-GSs/SPGE. Therefore, single-step chronoamperograms were recorded by setting the applied potential step at 0.5 V to measure the diffusion coefficient of bisphenol A on the surface of the modified electrode. Figure 6A shows the single-step chronoamperograms recorded for N-GSs/SPGE in the presence of different concentrations of bisphenol A. It can be seen that increase in the concentration of bisphenol A was accompanied by an increase of the anodic current. The experimental plots of current versus $t^{1/2}$ with the best fits for different concentrations of bisphenol A are shown in Figure 6B. The slopes of the resulting straight lines were then plotted versus the concentration of bisphenol A (Figure 6C). Using the Cottrell equation, the diffusion coefficient of bisphenol A was calculated, and it equals $2.7 \times 10^{-5}$ cm$^2$ s$^{-1}$.
\[ I = nFAD^{1/2} C_b t^{-1/2} \] (1)

In eq. (1), \( I \) is current (A), \( D \) is the diffusion coefficient (cm\(^2\) s\(^{-1}\)), \( C_b \) is the bulk concentration of analyte (mol cm\(^{-3}\)), \( A \) is the surface area of the electrode (cm\(^2\)), \( F \) is Faraday's constant, \( t \) is the time (s), and \( n \) is the number of electrons transferred.

**Figure 6.** (A) Chronoamperograms of N-GSS/SPGE in 0.1 M PBS, pH 7.0 for different concentrations of bisphenol A: (a–e refer to 0.1, 0.25, 0.4, 0.9, and 1.5 mM) (B) \( I \) versus \( t^{-1/2} \) obtained from chronoamperograms a to f. (C) slope plot vs. concentration of bisphenol A

**Detection of bisphenol A at N-GSS/SPGE by differential pulse voltammetry (DPV) technique**

To evaluate the linear range and limit of detection of the designed electrochemical sensor, the determination of bisphenol A at N-GSS/SPGE in different concentrations of standard solutions was performed by DPV performed with step potential of 0.01 V, and pulse amplitude = 0.025 V. DPV responses are shown in Figure 7A linear relation was observed between the current signal and the bisphenol A concentration in the range of 0.08–300.0 μM.

**Figure 7.** (A) DPV response of various concentrations of bisphenol A in 0.1 M PBS of pH 7.0 at N-GSS/SPGE (a–h refer to 0.08, 1.0, 10.0, 30.0, 70.0, 100.0, 200.0, and 300.0 μM); (B) calibration curve of DPV oxidation current peaks against concentration of bisphenol A

The calibration graph was defined according to the following equation: 
\[ I_{pa} = 0.1626 C_{\text{bisphenol A}} + 1.0948 \] with a correlation coefficient \( (R^2) \) of 0.9998 (Figure 7B). Also, the detection limit, \( C_m \), of bisphenol A was obtained using the following equation:

\[ C_m = 3s_b / m \] (2)
In the above equation, m is the slope of the calibration plot (0.1626 μA μM⁻¹) and s_b is the standard deviation of the blank response obtained from 14 replicate measurements of the blank solution. The detection limit achieved by the designed electrochemical sensor was 0.02 μM.

Reproducibility and stability of N-GSs/SPGE

In this section, the reproducibility of the modified electrode was examined using four different sensors (N-GSs/SPGE) that were fabricated in the same condition containing 50.0 μM bisphenol A by CV. The relative standard deviation (RSD) value for this compound was found to be 2.9 %. This RSD value for analysis of bisphenol A reflects that N-GSs/SPGE displayed good reproducibility properties.

For checking N-GSs/SPGE sensor stability, the sensor was kept within PBS, pH equal to 7.0 for 15 days. Then, CV was recorded in the solution consisting of 50.0 μM of bisphenol A and compared with CV observed before immersing. The oxidation peak of bisphenol A did not change much and compared to the earlier response, there was less than 2.9 % reduction in signal, reflecting acceptable stability of N-GSs/SPGE sensor.

Application for real sample analysis

The applicability of the fabricated N-GSs/SPGE sensor was explored via detection of bisphenol A present in tomato paste, chili sauce and water bottles. The results are shown in Table 1, indicating that recoveries for bisphenol A were in the range from 97.3 to 104.6 %, and relative standard deviations were less than 3.5 %. This demonstrates the efficiency of the proposed sensor in the detection of bisphenol A in real samples.

| Sample           | Concentrations of bisphenol A, μM | Recovery, % | RSD, % |
|------------------|-----------------------------------|-------------|--------|
|                  | Spiked               | Founded     |        |
| Tomato paste     | 0.0                  | 1.5         | -      | 3.5    |
|                  | 4.0                  | 5.4         | 98.2   | 1.7    |
|                  | 6.0                  | 6.8         | 104.6  | 2.4    |
|                  | 0.0                  | -           | -      | -      |
| Chilli sauce     | 5.0                  | 5.1         | 102.0  | 2.3    |
|                  | 7.5                  | 7.3         | 97.3   | 2.9    |
|                  | 0.0                  | -           | -      | -      |
| Water bottle     | 5.0                  | 4.9         | 98.0   | 3.4    |
|                  | 7.0                  | 7.2         | 102.9  | 2.1    |

Conclusion

A sensitive electrochemical sensor for the detection of bisphenol A was fabricated by utilizing N-GSs as a sensing platform. The N-GSs exhibited improved electrochemical performance when drop-casted onto the SPGE, forming N-GSs/SPGE sensor. DPV response of NGs/SPGE increased linearly with bisphenol A concentration in the range of 0.08 μM to 300.0 μM ($R^2= 0.9998$) with a low detection limit of 0.02 μM and high sensitivity of 0.1626 μA μM⁻¹. Furthermore, reasonable reproducibility and lengthy stability were also attained for the proposed sensor. Finally, the NGs/SPGE was successfully applied for the detection of bisphenol A in real samples with promising recoveries.

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