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A new molecularly imprinted polymer (MIP)-based electrochemical sensor for monitoring 2,4,6-trinitrotoluene (TNT) in natural waters and soil samples

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ABSTRACT

A high selective voltammetric sensor for 2,4,6-trinitrotoluene (TNT) was introduced. TNT selective MIP and non-imprinted polymer (NIP) were synthesized and then used for carbon paste (CP) electrode preparation. The MIP, incorporated in the carbon paste electrode, functioned as selectively recognition element and pre-concentrator agent for TNT determination. The prepared electrode was used for TNT measurement by the three steps procedure, including analyte extraction in the electrode, electrode washing and electrochemical measurement of TNT. The MIP-CP electrode showed very high recognition ability in comparison to NIP-CP. It was shown that electrode washing after TNT extraction led to enhanced selectivity. The response of square wave voltammetry for TNT determination by proposed electrode was higher than that of differential pulse voltammetry. Some parameters affecting sensor response were optimized and then a calibration curve plotted. A dynamic linear range of 5×10^{-9} to 1×10^{-6} mol l^{-1} was obtained. The detection limit of the sensor was calculated equal to 1.5×10^{-9} mol l^{-1}. This sensor was used successfully for TNT determination in different water and soil samples.

1. Introduction

Due to health, ecological and security risks caused by long- and short-term exposure to explosive compounds, there is considerable interest in their measurements in environmental samples. Extensive efforts have been devoted to the development of innovative and effective sensors, capable of monitoring explosives rapidly in the low concentration and complex matrixes. Electrochemical sensors are among the widely used devices for TNT determination because of inherent sensitivity of these methods (Saravanan et al., 2006; Zimmermann and Broekaert, 2005; Agu et al., 2005; Wang and Thongngamdee, 2003). In order to enhance the sensitivity and also selectivity, the use of chemically modified electrodes is a common option (Wang et al., 2004; Hrapovic et al., 2006; Wang and Pumera, 2006; Shi et al., 2007). However, the moderate selectivity of these sensors can be considered as a main problem in this case. The use of electrochemical biosensors is the proper way to overcome the moderate selectivity problem of the electrochemical sensors based on the chemically modified electrodes (Naal et al., 2002).

Immunosensor is another class of biosensors and it involves the use of antibodies as biosensing element. Reaction takes place between a target analyte and a specific antibody. These immunosensors can be used for the detection of explosive compounds. For instance, the use of a membrane-based continuous flow displacement immunoassay for detection of nanomolar quantities of explosives has been reported (Rabbany et al., 1998).

Although biological receptors have specific molecular affinity, have been widely used in diagnostic bioassays and chemo/biosensors, they are often produced via complex protocols with a high cost, and require specific handling conditions because of their poor stability. Also, the natural receptors for many detected analytes do not exist (Whitcombe et al., 2000; Wulff, 2002; Haupt and Mosbach, 2000; Ye and Haupt, 2004). Thus, there has been a strong driving force in synthesizing artificial recognition receptors. Molecular imprinting is one of the most efficient strategies that offers a synthetic route to artificial recognition systems by a template polymerization technique (Mosbach, 2006; Tao et al., 2006; Sun et al., 2004; Piletskaya et al., 2005; Huang et al., 2004; Hall et al., 2006).

The synthesis technique is simple and cheap, and the resultant MIP materials exhibit; high selectivity, excellent mechanical strength, durability to heat, acid and base conditions and better engineering possibility than biological counterparts (Ye and Haupt, 2004). Moreover, the introduction of synthetic design into molecular imprinting strategy can even make a host element suitable for the analyte, for which the natural receptor does not exist. These characteristics allow MIP materials as recognition elements to be used in a wide range of fields (Andersson et al., 1990; Spivak, 2005).

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Although the main applications continue to be in selective separation, MIP-based sensors for the detection of active molecules, pharmaceuticals and environmental pollutants, are perhaps the most challenging and have attracted considerable interest in recent years (Piletsky and Turner, 2002; Holthoff and Bright, 2007a,b; Stephenson and Shimizu, 2007). The sensors with MIP recognition element can identify and quantify the target species by converting the analyte-MIP binding event into a physically readable signal.

Some chemical sensors, designed for TNT determination based on the MIP technology, can be found in the literatures. A chemical sensor, with capability to detect TNT at ppb quantities, has been developed to detect 2,4,6-trinitrotoluene utilizing planar integrated optical waveguide (IOW) attenuated total reflection spectrometry (Walker et al., 2007). In that work submicron thick films of organically modified sol–gel polymers, containing imprinted selective sites for TNT molecules, were deposited on the waveguide surface as the sensing layer. Binding of TNT and subsequent conversion to the anion, results in the attenuation of light, propagating through the waveguide and creating a spectrophotometric sensing device.

In the other work TNT was measured by the quartz crystal microbalances (QCM) sensor coated with thin layer of MIP containing TNT selective sites (Bunte et al., 2007).

Recently, another electrochemical sensor for the detection of TNT with enhanced sensitivities by imprinting of structure-like picric acid as substituting templates in the composite film of Au nanoparticles and conductive polymers has been reported. The ppt level detection limit and high selectivity was reported for this sensor (Riskin et al., 2008).

These works are interesting particularly with respect to the reported low detection limit and high selectivity. However, complex instrumentation requirement and complicated sensor preparation protocol together with high cost of the methods are the main disadvantages of these works.

In this work, a new, cheap and simple method was applied for design and preparation of the high selective and sensitive electrochemical sensor for TNT determination at sub-ppb quantities (DL ~ 0.34 ppb). The MIP having recognition sites for TNT was used as a recognition element, in the carbon paste electrode. This biomimetic modifier functioned as selectivity increasing and preconcentrator agent for TNT determination. The prepared electrode was used for TNT determination by the three steps procedure, including analyte extraction in the electrode, electrode washing and electrochemical measurement of TNT. It was found that the washing step had the main effect on the selectivity improvement of the sensor by removing the weakly absorbed interferences from the electrode without considerable effect on the sensitivity of the sensor. The optimized sensor was used successfully for TNT determination in water and soil samples.

2. Experimental

2.1. Instruments and reagents

Electrochemical data were obtained with a three-electrode system using a potentiostat/galvanostat model PGSTAT302, Metrohm. The differently prepared MIP or NIP involved sensors were used as a working electrode. A platinum wire and an Ag/AgCl electrode were used as the counter and reference electrodes, respectively. Methacrylic acid (MAA), obtained from Sigma–Aldrich (Munich, Germany), was purified by passing them through a short column of neutral alumina, followed by distillation under reduced pressure. Ethylene glycol dimethacrylate (EDMA), obtained from Fluka (Buchs, Switzerland), was distilled under reduced pressure in the presence of hydroquinone inhibitor and stored at 4 °C until use. 2,4,6-trinitrotoluene, para-nitrophenol, aniline, phenol, nitrobenzene, n-eicosane and 2, 2′-azobisisobutyronitrile (AIBN) were supplied by Sigma–Aldrich (Munich, Germany), and used as received. Graphite powder was purchased from Fluka (Buchs, Switzerland). Other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Molecularly imprinted polymers preparation

In order to produce a molecularly imprinted polymer template molecule (1 mmol), methacrylic acid (4 mmmol) and 50 ml of dry chlorofom were placed into a 100 ml round-bottomed flask and the mixture was left in contact for 10 min. Subsequently, EDMA (24 mmol) and AIBN (0.2 mmol) were added. The flask was sealed and the mixture was purged with nitrogen for 15 min. Polymerization took place in a water bath at 60 °C for 24 h. The final polymer was simply powdered and the template was removed by Soxhlet extraction with methanol for 48 h. The complete removal of template from the polymer was traced by the square wave voltammetry method. The non-imprinted polymer (NIP) was prepared similar to the MIP, except that the template was not present in the polymerization media.

In order to obtain finer and smaller MIP particles, the obtained powder was sequentially immersed three times in acetonitrile, and the supernatant portions were collected for final use.

2.3. Preparation of the sensors

For construction of the sensor (MIP-CP or NIP-CP), 0.05 g graphite was homogenized in a mortar with 0.01 g of powdered 2,4,6-trinitrotoluene MIP or NIP for 10 min. Subsequently, n-eicosane, 0.03 g was melted in a dish in a water bath heated at 45–50 °C. The graphite/MIP blend was then added to the melted n-eicosane and mixed with a stainless steel spatula. The final paste was used to fill a hole (2.00 mm in diameter, 3 mm in depth) at the end of an electrode body previously heated at 45 °C. After cooling at room temperature, the excess of solidified material was removed with the aid of sand paper. The electrode can be reused after each experiment by moving the electrode surface on a paper in order to rub out a thin layer of the electrode surface.

2.4. TNT measurement in real samples

In order to determine the TNT in the real samples, the sensor was immersed into the spiked solution with pH 4.5, adjusted by acetate buffer solution (0.15 mol l^{-1}). After incubation for 10 min the sensor was washed by emplacing it in the water/acetonitrile (97:3) solution for 15 s, then, the electrode was transferred to the electrochemical cell containing 10 ml of HCl solution (0.07 mol l^{-1}). The pre-potential of −1.0 V was applied to the electrode for 30 s. Finally, the square wave voltammograms in the potential range of 0.0–1.0 V with SW potential amplitude of 50 mV and frequency of 150 Hz were recorded and the current peak was used for final determination.

3. Results and discussion

3.1. Cyclic voltammetry behavior of TNT

Electrochemical behavior of TNT in different mediums and various electrodes has been studied and reported (Schmelling et al., 1996; Plambeck, 1982; You et al., 1997; Wang et al., 1998). However, the cyclic voltammetry of TNT was investigated by using pure carbon paste electrodes having no MIP or NIP. The obtained voltammogram is shown in Fig. 1. As can be seen, three distinct reductions