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Molecularly Imprinted Polypyrrole for Sensor Design

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Nano-structured (molecularly imprinted) conducting polymer, polypyrrole (Ppy), has been synthesized and applied in design of chemical sensor sensitive to caffeine. Electrochemical potential pulls technique has been applied for synthesis of molecularly imprinted polypyrrole (mPpy) and its deposition on electrode surface. Caffeine was used as a template for molecular imprinting of polypyrrole. Pulsed amperometric detection (PAD) was applied for the control of dedoping/redoping of mPpy by caffeine and detection of caffeine binding to the mPpy. The evaluation of analytical signals and dedoping of molecularly imprinted polypyrrole has been tested and correspondingly described by exponential decay and exponential increase to maximum equations. The dependence of analytical signal on concentration of caffeine in the sample can be successfully described by hyperbola equation. The sensor was applied for detection of caffeine in diluted coffee samples.

Keywords: polymer chemistry, molecular recognition, qualitative analysis, electrochemistry, surface science, caffeine, nanotechnology.

INTRODUCTION

One of the most important continuing challenges for chemists is to devise new ways to manipulate molecules in order to create and manufacture useful new substances. Polymers are of the most important and beneficial substances that synthetic chemistry has brought to the human race. Usual polymers are insulators and they do not conduct electricity. But Alan J. Heeger, Alan G. MacDiarmid and Hideki Shirakawa have changed this view. They brought the unique properties of conjugated polymers to the fore in 1977 when they discovered that chemical doping of these materials resulted in increases in electronic conductivity over several orders of magnitude and could be made conductive almost like a metal [1]. Since then, electronically conducting materials based on conjugated (conducting) polymers have been applied in diverse items such as corrosion protection agents, light-emitting diodes, polymeric actuators, biomaterials, and sensors. Some conducting polymers like polyaniline, polytiophene or polypyrrole are biocompatible and cause minimal and reversible disturbance to the working environment and protect electrodes from fouling and/or interfering with electrochemically active materials [2]. They can be considered as effective immobilization materials [3]. The electrochemical formation of CP films has found increasing interest in the development of bio- [4] and immuno- [5] sensors since they allow non-manual reproducible formation of modified electrode surfaces with integrated biological recognition elements. The use of conducting polymers for immobilizing BC in sensor applications has the advantage, compared to conventional immobilization procedures, because the amount of deposited material can be readily controlled and the immobilizing matrix can conduct electricity allowing switch between conducting and isolating state. Polypyrrole is used most frequently in design of biosensors and affinity-sensors since it posses the best biocompatibility and the easy ways for immobilization of various biologically active compounds [6]. Moreover, useful copolymeric structures can be developed if differently modified monomers of polypyrrole are copolymerized [7 – 9].

Stabilization of the biological response is currently a major problem, with almost every reported sensor exhibiting a gradual degradation in the electrical signal during continuing measurements. It is due to instability of biomolecules used in design of biosensors. Resolution of this problem and the production of robust designs, vital for medical and environmental monitoring applications, can be based on creation of synthetic molecular recognition systems. Artificial receptors have been gaining importance as a possible alternative to immobilized biomolecule based systems. Molecular imprinting is increasingly becoming recognized as a versatile technique for the preparation of artificial receptors based on molecularly imprinted conducting polymers (MIPs) containing tailor-made recognition sites. MIP is another class of substances of great interest in the field of chemical sensor technology. These highly stable synthetic polymers possess molecular recognition properties due to cavities in the polymer matrix that are complementary to the analyte (ligand) both in shape and in positioning of functional groups [10]. It is the reason why development of synthetic recognition systems is of great interest to workers in the field of sensor technology. Moreover, some of these polymers have shown very high selectivity and affinity constants fully

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comparable to naturally occurring recognition systems, such as antibodies, which makes them especially suitable for use in artificial receptors. Overoxidized polypyrrole exhibits improved selectivity, which is attributed to the removal of positive charges from Ppy films due to introduction of oxygen functionality, such as carbonyl groups. The nano-pores and nano-cavities complementary to removed dopant can arise during dedoping process. Sensors based on mPpy for serotonin and 1-naphthalensufonate [11] were reported. Molecular imprinting is a technology for the manufacture of synthetic polymers with predetermined molecular recognition properties. The preparation of molecularly imprinted polymers requires polymerization around print species using monomers those are selected for their capacity to form specific and definable interactions with the print species. Within Ppy entrapped BC molecules can be removed by solvent extraction and the molecularly imprinted polymer is ready for use. Cavities are formed in the polymer matrix those are images of the size and shape of the print molecules. Furthermore, chemical functionalities of the monomer residues become spatially positioned around the cavity in a pattern those are complementary to the chemical structure of the print molecule [12]. These imprints constitute a permanent memory for the print species and enable the imprinted polymer to selectively rebind the print molecule from a mixture of closely related compounds. Finally, the print molecules are removed by solvent extraction and the molecularly imprinted polymer is ready to be used. In some instances very high selectivity and affinity constants have been reported, fully comparable to naturally occurring recognition system such as antibodies. Some of these synthetic polymers have been shown to be useful in sensor applications, exhibiting tolerance towards acid, base, high temperature and organic phases.

Caffeine (1,3,7-trimethylxanthine) is a wide range stimulant consumed on a worldwide basis. It is the most common psychoactive substance consumed in the world. Earlier our works has shown that caffeine activates immune system [13], increases activity of lysozime in the blood [14] and exhibit sufficient antibacterial activity [15]. Detection of this biologically active compound in human diet is very important challenge of analytical chemistry.

The aim of the present study was to apply mPpy based sensors for detection of caffeine in real samples.

EXPERIMENTAL

Chemicals, equipment and electrochemical setup

All chemicals were of analytical grade and used as received (except pyrrole, which was purified additionally by passing through 5 cm length Al_2O_3 filled column). Oxygen-free solutions were obtained by purging argon gas through the solution for at least 20 min. All electrochemical polymerization and analyte detection procedures were performed by Potentiostat-galvanostat "VoltaLab-80" Radiometer analytical (Villeurbanne Cedex, France). All electrochemical experiments were performed using conventional three-electrode system, all potentials were referred to an Ag/AgCl/3 M KCl reference electrode. Pulsed amperometric detection (PAD) was applied for the detection of dedoping/redoping Ppy by caffeine. The anodic current of Ppy modified electrode was recorded during the sequence of 5 potential pulses with pulls profile: 1 s – 0 mV and 1 s – +600 mV mV vs. Ag/AgCl.

Electrode pretreatment and synthesis of molecularly imprinted polypyrrole

A platinum wire was melted in soft glass exposing a disk electrode with a diameter of 1 mm. As cleaning and pretreatment procedures, the Pt electrodes were immersed in concentrated HNO_3 for 10 min in an ultrasonic bath, rinsed with water, and polished on a polishing cloth using alumina paste with 3, 1, and finally 0.3 μm grain size. Afterwards the electrodes were rinsed with water, and ultrasonically treated in 10 M NaOH, and 5 M H_2SO_4 for 10 min in each solution.

After mechanical and ultrasonic pretreatment potential cycling follows in 0.1 M H_2SO_4 with a scan rate of 100 mV s^{-1} from –300 to +1200 mV until the cyclic voltammogram displays the characteristic features of a bare platinum surface. The Pt electrode was platinized by cycling the electrode in 0.1 M KCl containing 0.8 mM H_2PtCl_6. It was performed by 5 potential cycles between +500 and –400 mV vs Ag/AgCl with a scan rate of 10 mVs^{-1}. Solution containing 50 mM of pyrrole 100 mM of KCl and 5 mM of caffeine was used for electrochemical formation of caffeine doped polypyrrole films. The electrochemical formation of the caffeine doped polymer film was carried out by application of 30 potential pulses between 950 (1 s) and 350 mV (for 10 s), to allow the caffeine and the pyrrole monomer to equilibrate in the neighborhood of the electrode. Electrodes modified with blank polypyrrole have been prepared using the same deposition conditions in the absence of caffeine during the film-formation protocol. During final mPpy preparation step caffeine was extracted from the caffeine doped Ppy polymeric backbone by phosphate buffer, pH 7.0.

RESULTS AND DISCUSSION

Caffeine plays a role of molecular template, during preparation of molecularly imprinted polypyrrole. After elution of the template, complementary binding sites are revealed allowing specific rebinding of analyte. The recognition sites obtained possess sufficient binding affinities. Pulsed amperometric detection was applied to investigate elution of caffeine from polypyrrole matrix (dedoping) and to detect binding of analyte to molecularly imprinted polypyrrole (redoping). For this purpose pulsed amperometric detection is applied during intended periods (e.g. before elution, during elution at particular periods, before and during incubation in analyte containing solution). The differences in electrochemical signals registered are calculated and used as the value indicating redoping/dedoping of mPpy. To facilitate evaluation of analytical signal the differences between anodic and cathodic peak-currents in potential pulls amperograms were calculated. Analytical signal generated/propagated during analyte binding at mPpy depends on the following steps: (i) diffusion of analyte towards the electrode, (ii)

migration of analyte through the polymer membrane, (iii) adsorption-desorption of analyte at the specific binding sites of Ppy. The slow rates of migration and adsorption/desorption process are considered to be the rate-determining steps, and in generally they can be described by exponential decay to minimum if caffeine is added into solution or exponential rise to maximum if caffeine is eluted from Ppy matrix by blank buffer.

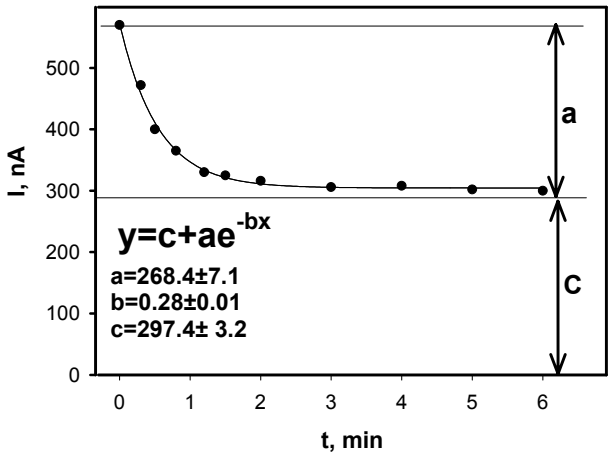

Fig. 1. Dependence of PAD current response on mPPy modified electrode incubation time obtained in 0.1 M phosphate buffer, pH 7.0, with 100 mM caffeine

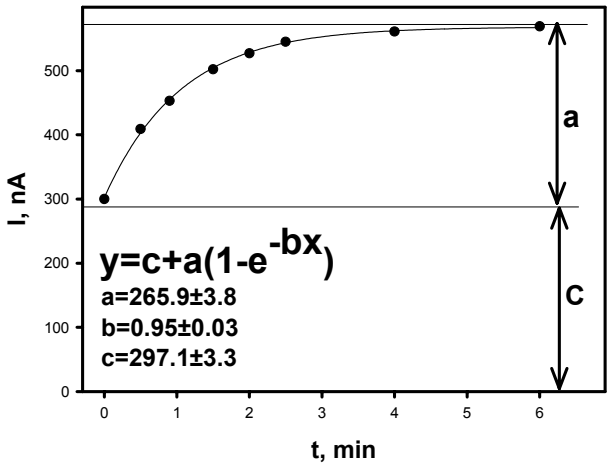

Fig. 2. Dependence of PAD current response on mPPy modified electrode incubation time obtained in blank 0.1 M phosphate buffer, pH 7.0

Exponential decay to minimum during detection of caffeine by mPpy based sensor in the sample (Fig. 1):

$$y = c + ae^{-bx}; \quad (1)$$

where parameters:
a – corresponds maximal possible amplitude for changes in analytical signal;
c – minimum of exponential decay to minimum; corresponds to the component of PAD signal which is not influenced by caffeine,
b – exponential parameter on which the decay in rate of calculated curve depends.

Exponential rise to maximum during elution of caffeine from Ppy matrix by blank buffer solution (Fig. 2):

$$y = c + a(1 - e^{-bx}), \quad (2)$$

where parameters:
a – corresponds maximal possible amplitude for changes in analytical signal;
c – from this value starts the exponential rise; corresponds to the component of PAD signal which is not influenced by caffeine;
b – exponential parameter on which the increase in rate of calculated curve depends.

Electrodes modified by blank Ppy were at least – 9 – 10 times less sensitive to caffeine than mPpy modified electrodes (data not shown).

Two methods were applied for estimation of analyte concentration: (i) using simple calibration curve, (ii) calculation of analyte concentration from the data obtained by standard addition method.

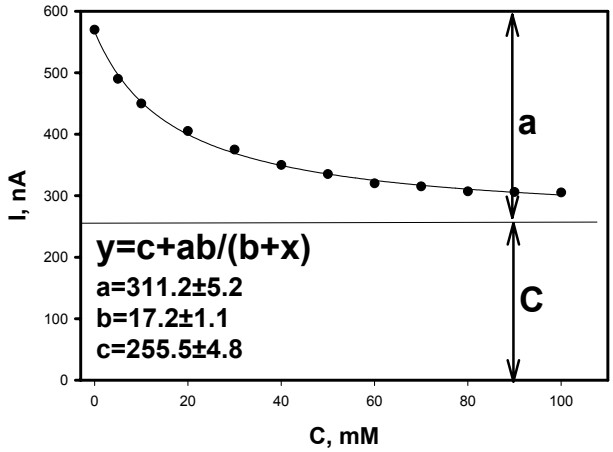

Fig. 3. The dependence of PAD current response on caffeine concentration in 0.1 M phosphate buffer, pH 7.0, obtained after 5 min. incubation

Calibration of caffeine sensor is performed by measuring analytical signal after incubation of mPpy modified electrode in different caffeine concentrations containing samples (Fig. 3). This curve is well approximated by hyperbola equation:

$$y = c + a \cdot b/(b + x), \quad (3)$$

where hyperbola parameters:
a – corresponds to maximal possible amplitude for changes in analytical signal;
c – corresponds the component of PAD signal which is not influenced by caffeine;
b – parameter on which the decay in rate of calculated curve depends.

Derivation of the presented equation, which is suitable for this particular caffeine; detection by mPpy case, is presented:

$$\text{Caffeine} + \text{mPpy} \leftarrow k_2 \;\; k_1 \rightarrow \text{Caffeine} - \text{Ppy}_{complex};$$

or schematically:

$$A + B \leftarrow k_2 \;\; k_1 \rightarrow A - B; \quad (4)$$

where:

A is the concentration of analyte;
B is the surface/volume concentration of free binding sites able to bind caffeine, change in analytical signal is directly proportional to this concentration.
C is the equilibrium concentration of $A - B$ complex;
D is the surface/volume concentration of all (free+engaged) binding sites able to bind caffeine;

$$D = B + C; \quad (5)$$

From (4) at equilibrium conditions:

$$k_1 \times A \times B = k_2 \times C; \quad (6)$$

From (6):

$$B = k_2 \times C / k_1 \times A;$$
$$K = k_2 / k_1;$$
$$B = K \times C / A; \quad (7)$$

From (5):

$$C = D - B; \quad (8)$$

From (7) and (8):

$$B = K \times (D - B) / A = K \times D / A - K \times B / A;$$
$$B + K \times B / A = K \times D / A;$$
$$B \times (1 + K / A) = K \times D / A;$$
$$B = (K \times D / A) / (1 + K / A) =$$
$$= (K \times D / A) / ((A + K) / A) = \mathrm{K{\times}D / (A{+}K)}; \quad (9)$$

PAD Signal (y) is superposition of two signals: B – which depends on caffeine concentration and constant c, which does not depends on concentration of caffeine in the sample:

$$y = c + B; \quad (10)$$

From (10) and (9):

$$y = c + K \times D / (K + A); \quad (11)$$

The symbols of this equation are transformed to more mathematically acceptable symbols: $K \rightarrow b$; $D \rightarrow a$; $A \rightarrow x$.
Form $(11)^{th}$ equation appears hyperbola equation (3).

After this, caffeine is again eluted from the Ppy matrix to form mPpy. PAD measurement is performed before and after 5 min. incubation of mPpy modified electrode in the caffeine containing sample. In our experiments 10 times by phosphate buffer diluted Jacobs coffee was investigated as analyte containing sample. The point '*X*' corresponding unknown caffeine concentration in the sample is presented in both plots (Fig. 4). It is reliable that coffee solution contains some other compounds that cannot specifically interact with mPpy and/or directly electrochemically interfere with analytical signal. To minimize this influence standard addition method is applied where known caffeine amounts are added into the same diluted coffee solution and analytical signals are measured after incubation (Fig. 4).

The points are approximated by the same hyperbola equation (3) and hyperbola constants are calculated. Then the value proportional to caffeine concentration in the coffee containing sample is calculated by using equation $y = c + a \times b / (b + x)$ derived from (3):

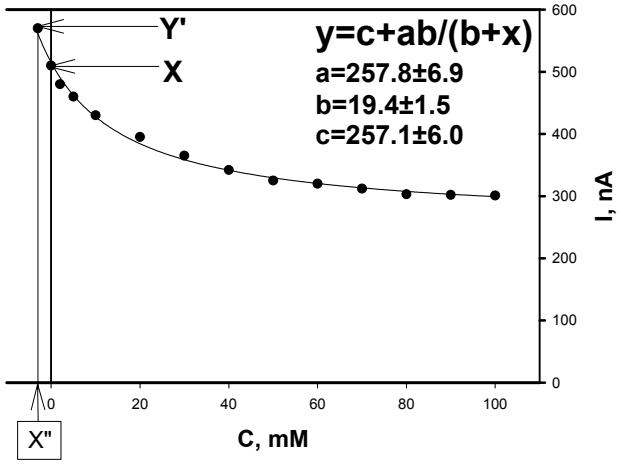

Fig. 4. Estimation of caffeine concentration in the diluted coffee sample by standard addition method. '*Y*' value was obtained in the blank 0.1 M phosphate buffer, pH 7.0; 'X' value was obtained after incubation of mPPy modified electrode in 0.1 M phospahe buffer, pH 7.0, containing 1/10 volume of coffee sample with unknown caffeine concentration. Other points were obtained after consecutive addition of fixed caffeine concentration into coffee sample containing phosphate buffer solution. *C*, mM represents known caffeine concentration added as standard additives

$$(b + x) = (a \times b) / (y - c);$$
$$x = (a \times b) / (y - c) - b; \quad (12)$$

where: a, b, c are hyperbola constants, y is value of analytical signal detected before incubation in caffeine containing sample.

Note, that the point *Y'*, which corresponds to 0 concentration of caffeine in the sample is located in the plot region that has negative concentration values. The value calculated from (12) has also negative meaning. Concentration of caffeine in the sample containing coffee should be calculated from difference obtained if from the caffeine concentration measured in the presence of coffee sample (point *X* in Figure 4) is deducted value calculated from (12):

$$X'' = X' - x; \quad (13)$$

where:
X'' – concentration calculated in the coffee containing sample;
X' – abscise value of point X which corresponds to the measurement of PAD signal after addition of coffee sample. Note, that this value is equal to 0, since it corresponds the point where no standard additives were added.
x – value calculated from equation (12). Please note that this value has a negative meaning according to position of *Y'* point in the plot.

Then (13) becomes conformation:

$$X'' = 0 - (-x); \quad (14)$$

It means that concentration of caffeine in coffee sample can be interpreted as a modulus of value calculated from (12):

$$|x| = (a \times b) / (y - c) - b \; ; \quad (15)$$

From results obtained it clearly seems that caffeine concentrations calculated by both methods differ – in the case of standard addition method caffeine concentration seems to be approximately 15 % lower. With the highest probability by using standard addition method it is possible to estimate and eliminate influence of other analytical signal distorting materials. The influence of ascorbic acid as very usual and very intense electrochemical interfering material was investigated. The results clearly show that the influence of ascorbic acid on mPpy coated electrode and Ppy is almost at the same level and at least 20 times smaller if compared with bare Pt electrode. Access of ascorbic acid to the electroactive surface of polypyrrole was prohibited in our system by highly cross-linked and overoxidized structure of the polymer, which limits the access of noncomplementary to mPpy and negatively charged ascorbic acid molecules.

The reproducibility of analytical signal was tested by application of series of 20 detections of 60 mM caffeine under to the same conditions as described in experimental section with the same sensor, variation coefficient calculated according to the corresponding equation [16]. In this case variation coefficient was found to be 3.2 %. But if the variation coefficient was calculated according the same equation for individual tests with 11 similarly produced sensors it would be 14.4 %. It shows that deposition of molecularly imprinted polypyrrole over the platinum electrode still needs some improvement to achieve more uniform molecularly imprinted polypyrrole layers. However such sensors can be successfully used after calibration and it is usual in construction of bioanalytical sensors.

The stability tests were performed within 9 day period. It was found that analytical signals constantly decrease during this period by approximately 9 % and can be described by exponential decay to minimum $y = c + ae^{-bx}$. Such character of inactivation is rather ordinary in the cases of chemical sensors and is determined by destruction of analyte sensitive layer. The parameter b which characterizes sensor inactivation velocity was found to be 7.2. It means that $\tau^{1/2}$ (half life period) of such sensor is 7.2 days. It can be due to constant degradation of polypyrrole film in the buffer solution and this analytical problem can be solved by recalibration of sensor based on molecularly imprinted polypyrrole every 4 – 6 hours.

The lower detection limit of the sensor was calculated from the calibration plot (Fig. 3) and it was found to be in the range of 0.8 mM of caffeine, according to (3) since according to calculations in the presence of such or higher concentration of caffeine, analytical signal measured is 3 times higher in comparison with average noise level of electrochemical system used. But according to our investigations significant decrease in lower detection limit of mPpy based analytical systems can be observed if the thickness of mPpy layer is significantly reduced by reduction of potential cycles number during electrochemical deposition of mPpy. Upper detection limit with 5 % reliability was found to be 14.5 mM of caffeine if linear regression [16] was applied instead of hyperbola decay equation presented in Figure 3. Upper detection limit can be extended up to 65 mM if hyperbola decay equation is applied for estimation of analytical signal. If concentration of caffeine is higher than 65 mM discrimination between the highest analytical signal and analytical signal measured in the presence of >65 mM caffeine does not exceed 5 %. Such significant increase of detection limit can be achieved by application of more appropriate hyperbola decay mathematical model (as it was demonstrated in this paper) in comparison with very usual, simple linear regression, since it incorrectly describes real processes taking place in the presented analytical system.

CONCLUSIONS AND FUTURE DEVELOPMENTS

The presented experimental results show that electrochemical affinity sensor based on molecularly imprinted polypyrrole could have a great potential for direct sensing. It is also expected that other organic template molecules can be imprinted/determined instead of caffeine. Registration of binding/desorption of template molecule and quantification of analyte can be performed by very simple PAD method. In the near future additional analytical characterisation (e.g. selectivity, reusability, stability etc.) of mPpy based sensors will be investigated. Future improvements can be expected through optimization of the measurement parameters and the use of a custom-made analyte displaying electrochemical characteristics as well as better and more specific binding to the imprinted polymer.

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