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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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ABSTRACT

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 diacetyl-monoxime 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$=5$ mHz^{-1} MHz, $\Delta E_{ac}=50$ mV and dc potential of 0.25 V (in the presence of redox couple of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$. 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$=0.28$ cm^2 and shroud area$=1.13$ 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 g .cm^{-3} (disk area$=0.28$ cm^2 and shroud area$=1.13$ 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 $\Delta E_{ac}=50$ 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:

$$C=\frac{\varepsilon_o \varepsilon A}{d} \quad (1)$$

where ε_o is the permittivity of the free space; ε_r is the dielectric constant of electrical double-layer that separates the electrode from the electrolyte; A is the surface area; d is the closest distance from the electrode to the electrolyte. As can be seen from Eq. (1), any insulating membrane adsorbed onto the electrode will change the thickness and/or the dielectric properties of the dielectric layer. The structure can be considered as a serial capacitor and the capacitance can be calculated as:

$$1/C_t = 1/C_i + 1/C_m \quad (2)$$

where C_t is the total capacitance; C_i is the initial capacitance; C_m is the capacitance of the insulating membrane. As C_i is much larger than C_m, C_t approximates to C_m. One difficulty with capacitive sensors is that their sensitivity depends on obtaining the proper thickness of the original sensing layer (Hu et al., 2002). If the original sensing layer is too thin, the underlying electrode surface may be partially exposed, allowing for non-specific interactions from interfering species. However, if the original sensing layer is too thick, the detected AC impedance current as a result of change in capacitance upon analyte binding is dramatically reduced.

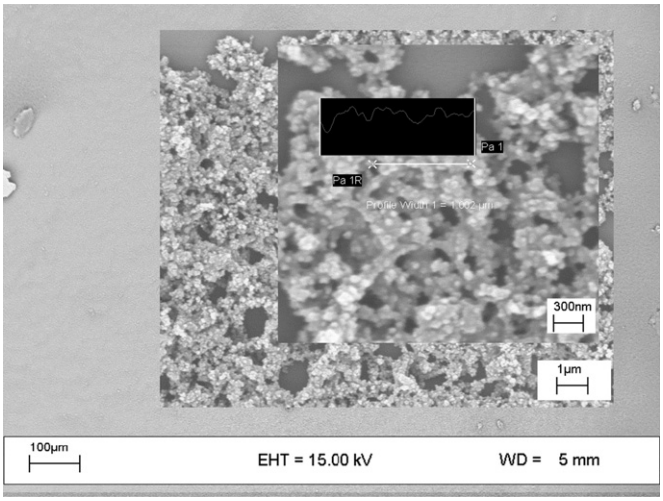

Fig. 1. Scanning electron microscopy image of the synthesized urea-imprinted polymer.

Here, in order to prepare a urea sensor, PVC/MIP blend was casted on a graphite electrode surface to yield a thin layer on the electrode surface. The nano-sized imprinted polymer enabled us to prepare a thin PVC/MIP layer on the electrode surface. In the absence of nano-sized MIP particles the coating of a thin composite layer on the electrode is not successful even if a thin layer of PVC can be layered on the electrode surface. Moreover, bulky-shaped MIP particles/PVC composite layer is not uniform and repeatable insulating layer cannot be prepared. As mentioned before, in addition to need to a thin sensing film on the electrode, a good insulation of the electrode surface is very important for acquiring a proper capacitive sensor. The insulating property of the composite layer was investigated by cyclic voltammetry in 10 mmol L^{-1} $K_3[Fe(CN)_6]$, as shown in Fig. 2(I). As can be seen, coating of a PVC layer and also casting of PVC/MIP composite on the graphite electrode surface had led to disappeared cyclic voltammetry signal of the redox couple. This indicates that the polymeric composite layer has insulated the graphite surface towards the redox couple, existing in the solution.

Electrochemical impedance spectroscopy was also applied to investigate the electrode surface coating. The Nyquist plot, shown in Fig. 2(II), indicates that the impedance of the electrode increases greatly, after casting of a thin layer of PVC on it. However, the impedance of the electrode decreases when the PVC/MIP layer is deposited on the graphite electrode; although, it

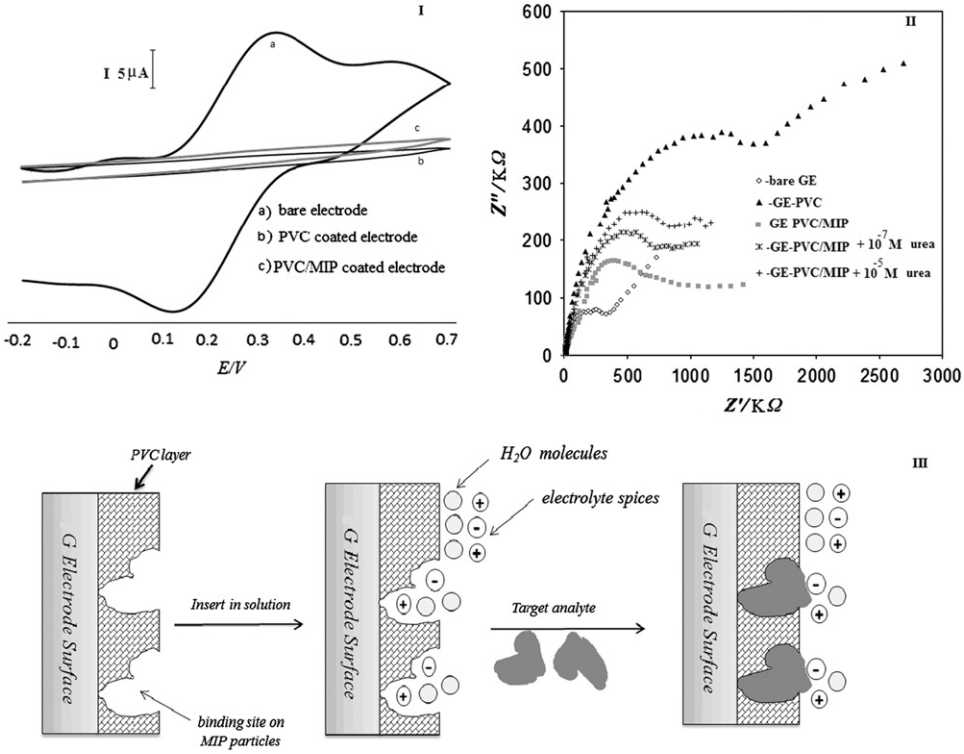

Fig. 2. (I) Cyclic voltammetry obtained for bare electrode (a), PVC coated (b) and PVC/MIP coated (c) electrodes, recorded in the solution of $[Fe(CN)_6^{3-}]$ (0.001 M) and phosphate buffer (0.1 M, $pH=3$); (II) Nyquist plots recorded for different electrodes and different urea concentration, frequency range = 5 mHz^{-1} MHz, ΔE_{ac} = 50 mV, electrode composition: MIP = 3 mg, PVC = 5 mg; (III) schematic representation of the proposed mechanism for signal generation in the developed MIP-based capacitive sensor.

is still much higher than the bare graphite electrode. This is likely due to the presence of MIP particles with considerable porosity which permit the water molecules and electrolyte ions to approach further to the electrode surface, compared to the case of PVC coated graphite electrode. According to the depicted Nyquist plots, immersing of the electrode coated with PVC/MIP composite, in the solution, containing urea molecules, increases again the electrode impedance. Moreover, it can be seen that the observed increase intensity in the impedance is directly proportional to the urea concentration. The mechanism proposed for the explanation of these observations is schematically shown in Fig. 2(III). It is supposed that the selective cavities of the MIP particles, existing in the PVC layer, are occupied with urea molecules as soon as the electrode is immersed in the urea solution. This event leads to removal of water molecules as well as the electrolyte ions from the cavities, resulting in intense decrease in impedance of the electrode. The capacitance of the sensor was found to considerably descend as a result of urea recognition. Since in such a phenomenon the water molecules with higher dielectric constant ($\varepsilon_{H2O}=80$) is replaced with urea molecules with lower dielectric constant ($\varepsilon_{urea}=3.5$), thus, one can expect considerable decrease in capacitance of the sensing film as a result of urea recognition. Moreover, the MIP particles can undergo swelling when interacting with target molecules, increasing thus the thickness of the insulating layer. This phenomenon can be also considered as another reason for the capacitance descent, observed after urea recognition.

3.3. Capacitive detection

In EIS, a small amplitude sinusoidal voltage signal applied to an electrochemical cell results in a measurable current response. Electrochemical changes, occurring at electrodes, are manifested either as the resistive or capacitive properties of materials, simply referred to as the impedance (May and Russell, 2002). The measured AC impedance can be expressed as:

$$Z = Z' - jZ'' \tag{3}$$

where Z' and Z'' are the real and imaginary parts of the measured impedance, respectively. Moreover, the imprinted polymer-coated electrode can represent a capacitive behavior. However, common situation is the non-ideal behavior of most capacitors in electrochemical systems under study, which results in impedimetric spectra where the semicircles of Nyquist diagrams presents a depressed and not completely symmetric shape. To fit better the experimental data to theoretical curves, the use of a constant phase element (CPE) instead of a capacitor is required (Kowino and Sadik, 2005; Sanchez et al., 2005; Suni, 2008). The impedance of a CPE is given by:

$$ZCPE = (j\omega)\alpha/C \tag{4}$$

where ω is the radial frequency, C the capacitance, and α an empirical coefficient. For a constant phase element the exponent $\alpha < 1$, since $\alpha = 1$ corresponds to the ideal capacitor.

The relationship between capacitance and impedance is given by the following equation:

$$C = -1/2\pi f Z'' \tag{5}$$

Here, f is the AC frequency (Hz). Capacitance values can be calculated directly using the above equation (Kowino and Sadik, 2005). The equivalent circuit of the MIP-based capacitive sensor can be determined by analyzing its Bode plots. Bode plots of impedance magnitude and phase angle as functions of frequency are given in Fig. 3(I) and (II). It can be seen that in the frequency range of 3–100 Hz the MIP/PVC coated graphite electrode shows capacitive behavior because of linearity of the frequency dependent impedance curve in this region. However, it is clear that the demonstrated capacitor is not ideal; because, the slope of linear curve is relatively far from 1. The deviation of ideality of the simulated capacitor can be assigned to the roughness of the polymer-coated surface which is enriched by addition of MIP

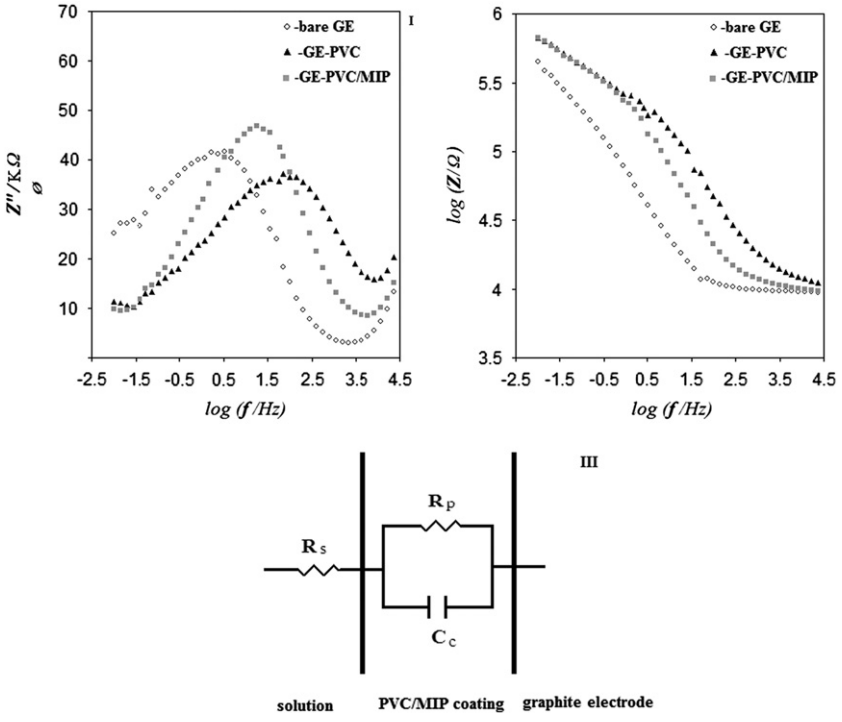

Fig. 3. (I) and (II) Bode plots calculated for bare graphite electrode, PVC coated electrode and PVC/MIP coated electrode, $\Delta E_{ac}=50$ mV, electrode composition: MIP=3 mg, PVC=5 mg; (III) simulated electrical circuit proposed for the PVC/MIP coated graphite electrode.

particles. Another Bode plot (phase angle as a function of frequency) indicates that the coating of the electrode surface with MIP/PVC composite makes the electrode to become better capacitor, compared to the electrode coated only with PVC; because, phase angle magnitude approaches more to the ideal value of 90° in the case of PVC/MIP coated electrode. In order to see the best capacitor characteristic for the aimed sensor, the frequency of about 10 Hz, giving the maximum phase angle, was chosen. According to the above mentioned expressions, a simple equivalent circuit, shown in Fig. 3(III), can be used to simulate the modified graphite electrode, coated with MIP/PVC film.

3.4. The effect of different parameters on the sensor performance

It is evident that the composition of the coating on the capacitive sensor has crucial effect on the final sensor performance. Because, the recognition site and it's availability to the target molecule is controlled by the MIP amount in the surface as well as the percent of PVC, blended with the MIP. Moreover, regardless to the recognition sites population, the capacitive sensor performance depends strongly on the coating thickness and its surface morphology which is certainly under control of the coating amount and its composition. Thus, the effect of the coating composition on the sensor performance was evaluated in detail. In order to investigate the effect of PVC or MIP content of the coating, different amounts of PVC and MIP were dissolved in tetrahydrofurane, while the volume of the solvent was kept constant. Then, 5 μL of the prepared solution was casted on the electrode surface. Fig. 4(I) represents the effect of the PVC amount in the coating on the capacitive sensor behavior. Increasing of PVC amount in the coating leads to an increase in the sensitivity of the capacitive sensor. However, higher than 4 mg, the sensitivity of the sensor for urea molecules declines considerably. Thus, 4 mg of PVC was chosen for final sensor fabrication. It seems that increasing of the PVC amount increases the insulation layer on the electrode, improving thus the efficiency of the simulated capacitor. However, further increase in PVC amount and thus in coating layer thickness decreases the sensitivity of the surface to urea recognition event. This is a usual disadvantage of the thick layer insulating coating. In the case of the MIP (Fig. 4(II)), it seems that increasing of the MIP improves the efficiency of the electrode surface, as its response increases with increasing of the MIP content of the surface. However, it was observed that higher amount of the MIP led to unrepeatable response for urea recognition. Moreover, because of porous nature of the MIP particles, the coating with higher content of MIP deviated strongly from ideal capacitor behavior. Therefore the MIP amount of the coating was chosen equal to 5 mg for the next experiments.

In this work, the nano-sized MIP particles were utilized for the preparation of sensing surface, because of their fast rebinding kinetic which allowed obtaining a short response time for the aimed sensor. However, with respect to the fact that considerable portion of the MIP sites were still situated far away the MIP particles surface, it was necessary to give enough time to reach adsorption equilibrium and thus steady state sensor response. Therefore the effect of sensor immersion time on its capacitive response magnitude was investigated. Fig. 4(III) shows the obtained results. It can be seen that the sensor response increases as the time increases; but, after 6 min the response of the electrode keeps constant. The recognition of any target molecule by the MIP materials is usually influenced by solution *pH* (Alizadeh, 2009; Alizadeh and Akhoundian, 2010). Fig. 4(IV) indicates that this developed sensor can be affected by the solution pH. It can be seen that $pH=5$ is the best condition for the sensor for responding to the urea molecules among the tested *pH* conditions. At further *pH* values, the sensor response to urea declined likely due to the deprotonation of the carboxylic groups of the binding sites of the MIP and thus weakening the binding site interaction with urea. Moreover deprotonation of the carboxylic acid functional groups makes the solvated positive ions to

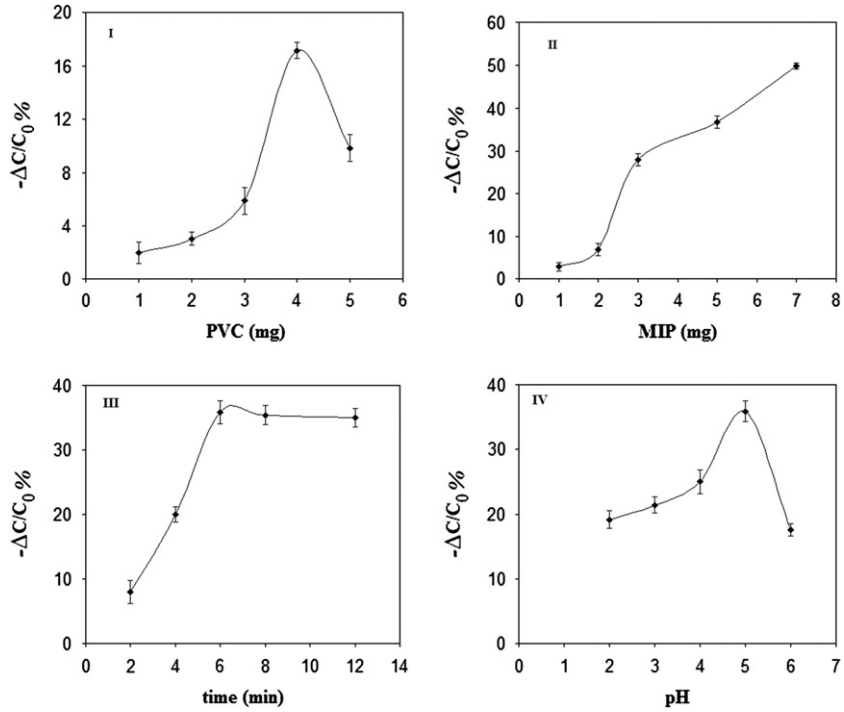

Fig. 4. Effects of different factors on the sensor performance; (I) variation of sensor signal as a function of PVC amount (mg) (MIP=2.5 mg, time=6 min, $pH=5$); (II) variation of sensor signal as a function of MIP amount (mg) (PVC=4 mg, time=6 min, $pH=5$); (III) variation of sensor signal as a function of equilibrium time (min) (PVC=4 mg, MIP=5 mg, $pH=5$); (IV) variation of sensor signal as a function of solution *pH* (PVC=4 mg, MIP=5 mg, time=6 min); EIS conditions for all experiments: frequency range=5 mHz^{-1} kHz, $\Delta E_{ac}=50$ mV.

penetrate into the sites and thus to swell the MIP particles. This phenomenon can be responsible for the losing of recognition characteristic of the MIP particles at higher *pH* values.

3.5. Evaluation of the selectivity and analytical characteristics of the designed final sensor

The optimized sensor was used for the evaluation of the sensor selectivity. For this aim the sensor was incubated in the solutions of different compound containing 1 μM of them.

Fig. 5(I) represents the obtained results. It can be seen that the response of the sensor for urea is greatly higher than that for its highly similar compound like thiourea. In the cases of other tested compounds including glucose, arginine, cysteine and tryptophan the same behavior can be observed, meaning that this sensor is meaningfully selective for urea against other similar and dissimilar compounds. In order to evaluate the effect of some common interfering agents on the sensor performance, the sensor response was checked for urea in the presence of other aimed interfering agents. The tolerance limit was established as the maximum concentrations of foreign species that caused a relative error of 7% in the analytical signal. The mole ratio of the interfering agent per urea that produced the defined error in the urea determination was considered as interference level. According to the obtained results, the developed determination method was not influenced in the presence of considerable amounts of thiourea, glucose, alanine, arginine, uric acid, tartaric acid, citric acid, creatinine, ascorbic acid, uric acid and dopamine. The interference levels of the described compounds were found to be 35, 50, 30, 50, 100, 80, 60, 60, 100,150 and 50, respectively. Moreover, the interference levels of cationic species like Fe^{3+}, Zn^{2+}, Cu^{2+} and NH^{4+} were 20, 30, 40 and 170, respectively. However, the presence of about 2- and 5-fold excess of Mg^{2+} and Ca^{2+}, respectively, was significantly influenced the sensor response to urea.

Calibration curve of the sensor showed that the sensor response for urea concentration is linear between concentration range of $1\times10^{-11}-1\times10^{-4}$ M. The calibration curve of the MIP-based capacitive sensor together with that obtained for the NIP-based sensor is shown in Fig. 5(II). As can be seen the MIP-based sensor responds to urea in a wide range of concentration, but the response of the NIP-based electrode for urea is not reasonable in a wide urea concentration range and its sensitivity to urea is not high enough, compared to that of the MIP electrode. This clearly indicates that the response of the MIP-based electrode for urea originates mainly from the selective cavities, crated during MIP preparation stage. The detection limit of this sensor was calculated equal to 3.7 picomolar (*S*/*N*). Five times determinations of a fixed amount of urea (1 μM) with a single sensor showed a standard error of 3.8%. Moreover, three replicated determinations of the same concentration of urea with three separately fabricated sensors resulted in 6.8%. These results are indicatives for satisfactory repeatability and reproducibility of the developed method.

The developed method was applied to assay urea content of some plasma and urine samples. As shown in the previous section, in order to get rid of the interference effect of some ions like Ca^{2+} and Mg^{2+}, which are usually present in biological samples, a strong acid cation-exchange resin was utilized to remove these agents from the samples. The determination results were summarized in Table 1. Moreover, in order to statistically verify the developed method, two serum sample, assayed previously by the developed method, were also analyzed by a HPLC

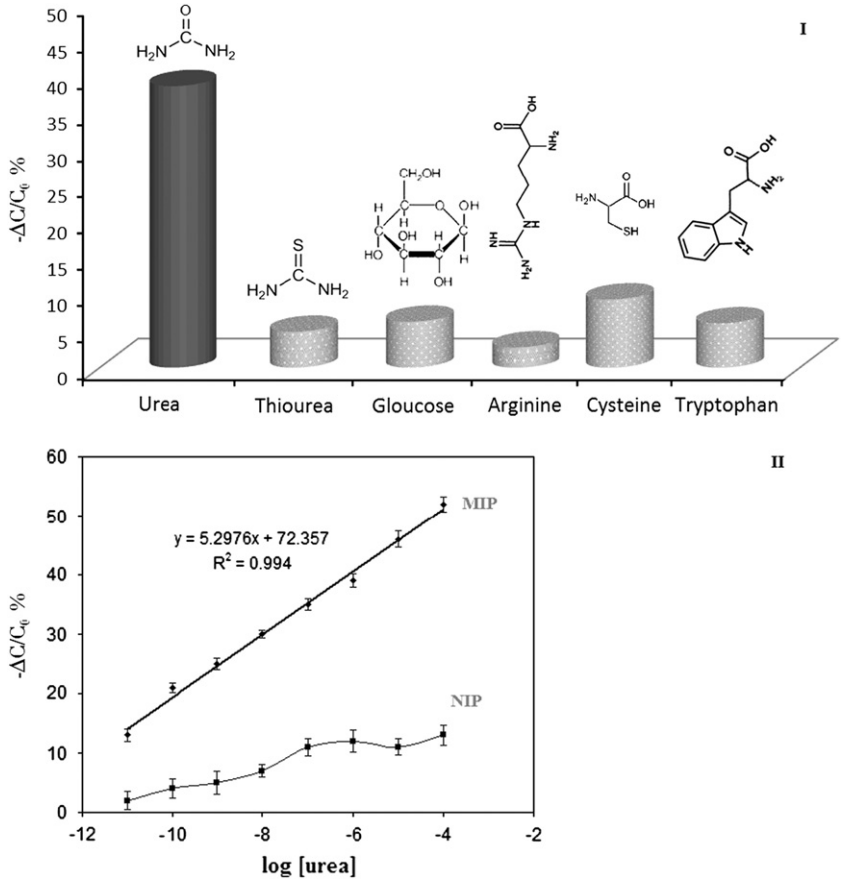

Fig. 5. Comparison of the urea response in the developed capacitive sensor with those of other structurally similar and dissimilar compounds at the same concentration; urea calibration curve of the capacitive sensor prepared by MIP or NIP at optimized conditions.

Table 1
Determination of urea in different real samples by the developed method ($n=3$) and reference HPLC technique ($n=4$).

Sample	Urea	Added (mmol L^{-1})	Found (mmol L^{-1})	Recovery (%)	HPLC
Serum1	6.2	1.0	7.0	97.2 (4.4)	6.4 (0.2)
Serum 2	2.8	1.0	4.1	108.0 (4.0)	–
Serum3	3.5	3.0	6.3	96.9 (6.3)	3.2(0.1)
Serum4	7.3	3.0	9.8	95.1 (5.5)	7.5 (0.2)
Serum5	5.5	–	–	–	5.2 (0.2)
Urine1	320.0	50.0	381.0	102.9 (3.9)	–
Urine 2	201.0	100.0	296.0	98.3 (5.0)	–
Urine 3	254.5	50.0	318.0	104.5 (3.6)	240.3 (9.4)
Urine 4	187.7	–	–	–	198.5 (7.5)
Water 1	–	0.50	0.44	88.0 (7.3)	–
Water 2	–	0.10	0.09	90.0 (5.7)	–

method. The obtained results of MIP-based and reference HPLC methods were statistically compared by the paired t-test. This test revealed the absence of significant differences between the results obtained by both methods (at 95% confidence level).

4. Conclusion

A new, cheap and simple strategy was proposed for the preparation of a capacitive sensor for urea with very low detection limit and high selectivity. Although, a real capacitor was not obtained for MIP/PVC coated on the graphite electrode; anyway, it was proved that a proper insulation on the electrode surface could be implemented by the nano-sized MIP particles, blended with a proper adhesive polymer like PVC. The effectiveness of the proposed technique was rationalized by demonstrating of a reasonable relationship between target molecule recognition event and the capacitive response of the sensor. The sensor kept its selectivity for urea in the presence of higher amount of other compounds, even very structurally similar compound like thiourea. However, some ionic agents like Mg^{2+} and Ca^{2+} annihilated the sensor urea selectivity. Treatment of aqueous samples with the ion-exchange resins was proposed to overcome this problem. Despite, the nano-sized MIP particles led to a relatively proper repeatability in sensor preparation; however, the repeatability in successive sensor preparation is still poor, thus further investigation in this case is required.

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