Analytical Methods

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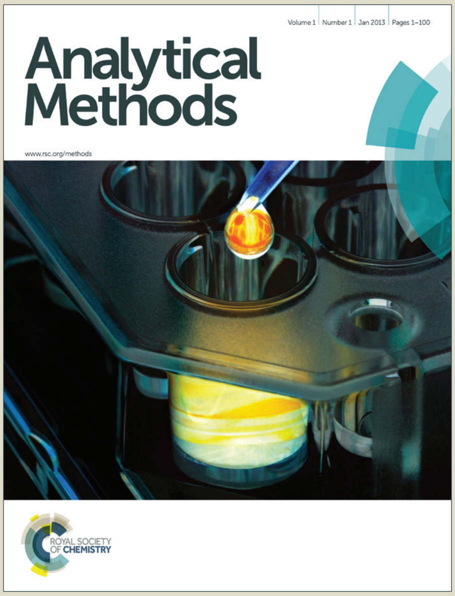

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A novel electrochemical sensor of 4-nonylphenol based on poly(ionic liquids) hollow nanospheres/gold nanoparticles composite modified glassy carbon electrode

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Abstract：A highly sensitive method for the determination of 4-NP based on a poly(ionic liquids) hollow nanospheres/gold nanoparticles composite modified glassy carbon electrode was proposed in this paper. The results showed that a well linear relationship between the peak current and the concentrations of 4-NP existed in the range of 0.1 μM-120 μM with a detection limit of 3.3×10^{-8}M (S/N=3) and a correlation coefficient of 0.9964. This method had lower detection limit and wider linear range. The effects of some foreign species on determination were also investigated. The proposed method has been successfully used to determine 4-NP in different water samples.

Keywords: poly(ionic liquids) hollow nanospheres, gold nanoparticles, 4-nonylphenol, modified electrode

1.Introduction

4-Nonylphenol (4-NP) is an important phenolic estrogenic compounds which is known in recent years for the adverse health effects on the endocrine system of human and animals and environmental toxicant [1-4]. The results of administration of 4-NP to rats indicated that it not only inhibits the growth of testes, but also affects the production and survival of sperms in rats[5]. In addition, many studies have indicated that 4-NP is ubiquitous fresh fruits and vegetables, human breast milk, livestock products, rice, and so on [6-8]. Therefore, it is very important to develop an accurate and rapid method for the detection of 4-NP in foods and environment.

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Several analytical techniques, such as gas chromatography-mass spectrometry (GS-MS)[9,10] high performance liquid chromatography(HPLC) [11], liquid chromatography- mass spectrometry (LC-MS)[12], liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS) [13] and immunosensors [14], *etc.* have been successfully developed for the separation and quantitative detection of 4-NP. Although these classic methods have reliable sensitivity, requiring complicated pretreatment procedures, very expensive and time-consuming limit their practicability. Therefore, different types of biosensors for detecting 4-NP in environmental and biological samples have been developed owing to its low cost, simple operation, high sensitivity and fast response[15-17].

A composite electrode was constructed by electrodepositing NiTPPs compound on a carbon nanotube-coated glassy carbon electrode(GCE) and was used to detect NP in water [18]. The detection limit is down to 260 nM. A imprinted sensor by immobilizing a molecularly imprinted polymer (MIP) sol-gel consisting of a multi-walled carbon nanotube (MWNT)-Nafion (NF) composite imprinted film on the GCE[19]. The imprinted sensor demonstrated higher affinity for target 2-NP than that of non-imprinted polymer (NIP) sensor. A novel electrochemical sensor with titanium oxide nanoparticles and gold nanoparticles for sensitive and fast determination of 4-NP was also developed[20]. The sensor was successfully employed to detect 4-NP in real samples.

Ionic liquids have increasingly been used as an excellent electrode material due to a number of advantages including high stability, high electrical conductivity and very low vapour pressure[21-23]. Bala *et al*[24] constructed a novel biosensors based on single-walled carbon nanotubes and 1-butyl-3-methylimidazolium hexafluorophosphate ionic liquid. The biosensor exhibits high sensitivity and good stability for detection of endocrine disrupters including 4 -nonylphenol.

In this work, a composite electrode was constructed based on poly(1- butyl imidazole -3- (2- ethyl methacrylate) tetrafluoroborate) ionic liquid hollow nanospheres and gold nanoparticles. Due to the large surface area and high electrical conductivity, both the poly ionic liquids hollow nanospheres and gold nanoparticles were used to enhance the electrochemical oxidation signals of NP and to minimize electrode fouling. This composite electrode was applied to detect low concentrations of nonylphenol in water samples.

2.Experimental

2.1 Chemicals

All regents were of analytical grade. 4-Nonylphenol and Chloroauric acid were purchased from Sigma-Aldrich(USA). Phosphate buffer solution was prepared by mixing the stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 and adjusting the pH with 0.1 M H_3PO_4 or 0.1 M NaOH. Double distilled water was used throughout the experents.

2.2 Apparatus

All electrochemical measurements were performed with CHI832 electrochemical workstation (Shanghai Chenhua Instrument Corporation,China) with a conventional three-electrode cell. A bare glassy carbon electrode (GCE, 3 mm diameter) or modified GCE was used as working electrode. A platinum sheet and a saturated calomel electrode (SCE) were used as the counter electrode and reference electrode, respectively. Scanning electron microscope (SEM) images were obtained using a ULTRA plus FESEM (Zeiss, Germany).

2.3 Elecrode preparation and modification

Poly(1- butyl imidazole -3- (2- ethyl methacrylate) tetrafluoroborate) ionic liquid hollow nanospheres(PILs) was prepared by following a previously reported procedure [25,26]. Gold nanoparticles/poly(ionic liquids) hollow nanospheres composite (AuNPs/PILs) was prepared by a simple synthetic method. Typically, PILs (10 mg) was dispersed in 10.0 mL water and 0.2 mL $HAuCl_4$ aqueous (1g/L) was added, the reaction mixture was stirred at room temperature for 24h. Then 0.2 mL cold $NaBH_4$ solution (1g/L) was added and stirred for 1h. Finally, SiO_2 was removed by HF solution (10%) and AuNPs/PILs hollow nanospheres were obtained.

Before the modification, GCE (3mm, diameter) were polished to a mirror with 0.3

μm and 0.05μm alumina slurries on a polishing cloth in sequence, rinsed thoroughly with doubly distilled water between each polishing step, finally sonicated in ethanol and double distilled water, and dried in air. For the preparation of modified electrode, 10 mg PILs or AuNPs/PILs was dispersed in 10 mL doubly distilled water and sonicated for 1 h. Then, 5 μL of the solutions were dropped on the pretreated GCEs with a microsyringe, respectively, and then dried for 24 h in air before use.

3. Results and discussion

3.1 Characterization of the modified electrode

Figure 1 shows scanning electron microscopic images of AuNPs/PILs hollow nanospheres and the GCE electrode surface modified by AuNPs/PILs. As shown in Figure 1a, the diameter of the resultant hollow nanosphere is less than 200nm and uniform in size. The hollow particles have a large surface area, which may facilitate the improvement of electrochemical signals. When the particles deposited on the GCE surface(as shown in Figure 1b), a well-packed film of AuNPs/PILs was formed, which provide a favorable environment for electrochemical detect of analytes.

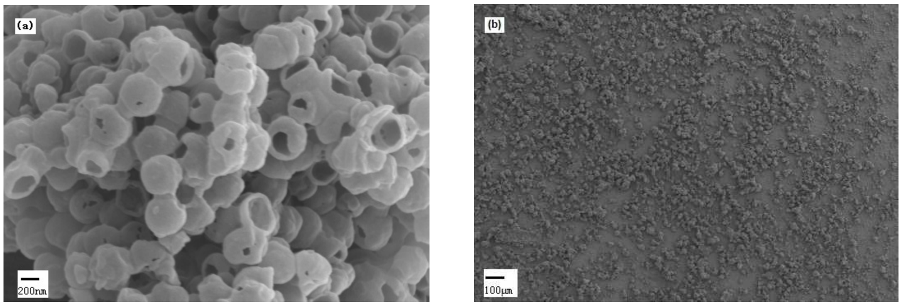

Figure 1. SEM images of AuNPs/PILs hollow nanospheres(a) and the GCE electrode surface modified by AuNPs/PILs(b).

3.2 Electrochemical properties of the modified electrode

The sensor based on PILs and AuNPs/PILs composites modified electrodes were investigated by cyclic voltammetry (CV) in aqueous solution containing 1mmol L^{-1} $K_3[Fe(CN)_6]$ as indicator substance and 1 mol L^{-1} KCl mixture solution in the range from -0.2 to 0.5V. Curve a showed a CV of the bare electrode in $K_3[Fe(CN)_6]$ solution, there were a pair of redox peaks. After modification of PILs, the redox peaks increased as shown in Curve b. When AuNPs/PILs composite was deposited onto the

GCE electrode, the redox peaks further increased (Curve c). These indicated that both PILs and AuNPs could enhance the electrochemical signal of the obtained sensor.

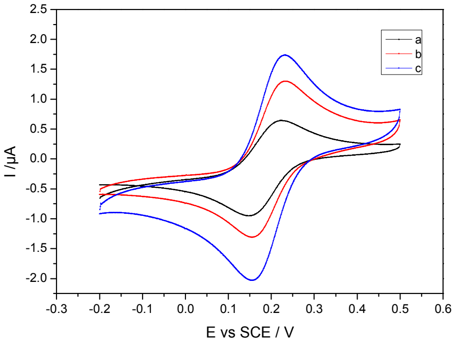

Figure 2. The CVs of 1mmol L^{-1} $K_3[Fe(CN)_6]$ on the bare electrode(a); PILs modified electrode(b); AuNPs/PILs modified electrode (c). Scan rate: 50 mV s^{-1}.

3.3 Electrochemical behavior of 4-nonylphenol at the modified electrodes

Figure 3 shows the CVs of 4-NP at bare GCE, PILs/GCE and AuNPs/PILs/GCE in 0.1mol L^{-1} PBS（pH=6.0）and 1 mol L^{-1} KCl, respectively. There is an irreversible oxidation peak at 0.7 V and no reduction peak in the reverse scan with the oxidation peak current is 0.3388 μA at the bare GCE(as shown in Curve a). At the PILs/GCE, the oxidation peak current increased and the peak potential negatively shifted to (Curve b). A well-defined cathodic peak was observed and the peak current increased obviously at the AuNPs/PILs/GCE(Curve c). The cathodic peak current signal was 5-fold increments when camparing the bare GCE. The CV's changes observed indicated that the AuNPs/PILs/GCE had a good catalytic activity for the oxidation of 4-NP.

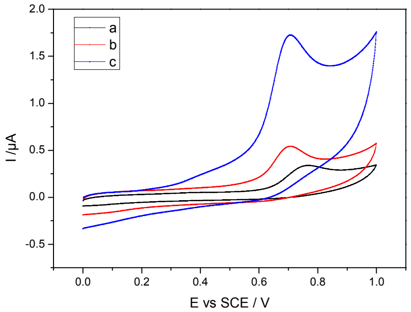

Figure 3. The CVs of 4-nonylphenol on the bare electrode(a); PILs modified electrode(b);

AuNPs/PILs modified electrode (c). Scan rate: 50 mV s^{-1}.

3.4 Optimization of analytical conditions

3.4.1 Effects of accumulation potential and time

In order to improve the amount of adsorption of the ananlyte at the electrode and enhance the sensitivity, accumulation is generally used in electrochemical ananlysis process. Figure 4A shows the effect of accumulation time on the peak current of 4-NP. It can be seen that with increasing accumulation time the peak current rapidly increased and then decreased with the further increase, and the maximum peak currents was obtained at 40 s. Therefore, the accumulation time of 60 s was chosen as the optimum value for further experiments. Figure 4B shows the influence of accumulation potential on the peak current after accumulation for 40 s. There was a dramatic increase of the peak current and then decrease as the potentials increased from 0 to 0.6 V. The maximal peak current was observed at 0.2 V. Therefore, 0.2 V was chosen as the optimal accumulation potential.

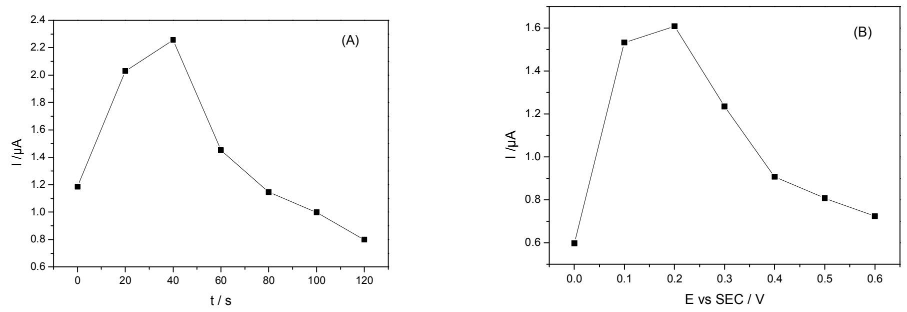

Figure 4. Effects of accumulation time (A) and accumulation potential (B) on the peaks current of 4-NP in 0.1M PBS. Scan rate: 50 mVs^{-1}. 4-NP concentration: 20μM.

3.4.2 Effect of the solution pH

Proton is always involved in the electrochemical reaction of organic compound and exerts significant impact on the reaction speed. Therefore, the effect of test solution pH on the peak current of 4-NP at the AuNPs/PILs modified electrode was investigated by CV in 0.1 M PBS over the range from pH 4.0 to 8.0. As illustrated in Figure 5A, the peak current increased with the increasing pH from 4.0 to 6.0. Then, it decreased when the pH value further increased, and the highest peak current was obtained at pH 6.0 Therefore, pH 6.0 was chosen for the subsequent analytical experiments. In addition, the peak potential for the oxidation of 4-NP shifted linearly and negatively with the increasing pH (as shown in Figure 5B), which indicated that protons had taken part in the electrode process.

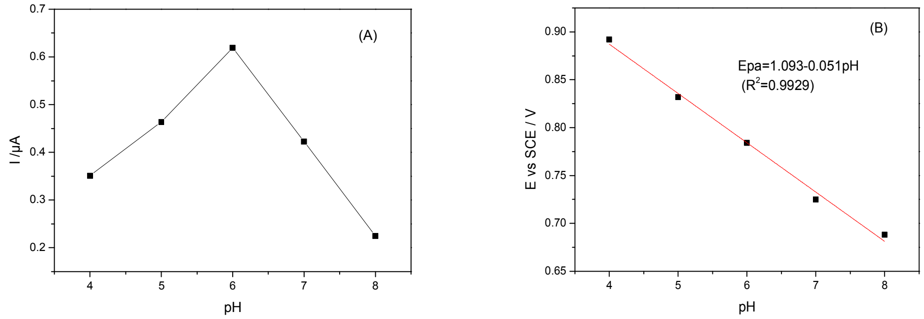

Figure 5. Effects of pH on the oxidation peak current (A) and potential (B) of 4-NP. 4-NP concentration: 20μM.

3.4.3 Effect of potential scan rate

In order to further investigate the electrochemical behavior of 4-NP at the AuNPs/PILs modified electrode, the effect of scan rate on the oxidative peak potential was investigated by CV in 0.1 M PBS. Figure 6A shows the CV of the 4-NP at the modified electrode at different scan rates. The peak current linearly increased with the scan rate over the range of 10-90 mV s^{-1}. The corresponding plot of peak currents vs. scan rates was shown in Figure 6B. The linearly relationship suggests that the electrochemical oxidation of 4-NP at the AuNPs/PILs modified electrode is an adsorption controlled process.

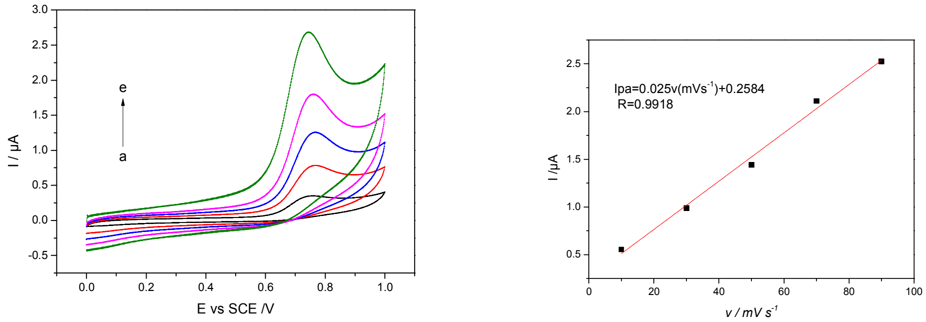

Figure 6. A: The cyclic voltammograms of 4-NP on AuNPs/PILs/GCE modified electrode at different scan rates in 0.1 M PBS (pH=6.0). Scan rates are 10, 30, 50, 70, 90 mV s^{-1} (from a to e curve), 4-NP concentration:20μM.   B: plots of peak currents vs. the scan rates.

3.5 Determination of 4-nonyl phenol

The differential pulse voltammetry (DPV) was used for the determination of 4-NP under the optimum conditions. The voltammograms of solutions with different concentrations of 4-NP were shown in Figure 7. The peak currents were proportional to 4-NP concentration in the range of 0.1 μM-120 μM with a detection limit of 3.3×10^{-8}M (S/N=3). This linear relationship can be represented by the expression,

$$I_{pa}(\mu A)= 0.0301c(\mu M) + 0.5426 \qquad (R=0.9964, N=9)$$

Compared with other electrochemical sensors for 4-NP (Table 1) reported in literature, the proposed sensor provided with a wider linear range. The limit of detection was lower than that most of other electrochemical sensor consisting of titanium oxide and gold nanomaterials .

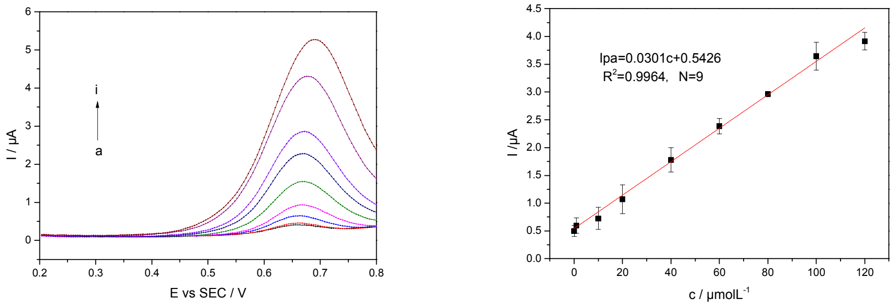

Figure 7. A: DPVs for various concentrations of 4-NP in the range of 0.1to120μM (from a to i) in 0.1 M PBS.B: Corresponding linear calibration curve of peak current vs. 4-NP concentration. Pulse amplitude: 50 mV, pulse width: 50 ms, pulse period: 0.2 s.

Table 1. Comparison of the proposed 4-NP sensor with other sensors based on differential matrices.

Electrode	Linear range(μM)	Detection limits (μM, S/N =3)	References
MIP/NGNRs-IL/GCE	0.04-6	0.008	[15]
NiTPPS[a]/CNT/GCE	0.1-4.0	0.26	[18]

HRP^b/SWCNT–IL/SPE	5.5-140	0.4	[24]
MIP@GNP^c/TiO$_2$/GE^d	0.95–480	0.32	[20]
MIP/MWCNTs-NF^e/GCE	0.2–360	0.06	[19]
AuNPs/PILs/GCE	0.1–120	0.033	This work

a Ni(II) tetrakis(4-sulfonatophenyl) porphyrin; b horseradish peroxidase; c gold nanoparticles; d gold electrode; e Nafion

3.6 Stability, reproducibility and selectivity of the modified electrode

The stability, reproducibility and selectivity are important parameters for evaluation an electrode. Therefore, three modified electrodes with the same fabrication procedure were prepared and used for the determination of 4-NP solution, and the RSD was 5.28%. Meanwhile, the reproducibility of the modified electrode was investigated and the relative standard deviation (RSD) of the sensor response was 3.25% for eight successive measurements. The modified electrode was stored at 4 ℃ a week and used to detect 4-NP solution, 93.3% of the initial current signal was obtained and after two-week storage, 95.2% signal remained. These results indicated that the biosensor had good detecting and fabrication reproducibility, and excellent long-term stability.

It is well known that the electrochemical system is highly vulnerable to the foreign species, so some substrates which may possibly be existed in water sample were investigated. The tolerable ratio (defined as the maximum amount of foreign species causing an error lower than ±5% in the determination of 10.0μM 4-NP.) were listed in Table 2. It can be seen that common inorganic ions have no influence on the determination of 4-NP, while affect slightly the determination.

Table 2: Effects of foreign species on the determination of 10.0μM 4-NP.

Foreign species	Tolerable ratio (foreign / epinephrine)
K^+ , Mg^{2+}, Na^+, Fe^{3+}, Cu^{2+}, SO_4^{2-}, NO_3^-, Cl^-	400
bisphenol A, phenol, 4- nitrophenol, 2, 4-2 nitro phenol, p-aminophenol	20

3.7Analytical applications

In order to evaluate the validity of the proposed method, the modified electrode was applied to determine 4-NP in different water samples. The sample was diluted 10-times with double distilled water and then appropriate amounts of the sample were transferred to an electrolytic cell for the determination using DPV. The analytical results are shown in Table S1. It can be seen that DA in the sample could be satisfactorily detected with a recovery of 97.8%–103.5%, and the RSD (n = 5) was less than 3.0%.

Table 3. Determination of 4-NP in different water samples (n= 3).

Sample	Added（μM）	Found（μM）	Recovery（%）	RSD(n=5)（%）
Tap water	10.0	9.86	98.6	2.08
	50.0	48.62	97.2	3.14
Yellow river water	10.0	10.34	103.4	2.59
	50.0	49.21	98.4	2.98
Weste water	10.0	10.42	104.2	3.02
	50.0	50.10	100.2	2.36

4. Conclusions

A novel and simple method for the sensitive detection of 4-NP at AuNPs/PILs hollow nanospheres modified electrode was described in this paper. The experimental results demonstrated that the AuNPs/PILs modified electrode provided with a good sensitivity, wide linear range and low detection limit compared with other 4-NP electrochemical sensors. In addition, it displayed a good recognition capacity for 4-NP in the presence of several phenol structural analogs and showed good detecting and fabrication reproducibility, and excellent long-term stability. Both poly(ionic liquids) hollow nanospheres and gold nanoparticles may facilitate the improvement the electrochemical behaviors of 4-NP at the modified electrode. Meanwhile, the proposed method was successfully applied to the determination of 4-NP in different water samples and had shown a great potential use in the environmental samples analysis.

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