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RESEARCH ARTICLE

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A sensitive nicotine sensor based on molecularly imprinted electropolymer of *o*-aminophenol

Abstract A sensitive nicotine sensor based on a molecularly imprinted electropolymer of o-aminophenol is proposed and its configuration and performance are studied in detail. On the condition of weak acidity, the sensitive layer was prepared by electropolymerization of o-aminophenol on a gold electrode in the presence of the template (nicotine). The sensor exhibits good selectivity and sensitivity to nicotine. The determination limit is 2.0×10^{-7} mol/L and a linear relationship between the current and concentration is found in the range of $4.0\times10^{-7} \sim 3.3 \times10^{-5}$ mol/L. The sensor has also been applied to the analysis of nicotine in tobacco samples with recovery rates ranging from 99.0 % to 102 %.

Keywords molecularly imprinting, electropolymerization, nicotine, poly (*o*-aminophenol)

1 Introduction

Nicotine is one of the very important alkaloids; it is not only a raw material of medicine and chemistry but also a natural pesticide with high performance and low toxicity. So the content analysis of nicotine is a major detection item in quality control of many tobacco and medicinal products [1].

In recent years, the analytical methods for nicotine have increasingly been developed. Besides the gravimetric method and chemical volumetric method, many instrumental methods, such as GC-MS, CE, AAS and chromatography, etc., have been applied to the nicotine assays [2]. But these methods are generally rather complicated and poorly selective, and the facilities used are expensive. The relatively simple and low-cost sensing methods have become more and more popular, and developing nicotine sensors with good selectivity and sensitivity is a significant subject.

Molecular imprinting is a means of synthesizing some polymers with selectivity to some particular molecules (template molecules) in recent years [3]. Molecularly imprinted polymers usually have some cavities with a given size and shape and arraying functional groups, so they lend some memorial function to the specific steric structures of template molecules, thus endowing their good recognizing capability. Molecularly imprinted polymers can be used as the sensitive membranes of sensors, but some films prepared by routine ways [4–6], such as casting, in situ polymerizing, spin coating, etc., are generally of a thickness of over micron and are poor homogeneous [7], influencing the sensitivity and the final design of sensors to a certain degree.

In this paper, we try to study an electrochemical sensor for nicotine based on the electropolymerization molecular imprinting polymer with o-aminophenol as monomer and nicotine as template. Compared to the nicotine imprinting membrane by means of methyl acrylic acid polymerization [2], the electropolymerization film of poly *o*-aminophenol has the following advantages: it is homogeneous, it possesses nano-meter thickness, it involves simple preparation techniques and requires low experiment conditions, and so on. On the condition of acidity, basicity or neutrality, the *o*-aminophenol can be homogeneously electropolymerized [8], and at the mean time, the electropolymerization film of *o*-aminophenol has more functional groups and better insulation than that of phenylamine. Thus it can be expected to yield better results in molecular imprinting techniques. In this experiment, using cyclic voltammetry, the sensitive layer was prepared on the condition of weak acidity by electropolymerization of *o*-aminophenol on a gold electrode in the presence of the template (nicotine). The sensor exhibits good selectivity and sensitivity to nicotine, and it

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can be applied in the detection of nicotine in tobacco.

2 Experimental

2.1 Reagents and Apparatus

O-aminophenol (OAP, analytical-reagent grade) was purchased from Tianjin Gungfu Fine Chemical Engineering Institute (Tianjin, China), Nicotine(>99%) was purchased from Merck-Schuchardt (Germany). All other chemicals used were of analytical-reagent grade and were used as received without further purification. All solutions were prepared with doubly distilled water.

Cyclic voltammetry, differential pulse voltammetry and amperometric experiments were performed using a PAR 283 potentiostat/galvanostat and model 270 software (EG & G Princeton Applied Research).

The three-electrode system consisted of an Au electrode modified by molecularly imprinted film as the working electrode, an SCE as the reference electrode and a Pt foil as the counter electrode. All experiments were carried out at ambient temperature. All potentials were measured and reported versus the SCE.

2.2 Pretreatment of the gold electrode

The base-working electrode was constructed by sealing a 1.00 mm diameter pure Au wire at one end of a thin glass tube. The other end of the tube was sealed with epoxy resin and connected with a copper conductor to accomplish the electric circuit. The Au electrode surface was polished with 1.0 μm, 0.3μm and 0.05 μm Al_2O_3 powers respectively, ultrasonically washed subsequently in the absolute ethyl alcohol and water each for 5 min, and then electrochemically polished by cyclic voltammetry in the solutions of 0.5 mol / L H_2SO_4 in the potential range of –0.3 to 1.5 V at a scan rate of 100 mV/s.

2.3 Preparation of imprinted polymer

Similar to that described elsewhere [9, 10], the electropolymerization was carried out by continuous cyclic potential scanning from –0.2 to 1.2 V in a 2.5 mmol/L nicotine solution containing 0.05 mmol/L OAP at a scan rate of 50 mV/s; 30 cycles were repeated.

The electropolymerization film on the surface of the Au electrode was rinsed in the 0.5 mol/L H_2SO_4 solutions for 12 h; the template molecule was removed from the polymer and the imprinted polymer film remaining cavitie structure cavities of template was obtained. In the same experimental conditions, the electropolymerization non-imprinted film as a contrast was also prepared in absence of nicotine.

3 Results and discussion

3.1 Molecular imprinting electropolymerization

Fig.1 displays the cyclic voltammogram of OAP electropolymerization in the presence of nicotine. It can be seen that there is a clear anode peak ($E_{p,a}$) at 0.632 V and the anode peak current ($i_{p,a}$) is –31.85 μA. And the current values rapidly fall down with the increment of cyclic times. These results suggest that the electropolymerization of OAP is an irreversible process, and the dense nonconductive electropolymerization coating on the surface of the electrode can distinctly inhibit the voltammetry response.

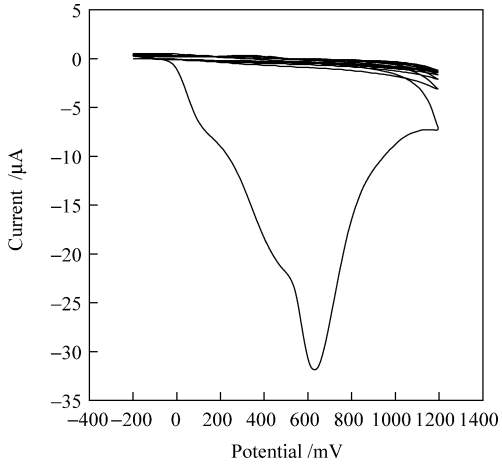

Fig. 1 Cyclic voltammograms of the electropolymerization of 50 mmol/L OAP at a gold electrode in $NaClO_4$ solution. Scan rate: 50 mV/s; number of scans: 30; nicotine concentration: 5 mmol/L; temperature: 25°C.

3.2 Conformation of the molecularly imprinted film

According to the literature [8], OAP can be electropolymerized to form a homogeneous and dense polymer film (POAP) in an acid solution, which is considered to have an open structure (Fig. 2).

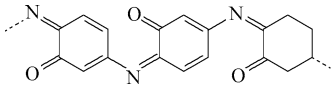

Fig. 2 Schematic structure of POAP

On the condition of pH≤3.0, pH=3.0~8.0 and pH≥8.0, nicotine is in forms of $NicH_2^{2+}$, $NicH^+$ and Nic, respectively[11]. Because the electropolymerization experiment was carried out in a weak acid solution, the nicotine molecules restricted in the steric cavities of the molecular imprinting polymer are dominantly in the form of $NicH^+$, and the nitrogen of nicotine should not be completely

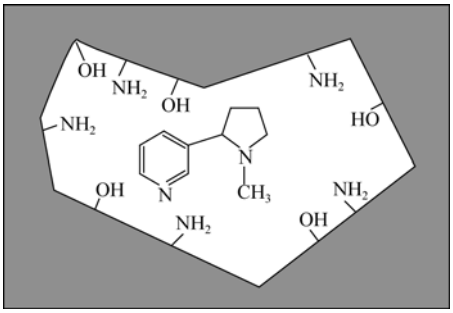

Fig. 3 Structure of nicotine-imprinted POAP membrane

protonized. Therefore, the hydrogen bond seems to be still an important interaction between the nicotine and the POAP in the imprinted film; its structure is schematically shown in Fig. 3

The nicotine template molecules are well eluted from the imprinting cavities in the H_2SO_4 solutions of 0.5 mol/L because the hydrogen bond can be broken in the strong acid solution. In a neutrality detecting solution, however, nicotine can enter into the imprinted film again by hydrogen bond interaction, generating a good recognizing response. The response process is displayed in Fig. 4.

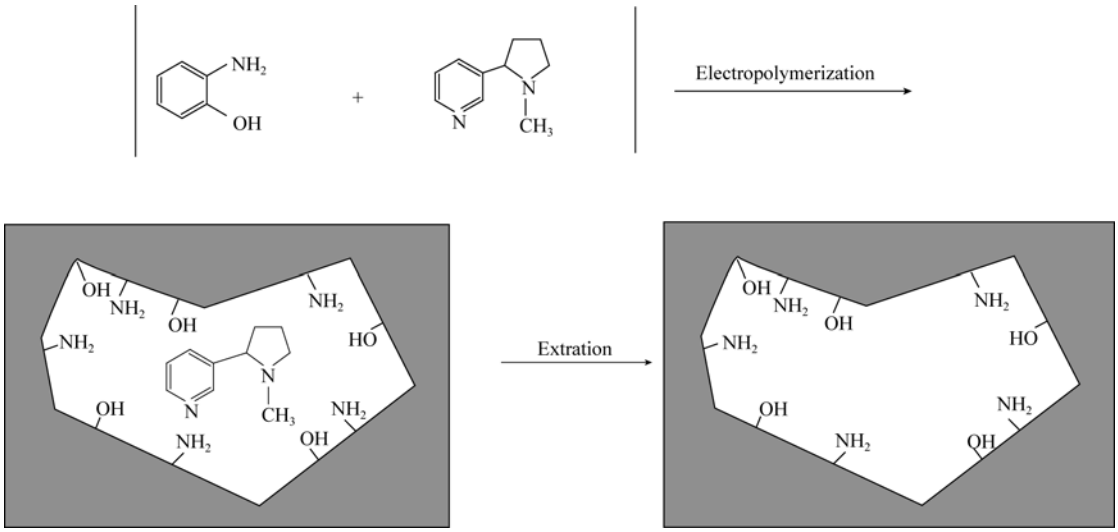

Fig. 4 Illustration of the imprinted site for nicotine

3.3 Characterization of the molecularly imprinted film

Differential pulse voltammetry (DPV) is more sensitive than cyclic voltammetry, and thus the DPV experiments of the Au electrode modified with or without the molecularly imprinted film are investigated, respectively, at the scan rate of 20 mV/s, the pulse amplitude of 50 mV and the pulse width of 50 ms. It can be observed that the peak current of the bare Au electrode in 0.01mol/L $K_3[Fe(CN)_6]$ solution is 11.57 μA (Fig.5 (a)). While there were no clear oxidation-reduction reaction on the surface of electrode modified with the molecularly imprinted film in the presence of nicotine (Fig. 5 (c)) or in the absence of nicotine (Fig. 5 (d)), indicating that these POAP films are nonconductive dense membrane. Moreover, the probe ions, $Fe(CN)_6^{3-}$, can be again transported to the surface of the Au electrode through the cavities in the imprinted film after eluting the template molecules, and the peak current decreases to 8.775 μA (Fig. 5(b)). These results show that the remaining special cavities in the imprinted film after eluting the template molecules enable the probe ions to freely reach the surface of the Au electrode, producing an excellent sensing response.

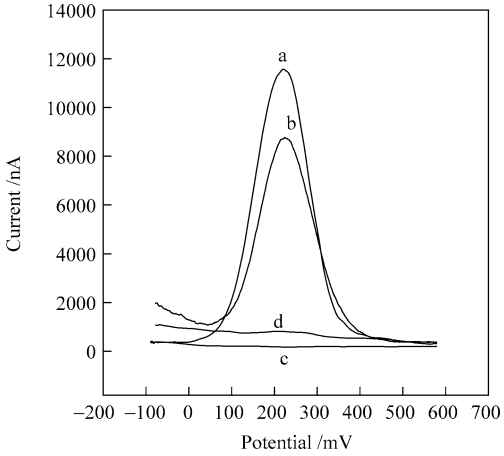

Fig. 5 Differential pulse voltammograms of four kinds of gold electrodes modified with different membranes in 0.01mol·L^{-1} $K_3[Fe(CN)_6]$ solutions. (**a**) bare gold electrode; (**b**) nicotine-imprinted POAP membrane after removing the template molecules;(**c**)nicotine-imprinted POAP membrane before removing the template molecules; (d) POAP

3.4 Sensor response characteristics

We studied the sensor response characteristics of the imprinted film to the nicotine in the solution of 0.01mol/L $K_3[Fe(CN)_6]$. From Figs. 6 (a) and 6 (b), a clear decrease in the peak current, 1.80 μA, is estimated when adding 4.0×10^{-4} mol/L nicotine because the nicotine molecules can occupy a part of the cavities in the imprinted film. For the POAP film, however, there is no current response under the same conditions [Figs.6 (c) and 6 (d)] because no imprinted cavities are available. These results show that the imprinted film remaining three-dimensional cavities can recognize the nicotine molecule, so the free diffusion of the probes, $Fe(CN)_6^{3-}$ ions to the surface of Au electrode can be obviously inhibited in the presence of nicotine, resulting in the decrease in the peak current.

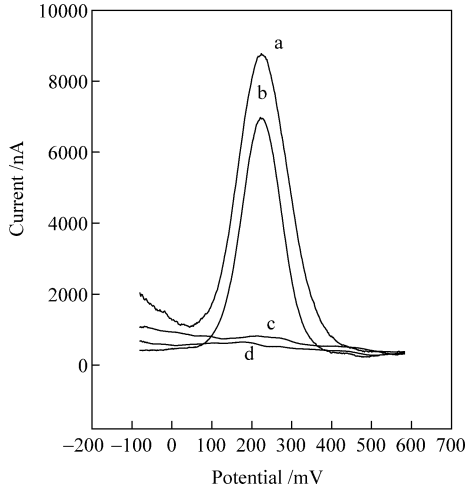

Fig. 6 Differential pulse voltammmetric responses of the gold electrodes in 0.01mol/L $K_3[Fe(CN)_6]$ solutions. (**a**) the gold electrode modified with nicotine-imprinted POAP membrane after removing the template molecules; (**b**) the (**a**) upon the addition of 4×10^{-4} mol/L nicotine; (**c**) the gold electrode modified with POAP membrane; (**d**) the (**c**) upon the addition of 4×10^{-4} mol/L nicotine

The responses of the imprinted film modified sensors to the nicotine solutions of different concentrations were detected by chronoamperometry, exhibiting rapid and sensitive response characteristics. The response time is less than 1min. And as can be seen from the calibration curve shown in Fig. 7, the sensor has a good linear response to nicotine in the concentration range of 4.0×10^{-7} to 3.3×10^{-5} mol/L. The detection limit is 2.0×10^{-7} mol/L at a signal to noise ratio of 3.

The selectivity of the sensor is also characterized by the selective factor K ($K= i/f$), where i is the current value of sensor responsing to 2.0×10^{-5} mol/L interferent solutions, f is the current value of sensor responsing to 2.0×10^{-5} mol/L nicotine solutions. The results as listed in Table 1 suggest that the other alkaloids, palmatine and berberine hydrochloride, have no interference, thus exhibiting good selectivity. However, atropine sulfate shows some interference, which seems to be related to the fact that it possesses a similar structure as nicotine.

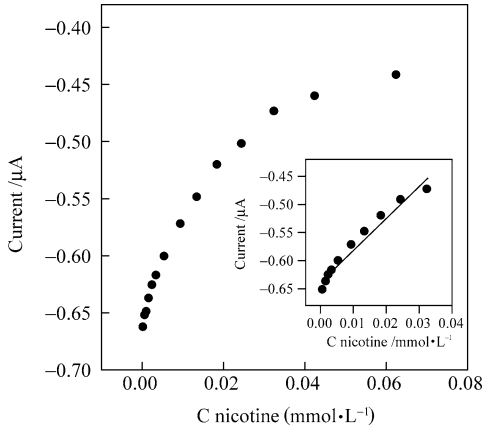

Fig. 7 Calibration curve of the sensor based on the nicotine-imprinted POAP membrane

Table. 1 Interferences of some compounds

Possible interference	$K = i / f$
Nicotine	1
Palmatine	No interference
Berberine Hydrochloride	No interference
Atropine Sulfate	0.13

3.5 Recovery rate tests

The responses of the sensors were tested when the standard nicotine solutions of different concentrations were added to the extracting solutions of tobacco. The results are listed in Table 2, and the recovery rates range from 99.0 to 102%.

Table. 2 Recovery experiments with calibration curve method

Sample	Added(mmol·L^{-1})	Founded(mmol·L^{-1})	Recovery (%)
1	0.01	0.0102	102
2	0.02	0.1983	99.1
3	0.03	0.0297	99.0

3.6 Repeatability and lifetime

The repeatability of the sensor responses was investigated at a nicotine concentration of 1.0×10^{-4} mol/L. The relative standard deviation is estimated to be 1.26 % for five successive assays (Table 3). The sensor still had good stability after it was used continuously for 7 days. The sensor should be stored in the doubly distilled water when it was not used.

Table. 3 Reproducibility of the imprinted electrode to 1.0×10^{-4}mol/L nicotine solution

Current（μA）	1	2	3	4	5	RSD(%)
Added before	1.176	1.310	1.223	1.295	1.380	
Added after	0.8487	0.9746	0.8944	0.9632	1.043	1.26
△I	0.3273	0.3354	0.3286	0.3318	0.3370	

4 Conclusions

A sensitive nicotine sensor based on the molecularly imprinted electropolymer of OAP was studied in this paper, exhibiting good selectivity, stability, repeatability and reproducibility. The sensor can be expected to be a detection technique as it is a low-cost procedure and is easy to fabricate.

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