Journal of Applied Polymer Science

Determination of Epigallocatechin-3-Gallate with a High-Efficiency Electrochemical Sensor Based on a Molecularly Imprinted Poly(*o*-phenylenediamine) Film

Yuqing Duan,[1] Xiaoping Luo,[1] Yu Qin,[1] Haihui Zhang,[1] Guibo Sun,[2] Xiaobo Sun,[2] Yongsheng Yan[1]

[1]School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China
[2]Institute of Medicinal Plants, Chinese Academy of Medical Sciences, Beijing 100193, China
Correspondence to: H. Zhang (E-mail: dyq101@ujs.edu.cn)

ABSTRACT: An electrochemical molecularly imprinted polymer (MIP) sensor for detecting the existence of epigallocatechin-3-gallate (EGCG) in tea and its products was successfully developed on the basis of a glassy carbon electrode modified with an electropolymerized nonconducting poly(*o*-phenylenediamine) film. The properties of the electrode were characterized by cyclic voltammetry, differential pulse voltammetry, and infrared spectroscopy. The template molecules could be rapidly and thoroughly removed by methanol/acetic acid. The linear response range for EGCG was 5.0×10^{-7}–1.0×10^{-4} mol/L, and the limit of detection was as low as 1.6×10^{-7} mol/L. The prepared MIP sensor could discriminate between EGCG and its analogs. In addition, satisfactory results were obtained in the detection of real tea samples. The results of our investigation indicate that the MIP sensor was useful for the determination of EGCG with excellent selectivity, high sensitivity, repeatability, and reproducibility. This MIP sensor provides the potential for monitoring the variation of EGCG content during the industrial processes and for predicting the quality of tea and its products.
 J. Appl. Polym. Sci. 129: 2882–2890, 2013

KEYWORDS: electrochemistry; films; molecular recognition

Received 13 August 2012; accepted 3 January 2013; published online 12 February 2013
DOI: 10.1002/app.39002

INTRODUCTION

Tea is one of the most popular beverages across the world and has an expanding market because of its wonderful taste, aroma, and health benefits. In the tea trade, the price depends on quality. In general, the quality of tea is evaluated through its appearance, scent, and flavor by a specially trained professional.[1] However, the results of professionals are highly subjective. Moreover, tea quality largely depends on the color differences of tea infusions and the components in the leaves, such as their free amino acids, caffeine, catechins, and ascorbic acid.[2] Therefore, it is important to develop precise methods for the objective evaluation of tea quality.

In the past few years, researchers have endeavored to develop chemical and physical methods for evaluating tea quality. Many advanced techniques, such as nuclear magnetic resonance,[3] electronic nose,[4] capillary electrophoresis,[5] near infrared spectroscopy,[6] high-performance liquid chromatography,[7] and electronic tongues[8] have been used to estimate the quality of tea. However, these methods have not been used broadly in commercial practices because of their high cost, the requirements of technical personnel, and experimental installation. In view of this, it is still necessary to develop simple and cheap methods for estimating tea quality.

Epigallocatechin-3-gallate (EGCG) is the most abundant catechin present in green tea,[9] and it is regarded as the source of the greatest biological activity in tea. In recent studies, it has been shown that EGCG has antioxidant, antimutagenic, and anticancer properties.[10] In addition, EGCG has been found to possess extensive biological activity, such as free-radical scavenging,[11] antiviral properties,[12] anti-inflammatory properties,[13] vascular disease prevention properties,[14] and radiation protection activity.[15] Therefore, the strength of the biological activity in tea and its products is directly determined by the content of EGCG, and the development of a method for detecting the content of EGCG in tea and its products would be significant. For EGCG analysis, many methods have been developed on the bases of, for example, liquid chromatography,[16] micellar electrokinetic chromatography,[17] and capillary electrophoresis.[18] However, the inherent limitations (time consuming and/or the high price of equipment) of those analyses have made it necessary for researchers to study more preferable methods for detecting

EGCG. Recently, an electrochemical method has been applied to detect the concentration of EGCG.[19,20] This method is fast, highly sensitive, and low cost. Nevertheless, low selectivity is still a problem, and this affects the accuracy of the methods used to detect EGCG. That is, the development of a low-cost method with excellent selectivity in the detection of EGCG in tea is still necessary.

Molecular imprinting technology (MIT) is a viable approach for designing molecularly imprinted polymers (MIPs) with molecular recognition cavities that have specific selectivity for the template molecules.[21] Since the initial example of MIT was reported by Wulff and Sarhan's[22] research group in 1972, MIT has attracted considerable attention, especially during the last 2 decades because of its importance in the preparation of antibody-like polymers. Because of their fine mechanical and chemical stability, high affinity, excellent template molecule recognition ability, and low cost of preparation, MIPs have been recently applied in many fields successfully. The field of application includes separations,[23] chiral resolution,[24] catalysis,[25] and sensoring.[26] In the application of MIPs as sensing components, the great majority of the reported MIP syntheses have been polymerizations with acrylic or vinylic types of monomers. Since the successful application of electropolymerized MIPs as sensor elements were established by Malitesta et al.[27] and Panasyuk et al.[28] in 1999, more and more electrochemical sensors based on MIP have been prepared successfully by electropolymerization.[29] This trend may be explained by the fact that this method has many advantages, including excellent selectivity, stability, and easy control of the thickness of the polymer films.[30,31] Furthermore, electropolymerization can form multilayer structures and fabricate fast-responding sensors with lessened interferences.[32,33]

In this study, we aimed to prepare an electrochemical sensor that could detect the concentration of EGCG by differential pulse voltammetry (DPV) in an electrolyte solution. Because *o*-phenylenediamine (*o*-PD) has been proven to be easily electropolymerized on various materials to form a nonconducting polymer film with good chemical and mechanical stability[34,35] and has been used as a functional monomer in many studies,[30,33,36] it was chosen as functional monomer of electropolymerization. The preparation conditions for the electrochemical sensor, suitable performing conditions, relationship of concentration and response, detection limit, selectivity, repeatability, reproducibility, and recovery of the prepared sensor were investigated and are discussed.

EXPERIMENTAL

Materials

EGCG used as the template and epicatechin gallate (ECG), epigallocatechin (EGC), and (+)-catechin (C) as analogs (>99%) were supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structures of EGCG and its analogs are shown in Figure 1. The functional monomer of *o*-PD and a probe of $K_3[Fe(CN)_6]$ were bought from Sinopharm Chemical Reagent Co. (Shanghai, China). Phosphate buffer solution (PBS; formed by the mixture of KH_2PO_4 with Na_2HPO_4 solutions and adjusted with sodium hydroxide or hydrochloric acid) was used as a supporting electrolyte solution. All other externally used chemicals were at least analytical grade, were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China), and were used without further purification.

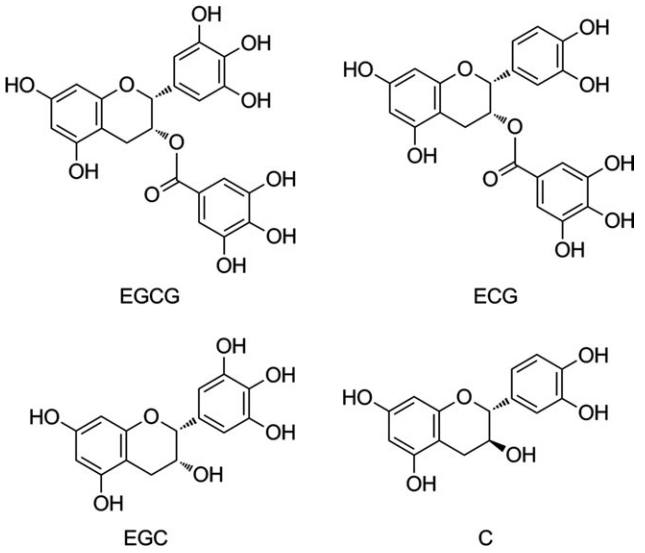

Figure 1. Structures of the template EGCG and its analogs.

Instruments

All electrochemical procedures such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), and alternating-current (ac) impedance were performed on a CHI 660D electrochemical workstation (Shanghai, China) with a standard three-electrode system controlled by a Lenovo computer. Electropolymerization was accomplished with a platinum counter electrode, an Ag/AgCl reference electrode, and a glassy carbon (GC) electrode with a diameter of 3.0 mm as the working electrode in electrolyte solution. Fourier transform infrared (FTIR) spectra of the poly(*o*-phenylenediamine) [poly(*o*-PD)] films were recorded in the range of 400–4000 cm^{-1} with a Nicolet Nexus 670 FTIR spectrometer (Madison, America).

Pretreatment of the GC Electrode

The surface of the GC electrode was polished with 0.3- and 0.05-mm alumina slurries on chamois leather consecutively until a shiny surface like a mirror appeared, and then it was sonicated with a ZF ultrasonic cleaner (Shanghai Zhifeng, China) in ethanol and double-distilled water for 3 min sequentially to remove the trace alumina. To ensure a clean electrode surface, the polished electrode was soaked in 0.5 mol/L H_2SO_4 solution and subjected to cyclic potential sweeps between 0 and 1.2 V until a stable cyclic voltammogram was obtained.[34]

Preparation of the MIP Electrode

Electropolymerization was done by CV (15 cycles) in the potential range 0–0.8 V at a scan rate 50 mV/s in PBS (pH 5.2), containing 5 mmol/L *o*-PD. For the preparation of the imprinted poly(*o*-PD) film, EGCG was also added at a concentration of 1 mmol/L. The electrode was then modified with the EGCG imprinted film and rinsed in methanol/acetic acid (90:10 v/v) for 13 min to remove EGCG entrapped in the polymeric matrix. After these steps, the MIP electrode was prepared. The NIP electrode used as a control electrode was gained in the same way

Applied Polymer SCIENCE

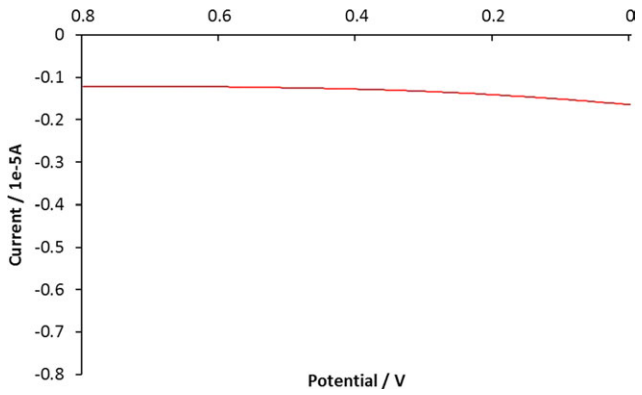

Figure 2. DPV of the bare GC electrode performed in PBS in the presence of EGCG. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

but without the addition of EGCG. Electrochemical solutions were thoroughly deoxygenated by N_2 bubbling before use.

Sample Preparation

Qiandaoyu tea, Longjing tea, and white tea were purchased from a market in Zhenjiang, China. Tea soup samples were obtained by the extraction of 0.200 g of dry tea leaves with 100 mL of boiled double-distilled water for 3 h. The obtained tea extract was filtered by a centrifuge for 5 min at 2000 rpm and then diluted 10 times by double-distilled water. The pH of the tea extract was adjusted to 5.0 with phosphate.

Electrochemical Measurements

Electrochemical measurements were performed with the three-electrode system, which was immersed in PBS (15 mL, pH 5.0) containing 5 mmol/L $K_3[Fe(CN)_6]$ and 0.2 mol/L KCl at room temperature. After each measurement, the MIP electrode was treated with methanol/acetic acid (90:10 v/v) for 13 min. When the MIP electrode was not in use, it was stored in PBS (pH 7.0) at 4°C.

CV was performed from 0.8–0.2 V with a scan rate at 100 mV/s. DPV was performed from 0.4–0.1 V, the pulse amplitude was 50 mV, the pulse width was 50 ms, the pulse period was 0.2 s, and the potential increment was 4 mV. ac impedance measurements were carried out by the application of a potential of 0.18 V over a frequency range from 0.01 Hz to 10,000 Hz with an alternating voltage of 5 mV.

RESULTS AND DISCUSSION

Electrochemical Stability of EGCG

The electrochemical stability of EGCG was evaluated by DPV in PBS (pH 5.2). Figure 2 shows the DPV of the bare GC electrode performed in the presence of EGCG. As shown in Figure 2, no peak current can be found, which indicates that EGCG has no electrochemical activity in the potential range of 0–0.8 V, meaning, the chemical structure of EGCG would not change during the process of CV scans.

Electropolymerization of *o*-PD

The EGCG-imprinted film covered on the surface of GC electrode was prepared by the electropolymerization of *o*-PD by CV scanning in PBS (pH 5.2) in the presence of 0.1 mmol/L EGCG

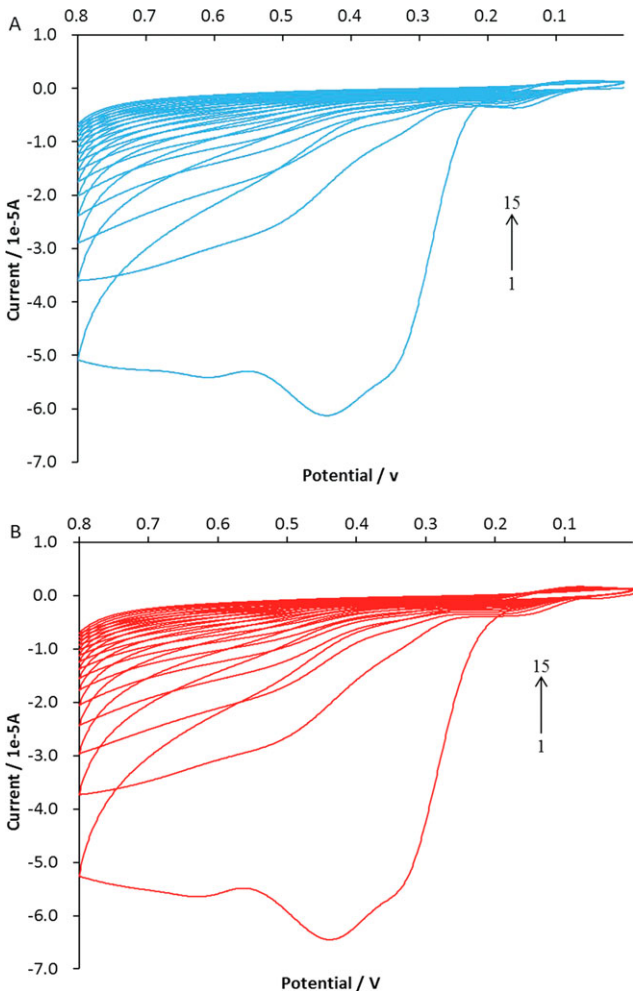

Figure 3. CV for the electropolymerization of 5 mmol/L *o*-PD on the GC electrode in PBS (pH 5.2) in the (A) presence of EGCG at a concentration of 1.0 mmol/L and (B) absence of EGCG (scan rate = 50 mV/s, sweep cycle = 20). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and 5 mmol/L *o*-PD. The typical cyclic voltammograms recorded during the preparation of poly(*o*-PD) film in the presence of EGCG are shown in Figure 3(A). As suggested by the CV, the oxidation reaction appeared to be completely irreversible. In the first scan, an *o*-PD oxidation peak current of -0.654×10^{-5} A appeared at 0.436 V. Then, the peak current of oxidation reaction decreased dramatically with each cycle, and finally, the peak current approached approximately zero; this indicated that a nonconducting polymer film was formed on the GC electrode surface and blocked the monomers from reaching the surface of the GC electrode.

Figure 3(B) shows the CV performed under the same conditions (*o*-PD concentration and pH) but in the absence of EGCG. By comparing Figure 3(A) with Figure 3(B), no remarkable differences were observed between the cyclic voltammograms obtained in the presence or absence of EGCG; we interpreted that to mean that EGCG did not have electroactivity on the GC electrode in the polymerization potential range 0–0.8 V. The

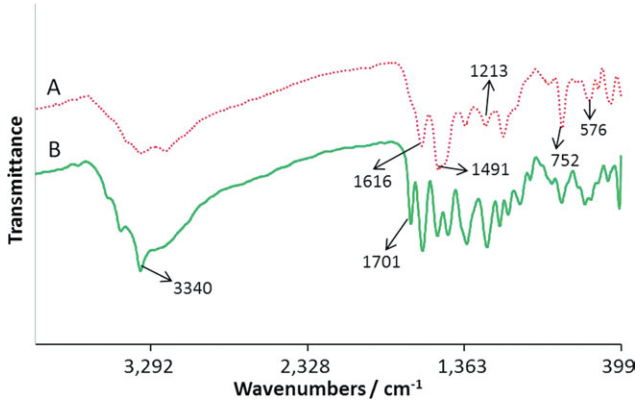

Figure 4. FTIR spectra of the (A) nonimprinted and (B) imprinted poly(*o*-PD) films. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

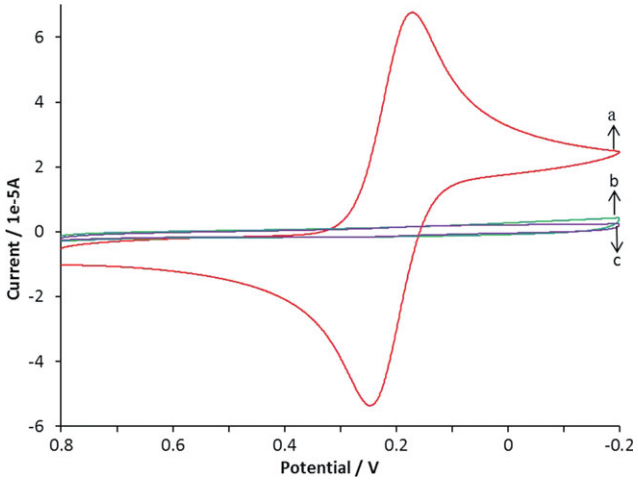

Figure 5. CV of different electrodes in PBS (pH 5.0) containing 5 mmol/L $K_3[Fe(CN)_6]$ and 0.2 mol/L KCl: (a) bare electrode, (b) GC electrode modified with the EGCG-imprinted film, and (c) GC electrode modified with the nonimprinted film (scan rate = 100 mV/s). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

result reveals that the structure of EGCG did not change during the electropolymerization process. Meanwhile, EGCG had no impact on the electropolymerization.

In addition, by comparison with Figure 3(B), it can be observed there was a feeble decrease in the peak current shown in Figure 3(A); this could be explained by the fact that EGCG mixed in the *o*-PD and accessed the surface of the electrode. This resulted in a reduced intensity.

FTIR Analysis

The FTIR spectra of the imprinted and nonimprinted poly (*o*-PD) films were measured. Figure 4 shows the FTIR spectra of the nonimprinted [Figure 4(A)] and imprinted [Figure 4(B)] poly(*o*-PD) films. As shown in Figure 4(A), the broad peak appearing between 3412 and 3155 cm^{-1} represented the absorption band of the N—H stretching vibration of the —NH— group. The peaks at 1616, 1491, and 1213 cm^{-1} were attributed to the C=N, C=C stretching vibrations in the phenazine ring along the polymer chain and the C—N—C stretching in the benzenoid units, respectively. Furthermore, strong bands at 752 and 576 cm^{-1}, which were characteristic of the C—H out-of-plane bending vibrations in the phenazine ring, were also obtained. The spectra of the imprinted poly(*o*-PD) film was similar to that of the nonimprinted film except for the strong peaks at 3340 and 1701 cm^{-1}, which contributed to the stretching vibration of —OH and —C—O, which existed in the EGCG molecules. The previous results suggest that EGCG was imprinted in the poly(*o*-PD) film during the electropolymerization of *o*-PD in the presence of EGCG.

Electrochemical Characterization of the MIP Electrode

To confirm the electrochemical properties of the different electrodes, CV experiments were carried out after each kind of electrode was prepared. Figure 5 shows the CV of three different electrodes in PBS (pH 5.0) containing 5 mmol/L $K_3[Fe(CN)_6]$ and 0.2 mol/L KCl at room temperature. Curve a shows a pair of typical redox peaks of ferricyanide occurring on the surface of the bare GC electrode. The difference in the peak potentials between the oxidative and reductive reactions was 74 mV, and the ratio of the peak currents was about 1:1, respectively; this indicated that the bare GC electrode was activated successfully. Curves b and c show the cyclic voltammograms with no peak appearing for the electrode modified with imprinted or nonimprinted films. In addition to this, we observed that the response of the modified electrode was lower than that of the bare GC electrode. The results indicate that a nonconductive poly(*o*-PD) film was successfully formed on the electrode surface and hindered ferricyanide from getting access to the surface of the GC electrode.

CV of the MIP electrode and NIP electrode was also done in PBS (pH 5.0) containing 5 mmol/L $K_3[Fe(CN)_6]$ and 0.2 mol/L KCl. To further verify whether EGCG was embedded in the imprinted film, EGCG was also added at a concentration of 0.1 mmol/L. Figure 6 shows a typical comparison of the CV between the MIP electrode and the NIP electrode. We found that there was no redox peak current [Figure 6(c)] for the NIP electrode; however, a standard pair of redox peaks was clearly observed in the curve for the MIP electrode [Figure 6(a)]. This result indicates that ferricyanide had access to the surface of the GC electrode modified with the imprinted film after removal of the template. This behavior was attributed to the presence of cavities formed after the entrapped template in the imprinted film was extracted with methanol/acetic acid (90:10 v/v). Figure 6(b) shows the CV response of the MIP electrode after incubation for 13 min in the presence of EGCG at 0.1 mmol/L. Through a comparison with curve a, the lower peak current could be found. This result implies that the MIP electrode could absorb the template, and this led to a decrease in the peak current in the presence of EGCG. As shown in curve d, the CV of the NIP electrode was obtained under the same conditions, and there was no current response observed. Meanwhile, a little smaller current is shown in curve d compared to that shown in curve c. The result means that the NIP electrode could not distinguish the template molecule, but it could absorb the template

Applied Polymer SCIENCE

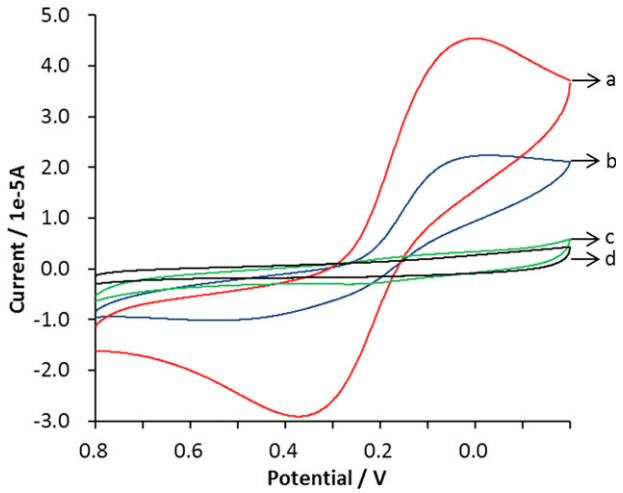

Figure 6. CV of the MIP and NIP electrodes in PBS (pH 5.0) containing 0.2 mol/L KCl and 5 mmol/L $K_3[Fe(CN)]_6$: (a,c) in the absence of EGCG and (b,d) in the presence of EGCG at 0.1 mmol/L. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

slightly by a process called *physisorption*. As suggested previously, the results of the DPV tests indicate that EGCG was embedded in the imprinted film covering the surface of the GC electrode, and this could be explained by the formation of hydrogen bonds between the hydroxyl of the EGCG molecule and the amino groups of *o*-PD. This led to the insertion of EGCG molecules into the poly(*o*-PD) film during the polymerization process. After removal of the template molecules, many cavities corresponding to the structure of the EGCG molecule in the poly(*o*-PD) film were left, and this allowed the ferricyanide to reach the surface of the electrode and led to the corresponding electrochemical response. This means that the MIP electrode, used as a component of the MIP sensor, was prepared correctly.

The method of ac impedance, which is efficient for studying the interface properties of surface-modified electrodes, was applied for the characterization of the electrodes modified with the imprinted poly(*o*-PD) film. Figure 7(a–c) illustrates the Nyquist impedance diagrams of the bare GC electrode and MIP electrode before and after the removal of EGCG, respectively, recorded in PBS containing 5 mmol/L $K_3[Fe(CN)_6]$ and 0.2 mol/L KCl. The semicircle diameter corresponds to the surface electron-transfer resistance (R_p). An almost straight line on the bare GC electrode [Figure 7(a)] was obtained, and a R_p value of 4230 ohm [Figure 7(b)] was also obtained from the MIP electrode without the removal of EGCG; this indicated that the coating of the poly(*o*-PD) film impeded the electron transfer. In addition, after EGCG was removed from the imprinted film, R_p decreased obviously to 2048 ohm [Figure 7(c)], and this further illustrated that the binding sites were formed and facilitated the diffusion of ferricyanide. These results were consistent with the CV tests.

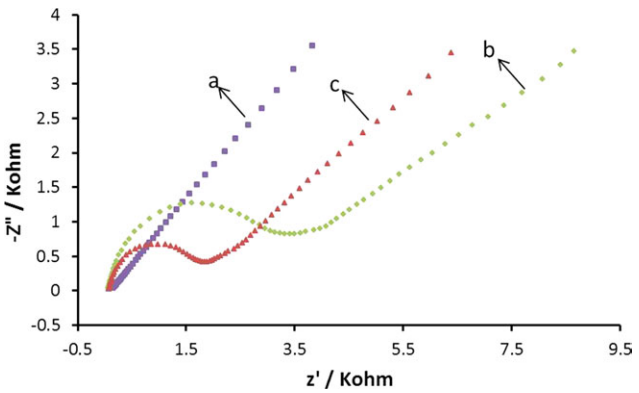

Figure 7. ac impedance spectroscopy of (a) bare GCE and the MIP electrode (b) before and (c) after the removal of EGCG performed in PBS (5.0) containing 5 mmol/L $K_3[Fe(CN)_6]$ and 0.2 mol/L KCl.

Effect of pH

On the basis of the determination of EGCG on the poly(*o*-PD) film, the current response of MIP electrode is largely affected by the pH value of the test solution. To research the influence of pH on the MIP sensor, DPV tests were carried out in PBS at different pH values. Because the EGCG molecule has been proved unstable in alkalinity solution,[37] seven different acid solutions were tested in this study. Figure 8 shows the DPV of MIP electrode performed in test solution at different pH values. When pH was less than 5, the peak current decreased with increasing solution pH. On the contrary, the peak current responses of the MIP electrode increased gradually when the pH was greater than 5. The minimum response current was obtained at a pH value of 5. Therefore, the test solution with a pH of 5 was chosen for all further experiments.

Template Removal Time

It is difficult to obtain an MIP electrode with satisfactory sensitivity, selectivity, and reproducibility if the template molecule cannot be removed thoroughly. The template removal treatment needs to be improved upon. Ethanol, double-distilled water, and methanol/acetic acid (90:10 v/v) were applied as eluents to

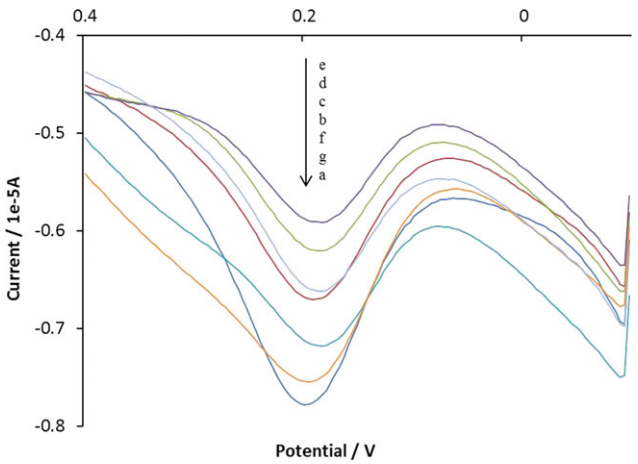

Figure 8. DPV of the MIP electrode in PBS containing 0.1 mmol/L EGCG at different pH levels: (a) 3.0, (b) 3.5, (c) 4.0, (d) 4.5, (e) 5.0, (f) 5.5, and (g) 6.0. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

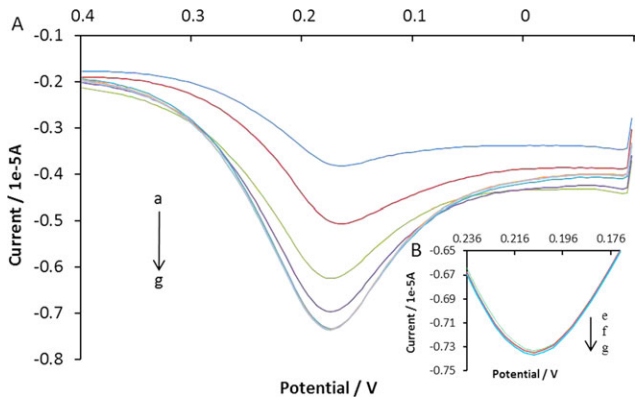

Figure 9. (A) DPV of different template removal times of (a) 1, (b) 4, (c) 7, (d) 10, (e) 13, (f) 16, and (g) 19 min (scan rate = 100 mV/s). (B) Amplifications of parts e, f, and g. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

remove the template molecules. Electrodes modified with the imprinted poly(*o*-PD) film obtained under the same conditions were each soaked in three kinds of eluent to remove the EGCG molecules, and then, the elution effects were determined by a comparison of the peak currents of the DPV tests performed with the eluted electrodes. The results indicate that ethanol and double-distilled water could only elute the EGCG template partially, whereas the methanol/acetic acid (90:10 v/v) solution could remove the template molecules quickly and thoroughly.

To determine the elution time to further enhance the elution effect, DPV was performed after the GC electrode modified with imprinted film was soaked in methanol/acetic acid (90:10 v/v) for different times. DPV with different removal times is shown in Figure 9. At the beginning, the peak current increased rapidly with increasing removal time. However, a stable peak current was obtained gradually after the removal time was longer than 13 min. The results of DPV demonstrate that the template molecules were removed thoroughly after extraction for 13 min. Therefore, dipping in methanol/acetic acid (90:10 v/v) for 13 min was chosen as the best condition for template removal in this study.

Incubation Time

The incubation step is usually an efficient way to enhance the sensitivity of an imprinted electrochemical sensor. After the EGCG molecules were removed from the imprinted poly(*o*-PD) film, the MIP electrode was incubated in PBS (pH 5.0) containing 0.1 mmol/L EGCG, 0.2 mol/L KCl, and 5 mmol/L $K_3[Fe(CN)_6]$. DPV was performed to record the change of the peak current with increasing incubation time at 2 min intervals. As shown in Figure 10, the peak current increased significantly with increasing incubation time in the beginning, and a stable peak current was gained after 11 min of incubation. This means that the absorption equilibrium was reached. Therefore, an incubation time of 11 min was chosen as the optimum incubation time before measurement in this study.

Detection of EGCG

EGCG can be detected by an indirect method with the MIP sensor. Ferricyanide was used as the probe between the MIP electrode and the supporting electrolyte solution containing EGCG. The peak current produced by ferricyanide decreased when the cavities in the imprinted film were occupied by EGCG. This means that the lower the obtained peak current was, the higher the concentration of EGCG was. In addition, the responses of the MIP sensor to EGCG were linearly proportional to the concentration of EGCG.

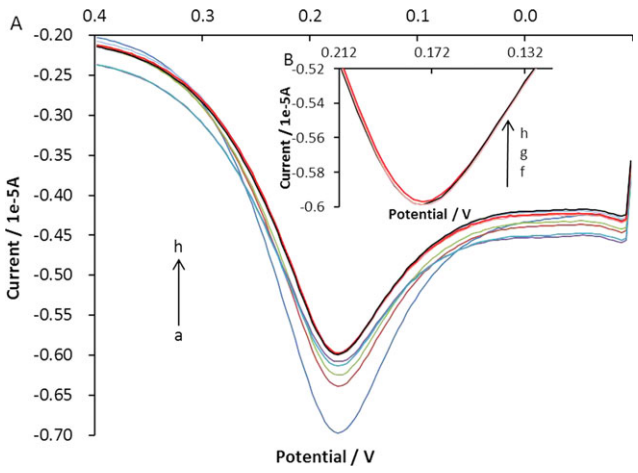

Figure 10. (A) DPV of different incubation times of (a) 1, (b) 3, (c) 5, (d) 7, (e) 9, (f) 11, (g) 13, and (h) 15 min (scan rate = 100 mV/s). (B) Amplifications of parts f, g, and h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Under the previously discussed optimal conditions, the MIP sensor was used to determine the concentration of EGCG. DPV was performed in PBS (pH 5.0) containing 0.2 mol/L KCl and 5 mmol/L $K_3[Fe(CN)_6]$ after the MIP electrode was incubated for 11 min. Figure 11(A) shows the responses of DPV. The peak current decreased with increasing EGCG concentration, and the peak current change tended to be stable within the high concentration range; this indicated that the imprinted cavities were

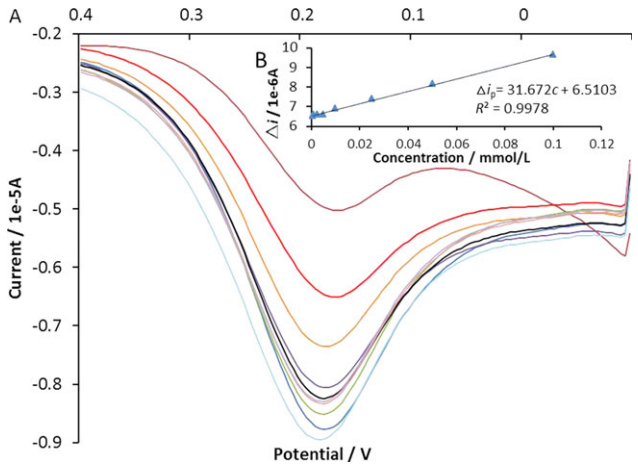

Figure 11. (A) DPV of EGCG at the MIP electrode in PBS (pH 5.0) with the EGCG concentrations ranging from 0.5 to 100 μmol/L. (B) The linear relationship between Δi_p and the concentration of EGCG. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Applied Polymer SCIENCE

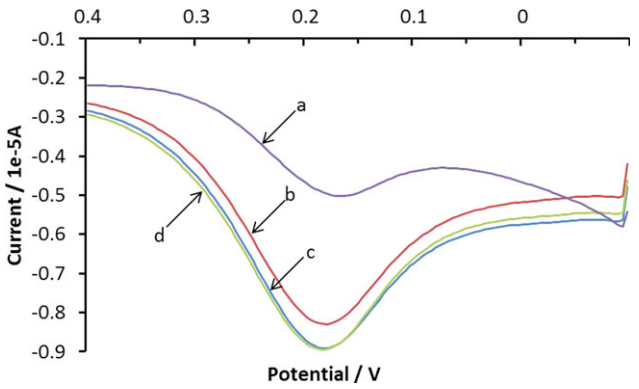

Figure 12. DPV of the MIP electrode after incubation in the test solution containing EGCG or its analogs for 11 min: (a) EGCG, (b) ECG, (c) C, and (d) EGC. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

gradually occupied by EGCG molecules and that the number of ferricyanide ions accessing the surface of GC electrode largely decreased. Figure 11(B) displays a calibration curve between the relative peak current (Δi_p) and the concentration (c) of EGCG in the range 5.0×10^{-7}–1.0×10^{-4} mol/L. Here, $\Delta i_p = i_0 - i_c$, where i_0 (11.020 μA) and i_c are the responses of the MIP sensor when the concentrations of EGCG were 0 mmol/L and c, respectively. The linear regression equation can be expressed as follows: Δi_p (μA) = 31.672c (mmol/L) + 6.510, and the correlation coefficient (R^2) was 0.9978. The limit of detection (LOD) was evaluated with the expression LOD = $(3\sigma)/s$,[38] where σ indicates the standard deviation of the seven consecutive measurements of the detection solution in the absence of EGCG, and s is the sensitivity obtained from the slope of the calibration curve. The value obtained was LOD = 1.6×10^{-7} mol/L. In addition, when the concentration of EGCG was higher than 1.0×10^{-4} mol/L, the peak current response of the MIP electrode in $K_3[Fe(CN)_6]$ solution became stable, and the linear relationship was destroyed.

Selectivity

As sensing elements of MIP sensors, MIP electrodes should have specific recognition to the template molecule, and the specific recognition is based on the molecular structure of the template and interactions between template and the imprinted cavities. To verify the specific selectivity of the MIP electrode for the detection of EGCG, interference experiments were performed by DPV. Before the analyses, analog solutions with the same concentration (0.1 mmol/L) were prepared with PBS (pH 5.0) containing 5 mmol/L $K_3[Fe(CN)_6]$ and 0.2 mol/L KCl, respectively. Figure 12 shows the results of the DPV tests performed with the MIP electrode for detection of EGCG and its analogs after 11 min of incubation. We found that the peak currents of the analogs were much larger than those of EGCG. The reason for this result was that the molecules of the analogs could not bind easily with the cavities in the imprinted film covered on the electrode. A great deal of ferricyanide accessed the surface of the GC electrode via the cavities and generated electricity. On the contrary, EGCG molecules occupied the cavities rapidly and led the peak current to decrease in abundance.

Table I. Responses of the MIP Electrode to EGCG and Its Analogues

Analyte	Concentration (mol/L)	Δi (μA)	Selectivity coefficient (k_{pc})
EGCG	1×10^{-4}	9.637	1
ECG	1×10^{-4}	6.286	1.533
C	1×10^{-4}	4.928	1.956
EGC	1×10^{-4}	4.780	2.016

To further confirm the selectivity of the MIP electrode, the response selectivity coefficient ($K_{pc} = \Delta i_p/\Delta i_c$)[39] was used in this study to demonstrate the selectivity of the MIP electrode, where Δi_p is the relative peak current change of the MIP electrode to 0.1 mmol/L EGCG and Δi_c is the corresponding relative current change of the MIP electrode to the 0.1 mmol/L analogs. As shown in Table I, the K_{pc} values of ECG, C, and EGC were 1.533, 1.956, and 2.016, respectively. We found that the analogs mentioned previously showed negligible interference with the determination of EGCG, and the reason was that the cavities matched the structure of the EGCG molecules existing in the poly(o-PD) film covering the surface of the MIP electrode. However, the structure of the interfering substances did not match the spatial structure of the cavities, so it could not fill the cavities to weaken the current response of the MIP electrode to the $K_3[Fe(CN)_6]$ solution. As suggested previously, the prepared EGCG MIP electrode had the specific capacity to recognize EGCG and could prevent the interference of analogs. This indicated that an MIP sensor based on a GC electrode modified with imprinted poly(o-PD) film for the detection of EGCG was prepared correctly.

Repeatability and Reproducibility

The repeatability of the MIP sensor was studied at an EGCG concentration of 7.5×10^{-2} mol/L with the same MIP electrode. Satisfactory repeatability was obtained with a standard deviation of 0.0177 (n = 5), and the peak currents of DPV were 2.314, 2.201, 2.121, 2.089, and 2.125 μA, respectively. From the linear regression equation, the concentration of EGCG was calculated to be 7.56×10^{-2} mmol/L. Through a comparison with the real concentration, an acceptable relative error of 0.8% was obtained. In addition, the reproducibility for the fabrication of five repeated MIP electrodes (independently prepared under the same conditions) was also investigated in the presence of a 7.5×10^{-2} mol/L EGCG solution. The results obtained were as follows: 2.348, 2.284, 2.015, 2.208, and 2.214

Table II. Detection of EGCG in Real Samples by the MIP Electrode

Variety	Weight (g)	Extract volume (mL)	Dilution factor (×)	Δi_p (μA)	c(mmol/L)
Qiandaoyu tea	0.2003	100	10	7.833	4.178×10^{-2}
Longjing tea	0.2010	100	10	7.978	4.633×10^{-2}
White tea	0.2005	100	10	7.481	3.065×10^{-2}

Table III. Determination of EGCG by the MIP Electrode in Real Samples

Variety	Added (mmol/L)	Found (mmol/L)	Recovery (%)	RSD (%)
Qiandaoyu tea	0.01	0.00986	98.6	2.38
Longjing tea	0.01	0.01022	102.2	2.21
White tea	0.01	0.00993	99.3	2.48

$n = 7$.

μA, and the relative standard deviation (RSD) was calculated to be 5.65%. This indicated acceptable interelectrode reproducibility.

Sample Analysis

To demonstrate the practical usage of the MIP sensor, three kinds of tea samples (20 mL, respectively) were assayed by DPV test performed with MIP electrode. The concentration of EGCG was calculated with the linear regression equation. As shown in Table II, Δi_p of the samples Qiandaoyu tea, Longjing tea and white tea were 7.833, 7.978 and 7.481 μA, and the concentrations of EGCG in the diluted samples were 4.178×10^{-2}, 4.633×10^{-2}, and 3.065×10^{-2} mmol/L, respectively. In addition, more than 9.555, 10.557, and 7.001% EGCG existed in the three kinds of dry tea leaves.

To further determine the performance and feasibility of the method in real samples, the MIP electrode was used to determine the EGCG content in spiked samples (20 mL each). As shown in Table III, the recovery values of the samples were in the range 98.6–102.2%, and the RSD was 2.21–2.48%. This indicated that the MIP sensor could be efficiently applied for the determination of EGCG in real samples.

CONCLUSIONS

In summary, the MIP electrode modified with an EGCG-imprinted nonconducting poly(*o*-PD) film was successfully synthesized in this study and was used as a sensing element of an MIP sensor for the determination of EGCG in aqueous solution. Because the MIP sensor could be prepared easily at low cost and showed excellent selectivity, sensitivity, repeatability, and prediction of the quality of tea and its products, we concluded that the combination of MIT with electrochemical techniques could be used to detect EGCG.

ACKNOWLEDGMENTS

Financial support was provided by grants from the National Natural Science Foundation of China (contract grant numbers 30970309 and 31201456), the China Postdoctoral Science Foundation (contract grant number 20100471379), the Natural Science Fund for Colleges and Universities in Jiangsu Province (contract grant number 05KJB550011), and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

REFERENCES

1. Liang, Y. R.; Lu, J. L.; Zhang, L. Y.; Wu, S.; Wu, Y. *J. Sci. Food Agric.* **2005**, *85*, 286.
2. Liang, Y. R.; Lu, J. L.; Zhang, L. Y.; Wu, S.; Wu, Y. *Food Chem.* **2003**, *80*, 283.
3. Tarachiwin, L.; Ute, K.; Kobayashi, A.; Fukusakii, E. *J. Agric. Food Chem.* **2007**, *55*, 9330.
4. Tudu, B.; Metla, A.; Das, B.; Bhattacharyya, N.; Jana, A.; Ghosh, D.; Bandyopadhyay, R. *IEEE. T. Instrum. Meas.* **2009**, *58*, 3069.
5. Hsieh, M. M.; Chen, S. M. *Talanta* **2007**, *73*, 326.
6. Ikeda, T.; Kanaya, S.; Yonetani, T.; Kobayashi, A.; Fukusaki, E. *J. Agric. Food Chem.* **2007**, *55*, 9908.
7. Sultana, T.; Stecher, G.; Mayer, R.; Trojer, L.; Qureshi, M. N.; Abel, G.; Popp, M.; Bonn, G. K. *J. Agric. Food Chem.* **2008**, *56*, 3444.
8. Ivarsson, P.; Kikkawa, Y.; Winquist, F.; Krantz-Rulcker, C.; Hojer, N. E.; Hayashi, K.; Toko, K.; Lundstrom, I. *Anal. Chim. Acta* **2001**, *449*, 59.
9. Shankar, S.; Ganapathy, S.; Srivastava, R. K. *Front. Biosci.* **2007**, *12*, 4881.
10. Yen, G.-C.; Chen, H.-Y. *J. Agric. Food Chem.* **1995**, *43*, 27.
11. Kondo, K.; Kurihara, M.; Miyata, N.; Suzuki, T.; Toyoda, M. *Free Radical Biol. Med.* **1999**, *27*, 855.
12. Ho, H. Y.; Cheng, M. L.; Weng, S. F.; Leu, Y. L.; Chiu, D. T. Y. *J. Agric. Food Chem.* **2009**, *57*, 6140.
13. Lambert, J. D.; Sang, S.; Hong, J.; Yang, C. S. *J. Agric. Food Chem.* **2010**, *58*, 10016.
14. Yang, J.; Han, Y.; Sun, H.; Chen, C.; He, D.; Guo, J.; Yu, C.; Jiang, B.; Zhou, L.; Zeng, C. *J. Agric. Food Chem.* **2011**, *59*, 11483.
15. Afaq, F.; Adhami, V. M.; Ahmad, N.; Mukhtar, H. *Oncogene* **2003**, *22*, 1035.
16. Fu, T.; Liang, J.; Han, G.; Lv, L.; Li, N. *J. Chromatogr. B* **2008**, *875*, 363.
17. Peres, R. G.; Tonin, F. G.; Tavares, M. F. M.; Rodriguez-Amaya, D. B. *Food Chem.* **2011**, *127*, 651.
18. Horie, H.; Mukai, T.; Kohata, K. *J. Chromatogr. A* **1997**, *758*, 332.
19. Fan, K.; Luo, X.; Ping, J.; Tang, W.; Wu, J.; Ying, Y.; Zhou, Q. *J. Agric. Food Chem.* **2012**, *60*, 6333.
20. Novak, I.; Seruga, M.; Komorsky-Lovric, S. *Electroanalysis* **2009**, *21*, 1019.
21. Gupta, R.; Kumar, A. *Biotechnol. Adv.* **2008**, *26*, 533.
22. Wulff, G.; Sarhan, A. *Angew. Chem. Int. Ed. Engl.* **1972**, *11*, 341.
23. Xu, Z. X.; Chen, S.; Huang, W.; Fang, G. Z.; Hua, P. Z.; Wang, S. *Anal. Bioanal. Chem.* **2009**, *393*, 1273.
24. Sueyoshi, Y.; Utsunomiya, A.; Yoshikawa, M.; Robertson, G. P.; Guiver, M. D. *J. Membrane. Sci.* **2012**, *401–402*, 89.
25. Resmini, M. *Anal. Bioanal. Chem.* **2012**, *402*, 3021.
26. Yang, Q. Y.; Sun, Q.; Zhou, T. S.; Shi, G. Y.; Jin, L. T. *J. Agric. Food Chem.* **2009**, *57*, 6558.

27. Malitesta, C.; Losito, I.; Zambonin, P. G. *Anal. Chem.* **1999**, *71*, 1366.
28. Panasyuk, T. L.; Mirsky, V. M.; Piletsky, S. A.; Wolfbeis, O. S. *Anal. Chem.* **1999**, *71*, 4609.
29. Sharma, P. S.; Pietrzyk-Le, A.; D′Souza, F.; Kutner, W. *Anal. Bioanal. Chem.* **2012**, *402*, 3177.
30. Weetall, H. H.; Rogers, K. R. *Talanta* **2004**, *62*, 329.
31. Holthoff, E. L.; Bright, F. V. *Anal. Chim. Acta* **2007**, *594*, 147.
32. Ulyanova, Y. V.; Blackwell, A. E.; Minteer, S. D. *Analyst* **2006**, *131*, 257.
33. Gomez-Caballero, A.; Goicolea, M. A.; Barrio, R. *J. Analyst* **2005**, *130*, 1012.
34. Yang, L.; Wei, W. Z.; Xia, J. J.; Tao, H.; Yang, P. H. *Electroanalysis* **2005**, *17*, 969.
35. Chirizzi, D.; Malitesta, C. *Sens. Actuators B* **2011**, *157*, 211.
36. Malitesta, C.; Mazzotta, E.; Picca, R. A.; Poma, A.; Chianella, I.; Piletsky, S. A. *Anal. Bioanal. Chem.* **2012**, *402*, 1827.
37. Zhu, Q. Y.; Zhang, A.; Tsang, D.; Huang, Y.; Chen, Z.-Y. *J. Agric. Food Chem.* **1997**, *45*, 4624.
38. Ng, S. M.; Narayanaswamy, R. *Anal. Bioanal. Chem.* **2006**, *386*, 1235.
39. Zheng, X.; Lin, R.; Zhou, X.; Zhang, L.; Lin, W. *Anal. Methods* **2012**, *4*, 482.