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A capacitive biosensor for ultra-trace level urea determination based on nano-sized urea-imprinted polymer receptors coated on graphite electrode surface

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A novel urea biosensor based on capacitive detection was developed using nano-sized molecularly imprinted polymers (nano-MIP). The sensitive layer was created by casting a thin layer of poly (vinyl chloride) (PVC)/nano-MIP composite on a graphite electrode surface. Cyclic voltammetry and impedance spectroscopy were used to monitor the electrode surface modification. The insulating properties of the layer were studied in the presence of $K_3Fe(CN)_6/K_4Fe(CN)_6$ redox couple by AC impedance measurements. The proposed capacitive sensor exhibited good selectivity for urea, compared to the chemicals with high resemblance to urea. The repeatability of the senor was found to be satisfactory. Very wide dynamic linear range (1×10^{-11}–1×10^{-4} M) as well as an ultra-trace detection limit equal to 5 picomolar was obtained for the sensor. The relevant experiments indicated satisfactory repeatability and reproducibility for the developed sensor. The results from sample analysis confirmed the applicability of the MIP-based sensor to quantitative analysis of urea in real samples.

1. Introduction

The determination of urea is important in a wide range of fields, including clinical diagnostics, environmental monitoring and food science. Several analytical methods including colorimetric method based on the reaction of urea and diacetylmonoxime derivatives (Fearon, 1993; Sullivan and Havlin, 1991; Goeyens et al., 1998). Infrared spectrometry (Lefier, 1996; Jensen et al., 2004) and high performance liquid chromatography (Clark et al., 2007) have been utilized for urea determination. Furthermore, electrochemical techniques such as potentiometry, conductimetry, amperometry and coulometry have also been extensively studied for the detection of the urease catalyzed hydrolysis products (Koncki et al., 2000; Ciana and Caputo, 1996; Lima et al., 1998; Lee et al., 2000). Urease is not ideally specific and it has been shown to catalyze the amido-hydrolysis of other compounds, such as formamide, acetamide and N-hydroxy urea (Dallet et al., 2000). Besides, ions such as sodium, potassium and fluoride inhibit the activity of urease. Also, urease-based analyses are subject to interference from ammonia existing in the sample (Francis et al., 2002).

Molecularly imprinted polymers have been introduced as interesting materials to act as counterparts for biological receptor (Alizadeha et al., 2010; Alizadeh and Amjadi, 2011; Alizadeh and Akhoundian, 2010; Alizadeh, 2009). This strategy has also been applied to prepare urea recognition materials. In the previously reported work, urea-imprinted polymer has been prepared on the gold electrode surface based on the phase inversion procedure (Huanga et al., 2009). Although, this technique gives recognition material capable to recognize the target compound; but, suffer from drawback of instability of the created selective sites of the imprinted layer.

Cross-linked imprinted polymers, however, are better options for the recognition purpose, regarding the stability and selectivity of the cavities created in the MIP. Anyway, in spite of this well-known advantage, the application of the cross-linked MIP as recognition element of the capacitive sensor has not already been reported. The reason can be addressed to the poor assembly ability of the commonly used bulky-shaped MIP particles at the surface of transducers (Holthoff and Bright, 2007). Moreover, it is not facile to prepare a uniform, repeatable and adequately thin MIP-based layer without destructive defects on the electrode surface by using common MIP. Nano-sized imprinted polymers, on the other hand, possess better assembly ability and can be used to prepare a thin layer on the aimed electrode surface without considerable defects.

Previously, we proposed a simple way for the preparation of cross-linked urea-imprinted polymer (Alizadeh, 2010). The polymer showed proper selectivity and recognition characteristics. The same formulation was used here for the MIP preparation. In order to decrease the MIP particle size, as far as possible, the method

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of suspension polymerization in silicon oil was utilized for the MIP synthesis. The nano-MIP particles, blended with poly (vinyl chloride), were casted on a graphite electrode to provide urea selective electrode. Impedance spectroscopy technique was applied to trace the recognition of urea molecules. Since urea is not an electroactive compound a capacitive sensor was used as a detection tool for urea.

2. Experimental

2.1. Apparatus and reagent

Electrochemical data were obtained with a three-electrode system using a potentiostat/galvanostat model PGSTAT302, Metrohm. The modified graphite disc electrodes ($d=3$ mm) were used as the working electrodes. A platinum wire and an Ag/AgCl electrode were used as the counter and reference electrodes, respectively. AUTOLAB PGSTAT302 electrochemical analysis system and GPES 4.9 software package were used for the electrochemical impedance spectroscopy. Impedance measurements were performed at frequency range$=5\ \mathrm{mHz}^{-1}$ MHz, $\Delta E_{\mathrm{ac}}=50$ mV and dc potential of 0.25 V (in the presence of redox couple of $\mathrm{Fe(CN)}_6^{3-}/\mathrm{Fe(CN)}_6^{4-}$. The measurements were performed in a three-electrode system: counter electrode (platinum), reference electrode (Ag/AgCl) and working electrode (MIP electrode).

Acrylic acid (AA) and ethylene glycol dimethacrylate (EGDMA), supplied by Sigma-Aldrich (Munich, Germany) were distilled under reduced pressure and used as functional monomer and cross-linker agent, respectively. 2,2-Azobisisobutyronitrile (AIBN), Sigma-Aldrich (Munich, Germany), was utilized as initiator. Urea and thiourea were from Fluka (Buchs, Switzerland). Arginine, cysteine and tryptophane were obtained from Merck (Germany). Amberlite 200C (Dow Chemicals) cation-exchange resin was pretreated with HCl (1 M) and then applied for real sample treatment. All other reagents were of analytical grade and were from Sigma-Aldrich (Munich, Germany).

2.2. Synthesis of nano-sized urea-imprinted polymer

The nano-sized urea-imprinted polymer was prepared according to the procedures described previously (Alizadeh, 2012). Briefly, In order to prepare nano-sized MIP by suspension polymerization in silicon oil, 5 mL of acetonitrile was mixed with 4 mmol of acrylic acid and 1 mmol of urea. The mixture was shaken for 10 min and after addition of 16 mmol of EGDMA, the shaking was continued again. When urea was completely dissolved, 0.01 g initiator (AIBN) was added. The described mixture, was poured into silicon oil (60 mL), purged previously with a stream of nitrogen gas for 15 min. Then, the polmerizable mixture was dispersed at 2000 rpm for 5 min. Next, the solution was further mixed by ultrasonic mixer in order to prepare smaller polymerizable droplets. The mixture was then purged with nitrogen gas for 10 min. Polymerization was carried out in a water bath, fixed at 65 °C, for 12 h. The synthesized particles were filtered and washed with petroleum ether and toluene several times. To extract the remained monomers and template from the polymer networks, the particles were successively washed with hot ethanol, ethanol/water and sulfuric acid solution (0.1 M). After every washing step, the polymer nanoparticles were separated from the solution by centrifuging method. Finally the polymer was washed with distilled water and dried in an oven overnight. The non-imprinted polymer (NIP) was prepared and treated similar to the MIP, except that the template was not present in the polymerization step.

2.3. Preparation of electrode and analysis method

A determined amount of PVC powder (4 mg) was dissolved in 5 mL of tetrahydrofurane solvent and shaken to dissolve it. Then, a known mass of MIP particles (5 mg) were suspended in the previous solution. The resulting mixture was sonicated for 15 min in order to obtain a homogenous suspension. Thereafter, 5 μL of resulting suspension was dropped on a homemade graphite disc electrode (disk area$=0.28\ \mathrm{cm}^2$ and shroud area$=1.13\ \mathrm{cm}^2$) and let evaporate the solvent. By this means, a thin layer of MIP/PVC composite was deposited on the electrode surface. This coating procedure leads to a film of about 2 μm thickness. The film thickness was calculated from the mass and the surface area of the coated film, assuming PVC density of $1.41\ \mathrm{g\,.cm}^{-3}$ (disk area$=0.28\ \mathrm{cm}^2$ and shroud area$=1.13\ \mathrm{cm}^2$).

In order to assay urea, the prepared electrode was immersed in 15 mL of electrolyte solution containing determined amount of urea. The solution was mixed via a magnetic stirrer for 6 min and afterwards, the stirring was stopped, followed by fulfilling the electrochemical impedance spectroscopy experiment.

2.4. Urea determination in real samples

0.5 mL of serum or urine sample was transferred to a beaker containing 3 g of pretreated cation-exchange resin and, followed by dilution to 50 mL. The mixture was shaken for 30 min and then 50 μL of the supernatant solution was transferred to the electrochemical cell containing 15 mL of acetate buffer solution ($pH=5$). The electrode, prepared according to the optimized conditions, was immersed in the solution. The solution was mixed by a magnetic stirrer for 10 min in order to complete urea adsorption in the MIP sites. Afterwards, the impedance measurement was performed at frequency range$=5$ mHz–1 kHz and $\Delta E_{\mathrm{ac}}=50$ mV. The capacitance of the electrode at the frequency of about 10 HZ was calculated and then subtracted from that calculated for the same electrode in the absence of urea and the resulting value was utilized for urea measurement according to the previously established calibration curve.

3. Result and discussion

3.1. Preparation of nano-sized MIP-based layer on the graphite electrode

Urea- imprinted polymer was synthesized according to the suspension polymerization in silicon oil. This technique can provide very small MIP particles (Alizadeh and Amjadi, 2011). Scanning electron microscopy image of the MIP particles, obtained by the described method, is represented in Fig. 1. As can be seen, the synthesized particles have nano-sized dimension with average size of about 50 nm (estimated from the SEM topographic map). The nano-scale dimensions of the MIP enabled us to make a thin layer of sensing composite on the electrode and led to fast binding kinetics for urea.

3.2. MIP-PVC blend as an insulating layer on the graphite electrode

When an electrode is immersed in the electrolyte, an electrical double-layer will come into being and the electrode/electrolyte interface behavior can be considered as a plate-capacitor. The capacitance can be expressed as the following equation:

$$C=\frac{\varepsilon_o \varepsilon A}{d} \tag{1}$$