Electrochemical Sensor Prepared from Molecularly Imprinted Polymer for Recognition of 1,3-Dinitrobenzene (DNB)

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An electrochemical sensor was modified with multi-wall carbon nanotubes (MWCNT) and molecularly imprinted polymer (MIP) material synthesized with acrylamide and ethylene glycol dimethacrylate (EGDMA) in the presence of 1,3-dinitrobenzene (DNB) as the template molecule. The MWCNT and MIP layers were successively modified on the surface of a glassy carbon electrode (GCE), of which the MIP film works as an artificial receptor due to its specific molecular recognition sites. The MIP material was characterized by FT-IR and electrochemical methods of square wave voltammetry (SWV). The interferences of other nitroaromatic compounds (NAC) such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB) and 2,4-dinitrotoluene (DNT) to DNB were also investigated by the prepared MIP/MWCNT electrode. Compared with other traditional sensors, the MIP/MWCNT modified electrode shows good selectivity and sensitivity. In addition, the current responses to DNB are linear with the concentration ranging from 4.5×10^{-8} to 8.5×10^{-6} mol/L with the detection limits of 2.5×10^{-8} (-0.58 V) and 1.5×10^{-8} mol/L (-0.69 V) ($S/N=3$). The construction process of MIP/MWCNT modified electrode was also studied as well. All results indicate that the MIP/MWCNT modified electrode established an improving way for simple, fast and selective analysis of DNB.

Keywords   MIP/MWCNT, electrochemical sensor, selectivity, DNB determination

Introduction

The nitroaromatic compounds (NAC) have attracted great attention for their serious harm during the past 20 years. The production, storage and testing of explosives and their uses in terrorist and war activities pose great threat to public security of the whole world.[1,2] As one kind of important pollutions, NAC also cause health problems in both animals and human beings, including anemia, abnormal liver function, cataract development and skin irritation.[3] Even after degradations, the by-products of the common explosives are still toxic and carcinogenic.[4,5] Therefore, there is an expanding interest among the public security specialists and environmental scientists for the identification and quantification of NAC.

Many GC and LC methods were most widely used for the determination of NAC.[6] In addition, mass spectrometry,[7] ion mobility spectrometry,[8] fluorescence[9] and chemiluminescence[10] have been also reported. Although each of these methods possesses certain advantages, many of them are time-consuming, labor-intensive and instrumentation-expensive. Electrochemical sensors modified with functional nanomaterials were constructed for the rapid and sensitive trace NAC determination.[11-15] However, these methods suffer from the limitation of the selectivity without which it is difficult to choose corresponding solution and not readily adaptable to field determination. Therefore, there is a great necessity for accurate, sensitive, rapid, and especially selective method to facilitate the detection, quantification and remediation of NAC.

Molecularly imprinted polymers (MIP) have been elucidated to work as an artificial receptor due to their superior selectivity, which typically involve the copolymerization of functional and cross-linking monomers in the presence of template molecules. Subsequent extraction of template creates specific molecular recognition sites in solid polymer, which are complementary to the shape, size, and functional group of the template. Furthermore, MIP possesses many other advantages, such as long-term stability, on-site and low cost etc. The MIP has been already successfully applied to molecular recognition of amino acids,[16,17] glucose[18-20] and protein epitope detection.[21,22] As reported in some articles, some scientists have already utilized the technique of MIP to

detect NAC. Murray presented a sensor for explosives with an MIP based on polymerisable porphyrin derivates coated on glass fibers.[23] NAC were detected by sensing the fluorescence properties, which was altered during the bonding of explosives. Bunte and his co-workers developed a specific sensor coating material on the basis of direct surface molecularly imprinted polymerization on quartz crystal microbalances (QCM).[24]

In this work, we synthesized a molecularly imprinted polymer (MIP) with DNB as the template molecule and modified the surface of a glassy carbon electrode with the MIP material to construct the DNB sensing system. In order to improve the electron transfer between the MIP layer and the surface of electrode, MWCNT were used to modify the electrode. Fast response time, a broad linear range, long-life stability and improved selectivity were achieved with the MIP/MWCNT modified electrode when it was applied to detect DNB. This method could open a possibility for the design of chemical sensors with different requirements.

Experimental

Reagents and apparatus

TNT (1000 μg/mL in acetonitrile), TNB, DNT, DNB, acrylamide and ethylene glycol dimethacrylate (EG-DMA) were all purchased from Sigma-Aldrich Chemical Co. Ltd. Azobisisobutyronitrile (AIBN) was obtained from the organic group of Department of Chemistry of East China Normal University. The solids of NAC of TNB, 2,4-DNT, and 1,3-DNB were dissolved in acetonitrile to form 1.0 g/L solutions. Electrochemical experiments were carried out on a CHI 830 electrochemical workstation (CH Instruments, USA) with a bare GCE (diameter 3 mm, BAS Co., Japan) or an MIP/MWCNT modified electrode as the working electrode, a Ag/AgCl (saturated KCl) electrode as the reference electrode and Pt wire as the auxiliary electrode. Infrared spectra were recorded on a Nicolet Nexus-670 FT-IR spectrometer. All electrochemical experiments were performed in a 5 mL voltammetric cell at room temperature (25 ± 1) ℃. All solutions were deoxygenated by bubbling with highly pure nitrogen for at least 20 min and maintained under nitrogen atmosphere during the measurements.

Synthesis of MIP for DNB

Figure 1a presents a scheme of the synthesis process for MIP material. Typically, Acrylamide, DNB, EG-DMA and AIBN were dissolved in 3 mL of acetonitrile to make a mixture solution which consists of 8 mmol of acrylamide, 48 mmol of EGDMA, 2 mmol of DNB and 5 mg of AIBN. This mixed solution was purged with nitrogen for 10 min in an ice bath. The prepolymerization was first done at 50 ℃ for 6 h, and the final polymerization was completed at 60 ℃ for 24 h. The products were further aged at 85 ℃ for 6 h for obtaining a high cross-linking density. At last, the solid material was ground with a mortar and sieved with a 500 sieve to get particles of micron size. The NIP material was synthesized by the same method only without the addition of the DNB.
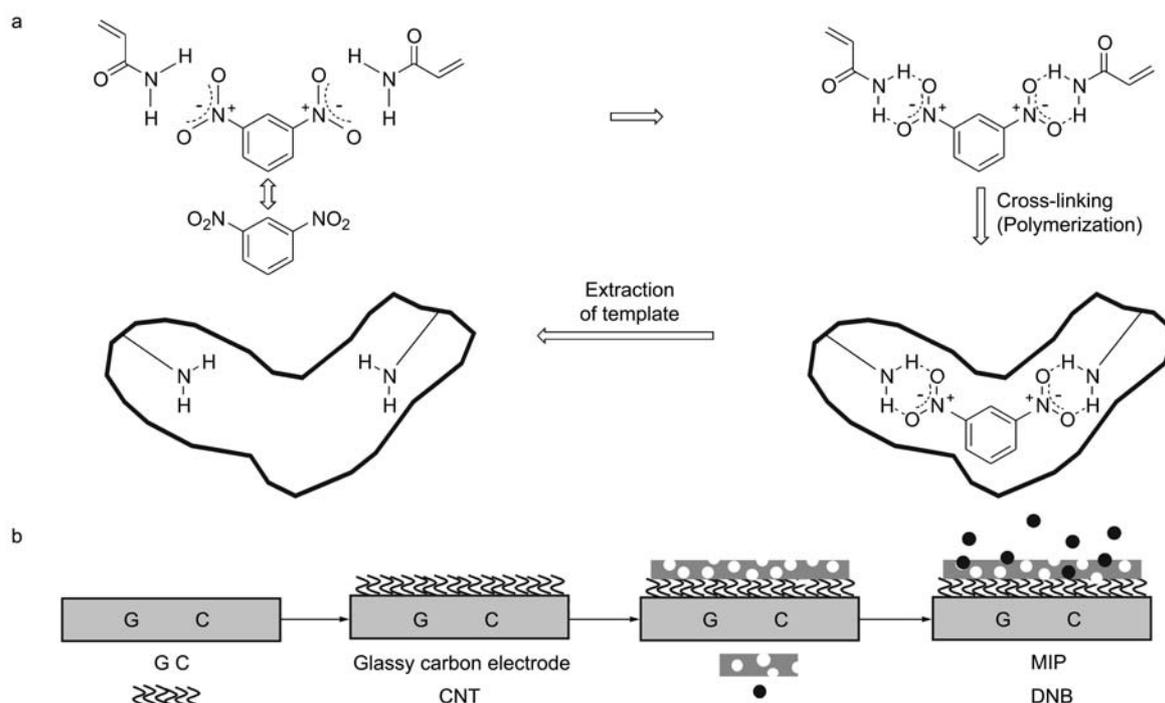

Figure 1 Synthesis of MIP (a) and construction of MIP/MWCNT modified electrode (b).

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Extraction of DNB from MIP

Original MIP particles were first suspended in a mixture solvent of $CH_3OH/HOAc$ ($V/V = 9/1$). After ultrasonic cleaning three times for 1 h each, DNB was extracted from MIP and the MIP material was dried for the electrode modification with MIP/MWCNT. The infrared spectra of MIP before and after extraction of DNB were recorded.

Fabrication and electrochemical behavior of MIP/MWCNT modified electrode

GC electrode was polished and cleaned as before.[12,13] As shown in Figure 1b, 2.0 μL of MWCNT solution (0.1 g/L in DMF) was dropped onto the clean GC electrode firstly and the MWCNT electrode was dried under the infrared light for 10 min. Then the MIP film was formed in the same way by dropping 2.0 μL of MIP solution (1 g/mL in chitosan solution of 0.5% in 0.1 mol/L HOAc) on the surface of electrode and drying for use.

Using the MIP/MWCNT modified electrode as the working electrode, SWV measurement was performed to investigate the electrochemical behavior of DNB and other NAC with the potential ranging from 0.1 to -1.0 V, amplitude of 50 mV, pulse width of 0.05 s, sampling width of 0.0167 and pulse period of 0.2 s. The selectivity of MIP/MWCNT modified electrode was also discussed in this work.

Results and discussion

Characterization of MIP

Figure 1 presents the synthesis process of MIP (a) and the construction of the modified electrode (b). As shown in Figure 1a, the molecularly imprinted polymer was synthesized with acrylamide as functional monomers and ethylene glycol dimethacrylate (EGDMA) as cross-linkers for imprinting the polymer with the template of DNB. In view of the fact that nitroaromatic components are weak hydrogen bond acceptors, the molecules of monomer acrylamide and template DNB spontaneously assemble perhaps due to the non-covalent hydrogen bonds between amino groups and electron-deficient nitroaromatics. The addition of EGDMA fixed the relations and the polymerization was initiated by AIBN. After the extraction of DNB molecules, the recognition sites were situated in the rigid material with stabilized binding sites and complementary shape.

The FT-IR spectra of DNB imprinted polymer and DNB-removed imprinted polymer are shown in Figure 2. The characteristic peaks of DNB molecules in Figure 2a at about 1380 and 1600 cm^{-1} were attributed to the stretching vibration of $N=O$ and deformation vibration of $C=C$, respectively. After extraction, it can be seen that the above absorption peaks are obviously reduced or even can not be seen in Figure 2b at all, which indicates that most of the template molecules have been removed though there are still a few left in the MIP polymer. This may be because the highly cross-linked rigid

structure does not allow the template molecules to move freely, and it is extremely difficult to extract the original molecules located in the central area of the bulk materials. As a result, the target DNB molecules that have been encased deeply in the rigid matrix can not be extracted easily.
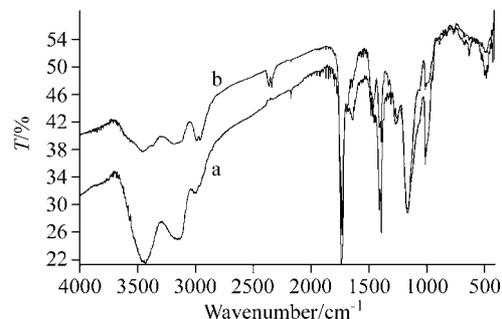

Figure 2 FT-IR spectra for (a) DNB imprinted polymer and (b) DNB-removed imprinted polymer.

In addition, the effect of washing time by $CH_3OH/HOAc$ ($V/V = 9/1$) on DNB was further investigated in this work. As shown in Figure 3, about 70% of the original templates of DNB in MIP material are removed after ultrasonic cleaning for about 60 min by $CH_3OH/HOAc$ ($V/V = 9/1$). Then the extraction reaches a stable value from 60 to 200 min. Therefore, 60 min was chosen as the optimal washing time in this work.
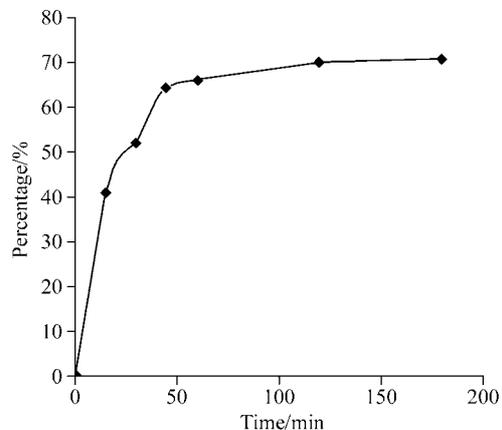

Figure 3 Effect of washing time on the extraction of template molecule DNB [1 mg of original MIP material was dissolved in 5 mL of a mixture solution of $CH_3OH/HOAc$ ($V:V = 9:1$)].

Capacity of MIP to DNB

Figure 4 shows the time-dependent evolution of the DNB amount bound by MIP and non-imprinted polymer (NIP). Before equilibrium adsorption is reached, the MIP particles take up DNB molecules from solution in a much faster rate than the NIP particles. The MIP particles take up 50% of the equilibrium amount during only 25 min and spend the equilibrium time period shorter than 70 min (Figure 4a), and it is obvious that the bind-

ing capacity of MIP is *ca.* 100 nmol per mg, which is about 5-fold of the capacity of NIP of 20 nmol per mg (Figure 4b). Both the longer equilibrium period and larger capacity could be attributed to the recognition cavity complementary to the shape and functional groups of the target molecule DNB. The more recognition sites located on the surface of MIP material result in the easy diffusion of target analyte into the recognition sites.
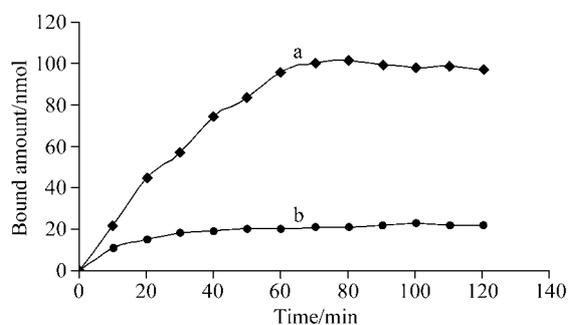

Figure 4 Amount of DNB molecules bound by imprinted particles (a) and nonimprinted particles (b). All measurements were carried out by suspending 1 mg of molecularly imprinted material in 5 mL of DNB solution.

Electrochemical behavior of MIP/MWCNT modified electrode

Figure 5 is the SWV current responses of MWCNT modified electrode (a), MIP/MWCNT modified electrode (b), bare electrode (c) and only MIP modified electrode (d) to the same concentration of 6.50×10^{-6} mol/L DNB solution. It illustrates that the MWCNT and MIP/MWCNT modified electrodes show higher responses than the bare electrode and only MIP modified electrode due to the MWCNT having more active centers and larger effective surface area, which can enhance the sensitivity to many electroactive substances.[25,26] Figure 5 also shows that the response of MIP modified electrode is even lower than that of a bare electrode
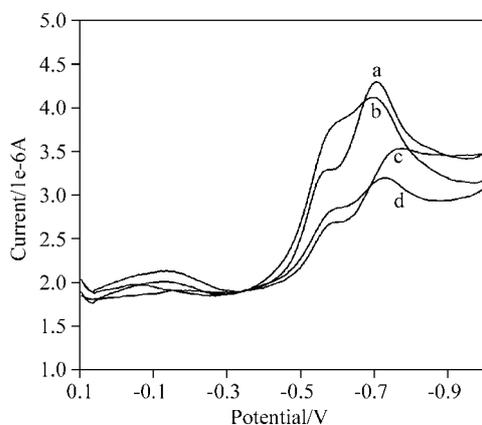

Figure 5 SWV current responses to the concentration of 6.50×10^{-6} mol/L of DNB solution of different electrodes. (a) MWCNT modified electrode, (b) MIP/MWCNT modified electrode, (c) bare electrode and (d) MIP modified electrode.

perhaps because MIP is a kind of polymer which may block the electron transfer from the NAC to the surface of glassy carbon electrode. It is the same reason that the response of MIP/MWCNT modified electrode is a little lower than that of MWCNT electrode.

Stability and reproducibility of the MIP/MWCNT electrode

The electrochemical activity of MIP/MWCNT modified electrode was examined in DNB solutions of 1.19×10^{-6}, 2.38×10^{-6}, 3.57×10^{-6} mol/L for six times respectively, with the RSD being lower than 4.5% (Figure 6). After one day, the sensor retained 93% of initial current response. One week, two weeks and a month later, the responses were still 89%, 80% and 75% of the initial response, respectively. In addition, the MIP/ MWCNT modified electrode also showed a good reproducibility. The five electrodes fabricated independently, showed an acceptable reproducibility in the current response to 5.5×10^{-6} mol/L DNB with RSD of 5.4%. These results show that MIP/MWCNT modified electrode is very stable for measurement of NAC.
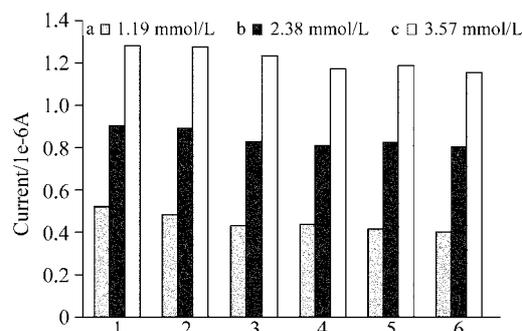

Figure 6 The stability of MIP/MWCNT modified electrode at the concentrations of the solution. (a) 1.19×10^{-6}, (b) 2.38×10^{-6}, and (c) 3.57×10^{-6} mol/L (After each series of detection, the modified electrode was immersed in distilled water for 30 min and rinsed by flowing distilled water for 10 min).

Selectivity of MIP/MWCNT modified electrode to DNB

The responses to other NAC (TNT, DNT and TNB) were examined in order to investigate the selectivity of the MIP/MWCNT modified GCE to DNB. In general, TNT, TNB and DNT could also be embedded in the imprinted sites for DNB due to their similar structures and functional groups, which establish the non-covalent hydrogen bonds with amino groups of the monomers left in the material. However, the volumes of the imprinted cavity sites created by target DNB molecules are smaller than those of molecules of TNB, DNT and especially TNT due to their comparatively large molecular cubage. It could result in the inability of the absorbability of the interferences and the decrease of the current responses to TNT, DNT and TNB with the MIP/MWCNT modified electrode.

Our experiments confirmed the hypothesis that the

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MIP/MWCNT modified electrode exhibited an improved selective response to DNB (Table 1) in this work. Given the response of MWCNT modified electrodes to 5.5×10^{-6} mol/L NAC solutions as 100%, it can be seen that the MIP/MWCNT modified electrodes show as much as the same response to DNB (98.4%) as the MWCNT electrode. The current responses of the MIP/MWCNT modified electrodes show only 46.2% and 39.8% of those of the MWCNT electrode to TNB and DNT, respectively. Moreover, the response to TNT is just one third (about 33.1%) of that of the MWCNT electrode. Therefore, the fact that the responses of MIP/MWCNT modified electrodes to TNT, TNB and DNT are even smaller than those of the bare glassy carbon electrode illuminates that the prepared MIP material can obstruct TNT, TNB and DNT from the electrode partly according their structure. In addition, a non-imprinted polymer modified electrode does not show any selective response to DNB, thus implying that the imprinted polymer acts as recognition element to target DNB. All of these results predict that the MIP/MWCNT modified electrode is suitable for not only sensitive but also selective detection of DNB.

Detection of DNB

Figure 7 shows the SWV response to a series of DNB solutions from 5.5×10^{-7} to 5.5×10^{-6} mol/L at the MIP/MWCNT modified electrode, alone with the blank voltammogram. With the increase of the DNB concentration, two continually increasing of reductive current peaks can be seen at the potential of -0.58 and -0.69 V around, respectively, which are consistent with the characteristic deoxidization peaks in the previous reference that the electron-transfer process involves an initial reduction of the nitro aromatic groups to hydroxylamines, followed by the conversion of the latter to amine groups. The current responses to DNB was in good linear range from 4.5×10^{-8} to 8.5×10^{-6} mol/L with the detection limits of 2.5×10^{-8} mol/L (-0.58 V) and 1.5×10^{-8} mol/L (-0.69 V) ($S/N=3$). The correlation coefficients are 0.9959 (-0.58 V) and 0.9986 (-0.69 V), respectively.

Herein, the saturation of the MIP material was also investigated. From the Eq. (1) and the research of capacity, it was estimated that 1 mg of the MIP particles modified on the electrode could hold *ca.* 30 nmol DNB

as expected.

$$n_{DNB} = \frac{10^{-3}}{m_0} \times n_0 \tag{1}$$

where m_0 is the total mass of imprinted material, and n_0 the total mole of DNB in the imprinted material.

In our experiment, the response current became stable at the capacity of 25.5 nmol, which was coincident with the theoretic datum of 30 nmol.
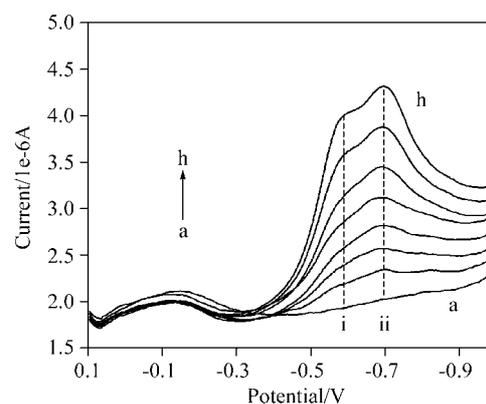

Figure 7 SWV responses to the solutions of (a) blank, (b) 0.55×10^{-6}, (c) 1.10×10^{-6}, (d) 1.65×10^{-6}, (e) 2.20×10^{-6}, (f) 3.29×10^{-6}, (g) 4.39×10^{-6} and (h) 5.50×10^{-6} mol/L DNB at the MIP/MWCNT modified electrode (i, 0.58 V and ii, 0.69 V).

Conclusion

An MIP/MWCNT modified electrode for the measurement of DNB has been demonstrated in this paper. The MIP possesses cavities that are complementary to the shape, size, and functional groups of the template. Thus, it exhibited better selectivity to DNB than other NAC. All the results indicated that the MIP/MWCNT modified electrode presented high sensitivity, good stability and improved selectivity for the electrochemical reduction of DNB. The proposed electrochemical sensing technology is thus to open new opportunities for selectively detecting NAC and will be undoubtedly of great promising for the development of miniaturized and field-portable detection kits, which we hope to demonstrate in the near future.

Table 1 Selectivity of CNT-MIP modified electrode (Given the response of the CNT-MIP modified electrode 100%)

Type of explosives	Current percentage (CNT modified electrode)	Current percentage (CNT modified electrode over MIP/CNT modified electrode)
DNB	100%	98.4%
DNT	100%	66.8%
TNB	100%	54.6%
TNT	100%	35.7%

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