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An amperometric sensor for the determination of benzophenone in food packaging materials based on the electropolymerized molecularly imprinted poly-*o*-phenylenediamine film

Haidong Li [a], Huaimin Guan [b,c], Hong Dai [a], Yuejin Tong [a,c,*], Xianen Zhao [d], Wenjing Qi [e,f], Saadat Majeed [e,g], Guobao Xu [e,**]

[a] College of Chemistry and Chemical Engineering, Fujian Normal University, Fuzhou 350007, China
[b] College of Materials Science and Engineering, Fujian Normal University, Fuzhou 350007, China
[c] Fujian Key Laboratory of Polymer Materials, Fuzhou 350007, China
[d] Changchun Center of Mass Spectrometry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
[e] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
[f] Graduate University of the Chinese Academy of Sciences, Chinese Academy of Sciences Changchun, Beijing 100864, China
[g] Department of Chemistry, Bahauddin Zakaryia University Multan, Pakistan, 60800

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ABSTRACT

Benzophenone is one of the most commonly used photoinitiators of UV-cured inks on food packaging materials and can migrate into foodstuffs. In this study, an amperometric benzophenone sensor based on molecularly imprinted polymer (MIP) was successfully constructed for the first time. The sensor was prepared by electropolymerizing *o*-phenylenediamine (*o*-PD) on a glassy carbon electrode (GCE) in the presence of template benzophenone, and then removing the template by immersing the poly-*o*-phenylenediamine film-modified GCE in ethanol. The molecularly imprinted sensor was tested in the presence or absence of benzophenone by cyclic voltammetry and linear sweep voltammetry to verify the changes in the redox peak currents of potassium ferricyanide. The sensor responded sensitively to benzophenone over a linear range of 0.05–5 μM with a detection limit of 10 nM. The imprinted sensor showed high recognition ability for benzophenone and was successfully applied to the determination of benzophenone in food packaging material samples.

1. Introduction

Benzophenone ($Ph_2C=O$, BP) is used as a photoinitiator, a fragrance enhancer, an ultraviolet curing agent, a flavor ingredient, and an additive in plastics, coatings, and adhesives [1]. Especially, BP is one of the most commonly used photoinitiators in the UV-cured inks [2]. However the photoinitiators are not always completely utilized or eliminated after the printing process. Consequently, BP, being a low molecular weight photoinitiator and applied on the outside of the food packaging material, might permeate through the printed material, and finally migrate into the foodstuffs [3–5]. What is more, BP may exist in any packaging made from recycled packaging materials without complete removal of BP, even though that packaging material itself has not been printed with UV-cured inks [5]. The tolerable daily intake for BP is 0.01 mg kg^{-1} body weight [6]. Studies have provided some evidence of the chronic toxicity of BP, such as carcinogenic activity [7], allergic contact dermatitis [8], estrogenic activity [9–11], and bad influence on the ecosystem [12]. Many attempts have been made to analyze BP in food samples. Current methods used to detect BP and its derivatives are mainly GC–MS [13], GC–MS^n [14], HPLC–UV [2,15,16], electrochemical analysis [17] and micellar electrokinetic capillary chromatography coupled with UV [18]. Although these well established methods are provided with a low detection limit, the bulky and expensive apparatus still hinder their practical use. As a result, the development of a convenient and sensitive analytical tool to detect BP is highly required.

Molecularly imprinting technology (MIT) is known as a method for creation of tailor-made binding sites with the memory of shape, size and functional groups toward the template molecules [19–21]. MIP sensors have been proven to have advantages such as strong affinity, excellent selectivity and toughness [22]. MIPs can be prepared through electropolymerization [23,24], self-assembled method [25], chemical grafting [26,27] and photochemical polymerization [28]. Studies on the electrochemical analysis have tended to concentrate on the electropolymerized imprinted film [29–32].

* Corresponding author at: College of Chemistry and Chemical Engineering, Fujian Normal University, Fuzhou 350007, China. Tel./fax: +86 591 83531762.
** Corresponding author. Tel./fax: +86 431 85262747.
E-mail addresses: tongyuejin@yahoo.com.cn (Y. Tong), guobaoxu@ciac.jl.cn (G. Xu).

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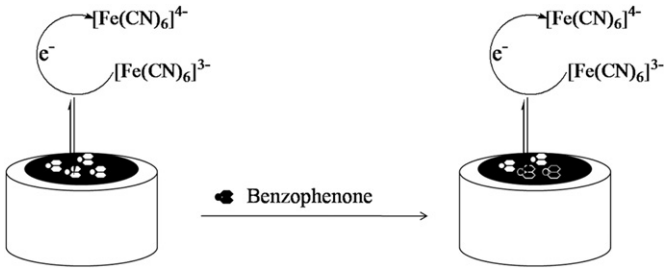


Fig. 1. BP partially blocks the accessibility of ferricyanide probe by selective adsorption in the molecularly imprinted cavities.

In this study, an amperometric BP molecularly imprinted sensor has been reported for the first time. Since poly-*o*-phenylenediamine (PPD) films grow compactly and rigidly and are regarded as mechanically stable [33–35], the sensor was prepared by electropolymerizing *o*-phenylenediamine (*o*-PD) on a glassy carbon electrode (GCE) in the presence of template BP, and then removing the template by immersing PPD film-modified GCE in ethanol. Considering that BP is electro-inactive over the studied potential range, an electroactive substance, potassium ferricyanide, was used as the redox probe of the imprinted film modified electrodes in solutions containing analyte. The imprinted cavities were used as access holes for potassium ferricyanide. BP can occupy the imprinted cavities in the imprinted PPD film (Fig. 1), and block electron transfer of redox probe, resulting in a decrease in current. This decrease in current was then applied to quantify BP concentrations.

2. Experimental

2.1. Materials

o-Phenylenediamine, BP and other chemicals were obtained from Sinopharm Chemical Reagent Company (Shanghai, China). All chemicals were of reagent grade and were used as received. Acetate buffer (pH 5.2) solution was made up from CH_3COONa and adjusted to the desired pH by adding 1.0 M NaOH or CH_3COOH solution. All solutions were prepared with double distilled water.

2.2. Apparatus

All the voltammetric measurements were performed using a CHI800B electrochemical workstation (CH Instruments, Chenhua Co., Shanghai, China) connected to a three-electrode cell. A conventional three-electrode system consisted of a KCl saturated Ag/AgCl electrode as the reference electrode, a platinum wire electrode as the auxiliary electrode and a polymer-modified glassy carbon electrode (3 mm in diameter) as the working electrode. Prior to modification, GCEs were polished with 0.3 and 0.05 μm alumina slurry, respectively, rinsed thoroughly with water between each polishing step, and sonicated in ethanol and water.

2.3. Preparation of imprinted and non-imprinted film modified electrodes

In our process, electropolymerization was performed for MIP preparation. Briefly, the GCE modified with PPD film containing BP was prepared by 30 cycles of cyclic voltammetric measurements in the range 0–0.8 V (scan rate 50 mV s^{-1}) in acetate buffer (pH 5.2) containing 5 mM *o*-PD, 3 mM BP and 2% volume of ethanol. After the electropolymerization, molecularly imprinted polymers modified GCE (MIP-GCE) was obtained by placing the resulting modified GCE in 50 mL ethanol for 15 min to remove BP from the electrode surface. Non-imprinted polymers modified GCE (NIP-GCE) was prepared under the same conditions in the absence of BP.

2.4. Electroanalytical measurements

Amperometric measurements of the imprinted and non-imprinted electrodes were carried out in 1:1 (v/v) aqueous ethanol containing 0.1 M KCl and 1 mM $K_3Fe(CN)_6$. Cyclic voltammetry was performed by potential cycling between −0.2 V and 0.6 V at a scan rate of 50 mV s^{-1}. Linear sweep voltammograms were recorded with scan potential from 0 to 0.6 V at a scan rate of 50 mV s^{-1}. After each experimental run, the sensor was washed in ethanol for 15 min to remove BP on the electrode surface. The electrode is reusable after this cleaning process.

3. Results and discussion

3.1. Cyclic voltammetry study of the electropolymerization of the imprinted and non-imprinted PPD film

Fig. 2A and B shows the typical cyclic voltammograms recorded during the electropolymerization of *o*-PD on GCE in

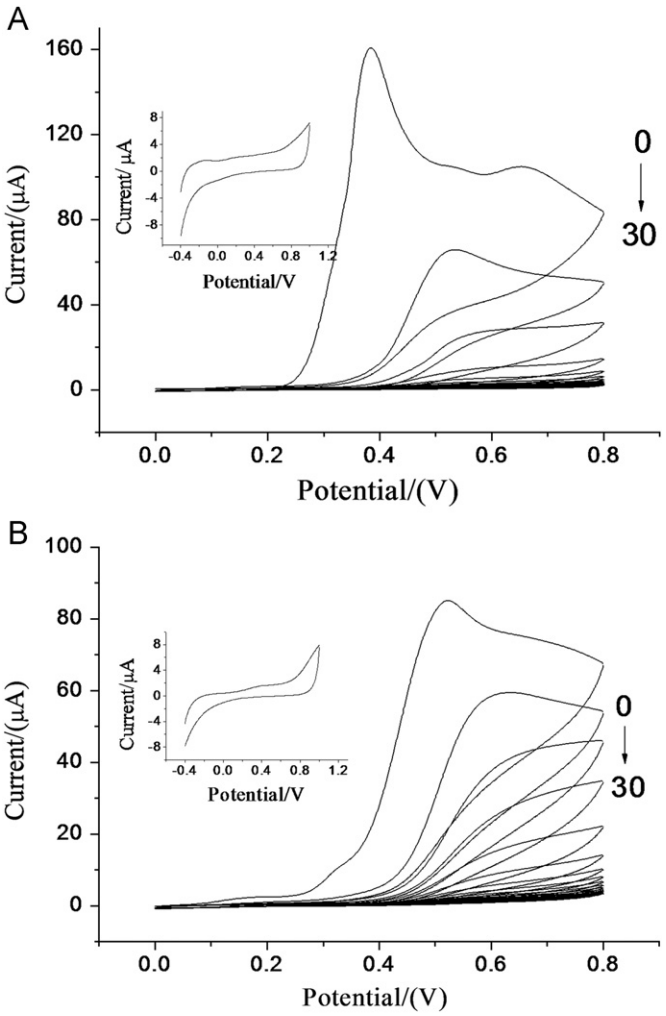
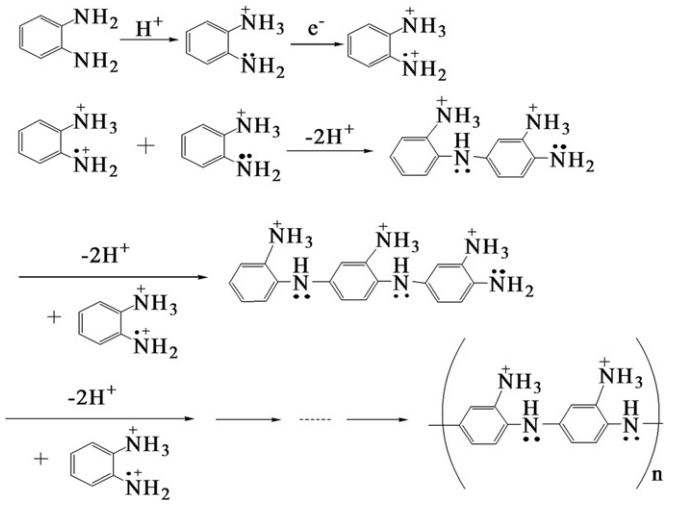


Fig. 2. Cyclic voltammograms for the electropolymerization of *o*-PD in the absence of BP (A) and in the presence of BP (B). Inset is the cyclic voltammograms of poly-*o*-PD electrodes obtained in 0.2 M pH 6.0 phosphate buffer solutions.



Scheme 1. Electropolymerization reaction of *o*-PD.

the absence of BP and presence of BP, respectively. The oxidation wave appears completely irreversible. Scheme 1 shows the electropolymerization reaction of *o*-PD [35]. The peak currents decrease with increasing number of cycles as shown in both Fig. 2A and B. When the number of cycles approached 30, the current of the oxidation peak became much smaller, indicating the formation of nonconductive film on the electrode surface. No significant difference was observed between the cyclic voltammograms obtained in the presence of BP and in its absence during the polymerization. These results demonstrate that BP does not have electroactivity on the GCE.

3.2. Electrochemical characterization of the MIP-GCE and NIP-GCE

Fig. 3A shows the cyclic voltammograms of different modified electrodes recorded in 0.1 M KCl solutions containing 1 mM $K_3Fe(CN)_6$. After the electropolymerization of *o*-PD in the presence of BP on the GCE surface, a dramatical decrease in oxidation–reduction peak current from curve a to curve b was observed because the modified PPD film has partly blocked reactant access to the electrode surface. An apparent increase in peak current was then observed after the template removal step (curve c), indicating the successful generation of imprinted cavities. The decrease in peak current from curve c to curve d can be attributed to the occupation of imprinted cavities by BP after the rebinding step. As for the NIP-GCE (Fig. 3B), the current decreased from curve a to curve e, because the electropolymerization of PPD film in the absence of BP covered the surface of the GCE. The change of current was negligible from curve e to curve f, because no imprinted cavities were obtained after immersing the NIP-GCE in ethanol.

3.3. Effect of the template removing time on the response of the MIP-GCE

With the purpose of obtaining a satisfactory sensitivity, selectivity, and reproducibility, it is very important to elute the template completely. BP has good solubility in ethanol and so ethanol was used to remove the template BP. Cyclic voltammograms were recorded after the MIP-GCE was dipped in ethanol for different time intervals. Fig. 4 shows the relationship between the soaking times and response current. The response currents increase with the increase in soaking time, but gradually approach a stable state after the soaking time is more than

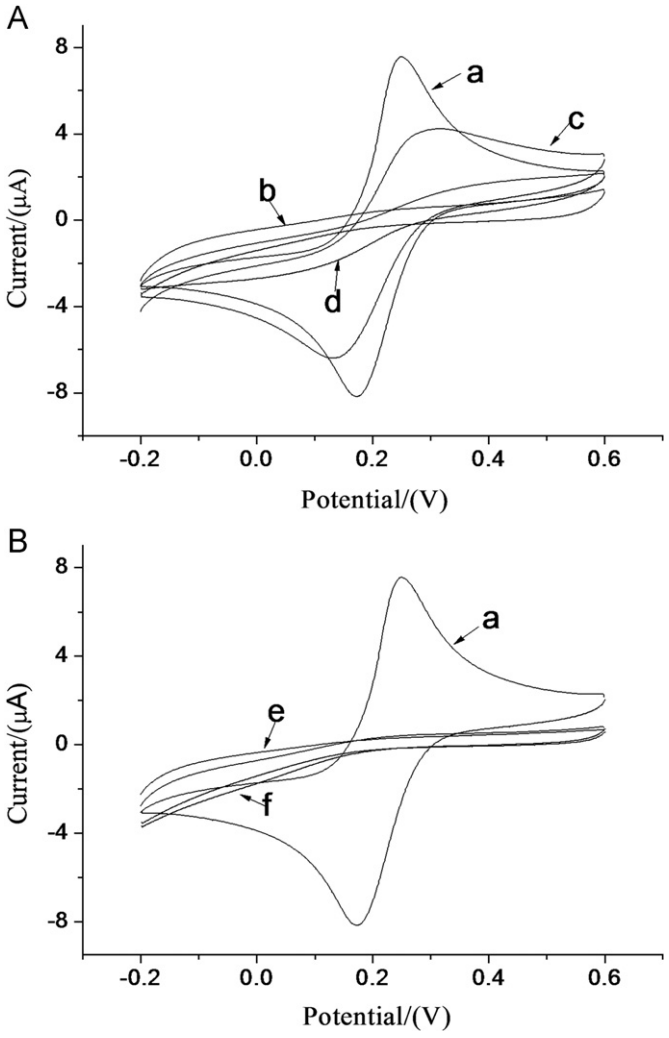


Fig. 3. Cyclic voltammetry results of the electrode in 1:1 (v/v) aqueous ethanol containing 1 mM $K_3Fe(CN)_6$ and 0.1 M KCl: (a) bare GCE, (b) MIP-GCE before template removal, (c) MIP-GCE after template removal, (d) MIP-GCE after rebinding, (e) NIP-GCE, and (f) NIP-GCE after the template removal step.

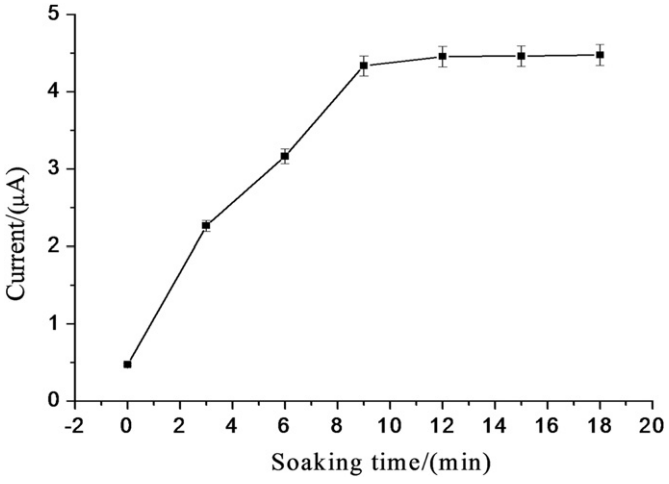


Fig. 4. Effect of soaking time on the response current of the MIP-GCE. Other conditions are as in Fig. 3.

9 min. In order to obtain the highest imprinting efficiency, 15 min was chosen as the optimum soaking time for template removal.

3.4. Effect of the incubation time on the response of the MIP-GCE

After template was removed from the film, the MIP-GCE was immersed in 1:1 (v/v) aqueous ethanol containing 1 mM $K_3Fe(CN)_6$, 0.1 M KCl and 3 μM BP for different time intervals. Then the corresponding response current was measured by cyclic voltammetry. As shown in Fig. 5, the peak currents decrease sharply with the incubation time up to 15 min and level off subsequently. Taking into account the improvement of sensitivity and dependability, 18 min was used for the determination of BP.

3.5. Determination of BP

Using the optimized conditions for the proposed method, linear sweep voltammograms of BP of different concentrations were recorded at the MIP-GCE. Fig. 6 shows the dependence of the reduction current on the concentrations of BP. The peak currents decrease with the increase in BP concentrations due to the occupation of imprinted cavities by BP. The inset in Fig. 6 shows the relationship between the relative current change ($\Delta i/i_0$) and the concentrations of BP. Herein, $\Delta i = i_0 - i_c$, i_0 and i_c are the currents when the concentrations of BP are 0 and c μM, respectively [36]. The relative current change increased with increasing concentrations of BP, and tended to be stable at the high concentration of BP. The relative current changes increase linearly with the concentration of BP in the range 0.05–5.0 μM. The linear calibration curve of ($\Delta i/i_0$) versus concentration of BP (c) can be described by the following equation, $y = \Delta i/i_0 = 0.108c\ \mu M + 0.0115$, and the calculated correlation coefficient is 0.9974. The detection limit is 0.01 μM by the relation $3S_b/S$, where S_b represents the standard deviation of the peak currents of the blank ($n=6$) and S represents the slope of the calibration curve for BP. Compared to other methods [13–16], the MIP sensor has shown high sensitivity for the determination of BP. The comparisons were listed in Table 1.

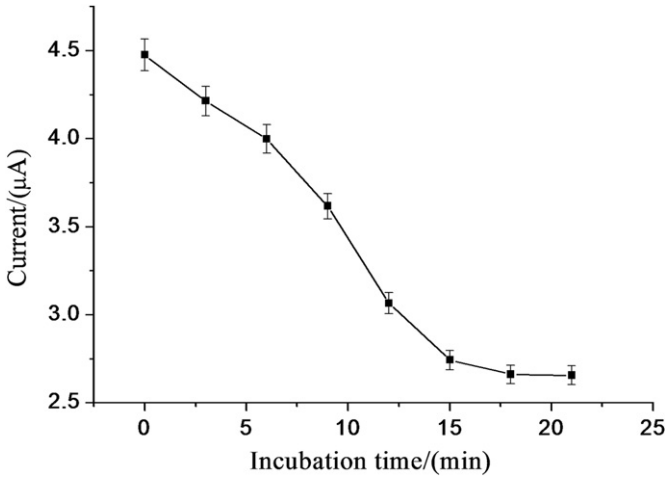


Fig. 5. Effect of incubation time on the response current of the MIP-GCE.

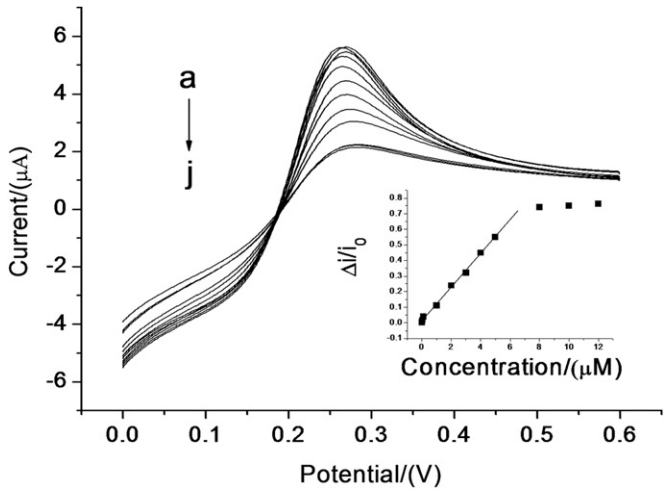


Fig. 6. Linear sweep voltammetry of the imprinted MIP-GCE in 1:1 (v/v) aqueous ethanol solutions containing 1 mM $K_3Fe(CN)_6$, 0.1 M KCl and BP of different concentrations after incubation for 18 min. (a) 0, (b) 0.01, (c) 0.05, (d) 0.1, (e) 1.0, (f) 2.0, (g) 3.0, (h) 4.0, (i) 5.0, (j) 8.0, (k) 10.0 and (l) 12.0 μM. Inset figure is the calibration curve.

3.6. Selectivity, repeatability, and stability of the MIP-GCE

To demonstrate the specificity of the MIP-GCE toward BP, the selectivity of the imprinted sensor was evaluated by testing the cyclic voltammetric response of BP in the presence of some analogs (Fig. 7), including diphenylamine (DPA) and diphenyl ether (DPE). Fig. 8 shows that 5 μM DPA, 5 μM DPE, as well as 5 μM DPA and 5 μM DPE have little effect on the determination of 1 μM BP.

To investigate repeatability, 1 μM BP was determined using the same MIP-GCE. The calculated RSD was about 3.2% ($n=6$). This good repeatability reveals that rebinding of BP is reversible and the MIP-GCE could be regenerated and reused. After the electrode was exposed to air for 15 days at room temperature and used at least 50 times, it reserved 91.25% of its original response, suggesting excellent storage stability.

3.7. Sample analysis

The MIP-GCE was applied to determine BP in food packaging material for practical application. All the analyzed samples were collected from local supermarkets (Table 2) with colorful printing patterns on the surface. Different test samples of 0.5 dm^2 were prepared from each packaging material. Then each specimen was cut into small pieces and placed into hermetically closed vials and extracted with 20 mL ethanol for 24 h at 70 °C. The extracts were simply filtered though microfiltration membrane (0.45 μm) and measured by MIP-GCE after dilution, and by HPLC–UV as a comparison, because HPLC–UV was a widely used method for BP determination only [2]. HPLC analysis was performed on a Waters 2695 system (Milford, MA) with a 2996 photodiode array detector. Analytes were separated on a Diamonsil C18 column (250 mm × 4.6 mm i.d., 5 μm, Dikma, USA). The column temperature was kept at 30 °C. The gradient solvent system consisted of solvent A (acetonitrile) and solvent B (water). Solvent A content of the mobile phase for HPLC separation was increased linearly from 70% to 100% within 10 min. The injection volume was 20 μL for standard and samples. The HPLC–PDA detection wavelength was set at 256 nm for BP. The analytical results are listed in Table 2. The amperometric BP MIP sensor shows comparable results with the HPLC–UV method, indicating that the proposed MIP sensor is effective for the determination of BP in real samples.

4. Conclusion

In this work, a sensitive amperometric BP MIP sensor was implemented via electropolymerizing BP imprinted PPD film on a glassy carbon electrode. The electropolymerization procedure was very simple, rapid, and controllable. The MIP-GCEs have been proved to be selective, repeatable, and stable for the determination of BP. Moreover, it has been applied successfully to the

Table 1
Comparison of the proposed sensor for BP detection with other methods.

Methods	Linear range	Detection limit	Recovery (%)	RSD (%)	References
Amperometric (MIP-GCE)	0.05–5 μM	10 nM	–	3.2	This work
GC–MS	2–10^4 ng L^{-1}	0.5 ng L^{-1}	101.0	4.5	[13]
GC–MS^n	100–1200 μg kg^{-1}	2 μg kg^{-1}	74–98	–	[14]
HPLC–UV	4.5–63 mg L^{-1}	0.017 mg L^{-1}	–	–	[2]
HPLC–UV	0.46–118.4 mg L^{-1}	0.046 mg L^{-1}	–	–	[15]

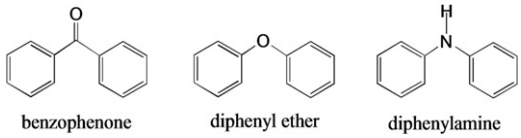


Fig. 7. Chemical structures of benzophenone, diphenyl ether, and diphenylamine.

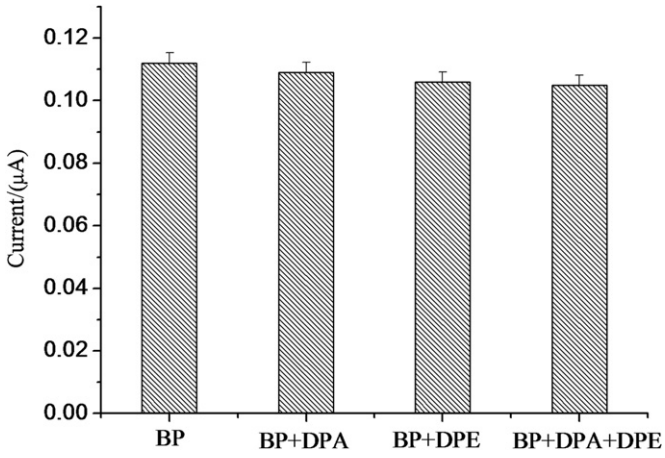


Fig. 8. Peak current response of MIP-GCE incubated in 1:1 (v/v) aqueous ethanol solutions containing 1 mM $K_3[Fe(CN)_6]$, 0.1 M KCl and (a) 1 μM BP, (b) 1 μM BP+5 μM DPA, (c) 1 μM BP+5 μM DPE, and (d) 1 μM BP+5 μM DPA+5 μM DPE. Incubation time: 18 min.

Table 2
Application of the proposed method to detect BP in food packaging plastics for cake, bakery product, and milk.

Sample	Food type	Component	Layer number	BP (mg dm^{-2})	
				Amperometric (MIP-GCE)	HPLC–UV
1	Cake	Plastic	1	0.21	0.23
2	Cake	Plastic	2	0.40	0.39
3	Bakery product	Plastic	1	0.24	0.28
4	Milk	Plastic	1	0.23	0.21
5	Milk	Paper and plastic	2	0.33	0.34

determination of BP in food packaging materials. The method is an attractive alternative to GC–MS, GC–MS^n, and HPLC–UV for the determination of BP.

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