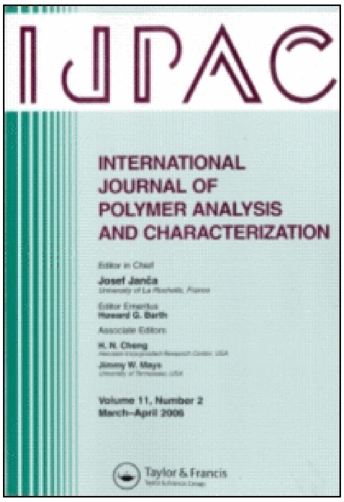

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Capacitive Detection of Theophylline Based on Electropolymerized Molecularly Imprinted Polymer

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Abstract: The capacitive sensor was prepared by electropolymerization of phenol onto the surface of gold electrode. By using AC impedance measurements, the sensor is based on the decrease in capacitance caused by the analyte used as the template in the formulation of an electropolymerized molecularly imprinted polymer as receptor layer. Improvement of the insulating properties of the sensor was investigated in detail. Electrochemical impedance (EI) was used to characterize the modified gold electrodes. Unlike the capacitive sensors reported previously, the present sensors were not treated with alkanethiol after the electropolymerization and show even more satisfactory performance.

Keywords: Capacitive sensor; Molecularly imprinted polymers; Theophylline

INTRODUCTION

During the past decade, molecular imprinting technology (MIT) has become a well-established analytical tool. Non-covalent imprinting, in particular, has found a great range of applications because of the theoretical lack of restrictions on size, shape, or chemical character of the imprinted molecule. The possibility of tailor-made, highly selective

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artificial receptors at low cost, with good mechanical, thermal, and chemical properties, makes these synthetic materials appear ideal chemoreceptors. There are great hopes for development of a new generation of chemical sensors using these novel synthetic materials as recognition elements.[1,2]

A review of the literature allows us to observe that MIT offers advantages by providing polymers capable of molecular recognition and analysis. Moreover, it has been successfully used in solid phase extraction,[3–10] enzymemimic catalysis,[11,12] binding assays,[13,14] affinity chromatography or capillary electrophoresis,[15–19] sensors,[20–32] and drug detection.[33–35] Despite the large amount of data available to date on formulas for molecularly imprinted polymers (MIPs), the development of electrochemical sensors, in particular, has been significantly slower. Electrochemical sensing could offer good limits of detection (LOD), at low cost, with the possibility of easy miniaturization and automation. This type of transduction is especially attractive for making a readily available range of small devices based on recognition by templating effect in relevant applications, such as biomarkers in clinical chemistry, environmental control in the field, on-line quality control in the pharmaceutical industry, and detection of food fraud.

The capacitance transducer is based on the theory of the electrical double layer. A metal electrode immersed in an electrolyte solution can generally be described as resembling a capacitor in its ability to store charge. The capacitance (C) can be described by the equation:

$$C = \frac{A\varepsilon_0\varepsilon_r}{d} \tag{1}$$

where ε_r is the dielectric constant of the material between the plates, ε_0 is the dielectric constant for a vacuum, A is the area, and d is the distance between the plates. The capacitance changes when the distance varies because of adsorption or desorption of material. In most cases, the capacitance is measured at the metal/solution interface in the electrochemical system. The ions and dipoles are ordered outside a metal electrode in such a way that the charges in the metal are balanced, thereby forming the electrical double layer. Since capacitive measurements give information about the metal-solution interface, a chemical modification of this structure will lead to a change in capacitance. The degree of variation is determined by the nature and coverage of the modification material.

In contrast to the numerous MIP-based sensors employing other signal transducing mechanisms reported in the literature, capacitive sensors are relatively less available. For the construction of a capacitive sensor, an ultrathin membrane of perfect insulating properties is very important. Hence, alkanethiol was usually used to fill the defects of the membrane after the electropolymerization[23,31] to enhance the insulating property. But this process is time-consuming (usually more than 12 h). Also, the response time of the sensor is rather long (usually longer than 1 h). In the present study, alkanethiol was not used to fill the defects of the membrane. To acquire satisfactory insulating properties of the membrane, a smaller potential scan rate (0.005 V/s) was employed during the electropolymerization. Thus, the sensors can be fabricated more easily and quickly. At the same time, the sensors show satisfactory performance.

Asthma is an inflammatory disease characterized by bronchial hyperresponsiveness that can proceed to life-threatening airway obstruction. Theophylline is a bronchodilator that is widely used in the treatment of asthma and bronchospasm in adults. It is one of the most commonly prescribed pharmaceuticals[36] and was one of the three drugs most frequently monitored by pharmacokinetics services in the U.S. Veterans Affairs medical centers. A developmental study has found that theophylline caused clastogenic but not genotoxic effects in human lymphocytes after long-term exposure. As a phosphodiesterase inhibitor, theophylline synergized with chlorambucil in inducing apoptosis of b-chronic lymphocytic leukemia cells, which suggested that the combination might have therapeutic value.[37] Theophylline was demonstrated to inhibit the repair of potentially lethal DNA lesions.[38] Thus, the determination of theophylline is very important.

In the present study, a capacitive sensor for theophylline detection has been developed by electropolymerizingphenol onto the gold electrode in the presence of the template. Cyclic voltammetry and electrochemical impedance experiments were performed to verify the imprinting effect.

EXPERIMENTAL SECTION

Reagents

Phenol was recrystallized three times before use. All chemicals were analytical grade and were used as supplied without further purification. High-quality nitrogen was used for deaeration. Double-distilled water was used throughout all the experiments. All measurements were performed at room temperature.

Apparatus

Cyclic voltammetry and electrochemical impedance experiments were performed with CHI-660A electrochemical work station (USA). A standard three-electrode configuration was used. A molecularly imprinted polymer (MIP) coated gold electrode acted as working electrode, a platinum wire was employed as counter electrode, and a Ag|AgCl|KCl_{sat} electrode was used as reference electrode.

Preparation of Polymer-Coated Electrodes

Before modification, the surface of gold electrodes was prepared by polishing on a metallographic polishing cloth with aqueous alumina slurries with successive decrease in particle size (1 μm to 0.3 μm). The remaining particles on the surface were removed by ultrasonic treatment in distilled water for a few minutes. Then the electrodes were cleaned with a 1:3 mixture of 30% H_2O_2/concentrated H_2SO_4, rinsed with double-distilled water.

After deoxygenating the solution by bubbling nitrogen gas for about 15 min, the electropolymerization was performed by cyclic voltammetry (10 cycles) in the potential range 0.2 to 0.8 V at a sweep rate of 5 mV/s in 0.05 mol/L borate buffer solution containing monomers and template molecule. After the electropolymerization, the electrodes were washed with double-distilled water to remove the adsorbed molecules. In this way, the electrodes modified with imprinted membrane were prepared.

A control electrode was prepared following the same procedure but without the template molecule. The control electrode was treated by the same procedure as the imprinted electrode to ensure that the effects observed are due only to the imprinting features and not to the subsequent treatments undergone by the electrode.

Measurement

Cyclic voltammetry measurements were performed in the presence of 5 mM $K_3[Fe(CN)_6]$ and 0.2 mol/LKCl solution.

Alternating current (AC) impedance measurement was performed between 1 and 1000 HZ in 0.05 mol/L borate buffer solution, using pulse amplitude of 5 mV and quiet time of 2 s. After recording the impedance spectra, the electrode was washed again in the stirred ethanol:water (5:1) solution for 10 min for the next rebinding. All the capacitance (C) values were calculated directly from the imaginary part of Z'' by using:

$$C = \frac{-1}{(2\pi f Z'')} \tag{2}$$

RESULTS AND DISCUSSION

Formation of Polymeric Film

A typical cyclic voltammogram recorded during the electropolymerization in the presence of phenol and the theophylline is shown in Figure 1. There is a significant difference in comparison with voltammograms obtained under the same conditions but without the template. The

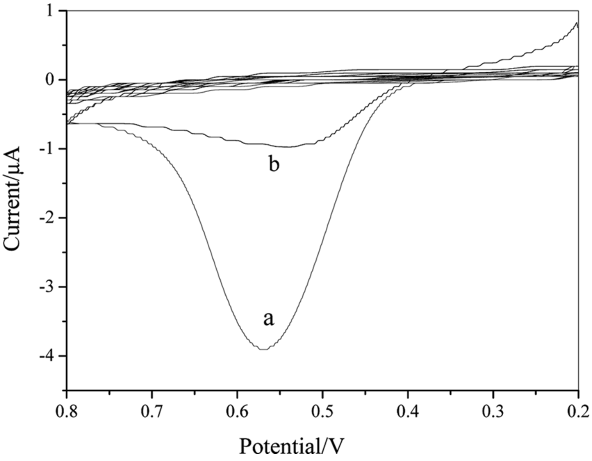

Figure 1. Cyclic voltammograms for the electropolymerization of 0.1 mol/L phenol at a gold electrode (a) in the presence of 9.0 mmol/L theophylline and (b) in the absence of theophylline.

oxidation wave appears completely irreversible. In the first cycle, a sensitive peak is observed at approximately 0.55 V. The peak current in the presence of 9.0 mmol/L theophylline is larger than that obtained in the absence of theophylline, indicating that there are interactions between theophylline and phenol; in the second cycle, the peak disappeared, which means that a nonconductive film was formed during the electrochemical recycle to block the access of the monomers to the electrode surface. From the total charge resulting from oxidation, the thickness of the polymer film can be estimated:

$$d = \frac{mQ}{(FA\rho)} \tag{3}$$

where d is the thickness of the membrane, m is the molecular weight of the phenol monomer, Q is the total charge transferred during the electropolymerization, F is the Faraday constant, A is the surface area of the electrode, and ρ is the density of the polymer. Under the electropolymerization conditions described above, Q is about 6.0×10^{-5} C, and the thickness of the resulting membrane is about 70 nm, which is suitable for capacitive detection.

Characteristics of Polymeric Film

The property of the polymeric layer was investigated by cyclic voltammetry in 5 mmol/L $K_3[Fe(CN)_6]$ and 0.2 mol/L KCl solution; the results are

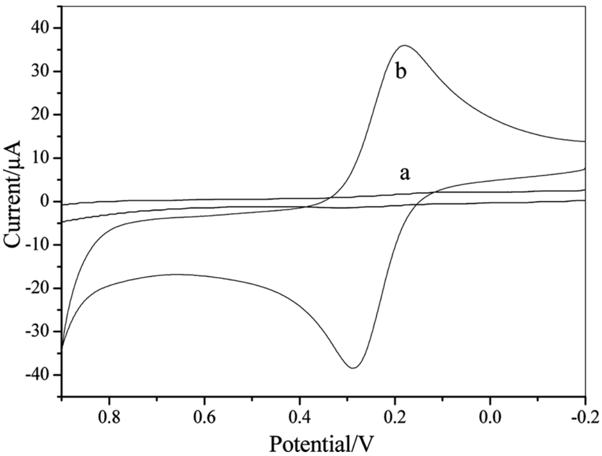

Figure 2. Cyclic voltammograms recorded in 5 mmol/L $[Fe(CN)_6]^{3-}$ + 0.2 mol/LKCl solution when the measuring electrode was (a) imprinted polymer-coated gold electrode and (b) bare gold electrode.

shown in Figure 2. It was found that the polymeric layer insulated the gold surface towards the redox couple presented in the solution. Thus, the polymer film exhibits good insulation in such medium. There was no obvious difference between the imprinted polymer-coated electrode and non-imprinted polymer-coated electrode.

The imprinted polymer-coated electrode was incubated in the stirred ethanol:water (5:1) solution for 10 min and then washed with double-distilled water. Impedance responses in 0.05 mol/L borate solution are shown in Figure 3. The impedance values (Z″) were lower than those of the imprinted polymer-coated electrode at the same frequency, indicating that theophylline was removed. When the incubation time went beyond 10 min, the impedance values kept stable, so we chose 10 min as the incubation time.

Figure 4 shows the Bode plot obtained with imprinted polymer-coated gold electrodes at different scan rates in borate buffer solution containing 0.01 mol · L^{-1} $KClO_4$ (pH 9.2). Curve a shows that the slope of the impedance is–0.9993 and the corresponding phase angle is nearly 83°; curve b shows that the slope of the impedance is –0.9995 and the corresponding phase angle is nearly 88°; curve c shows that the slope of the impedance is –0.9995 and the corresponding phase angle is nearly 87.5°. This indicates that the electrodes prepared at 0.005 V/s had satisfactory insulating properties and were not treated with alkanethiol after electropolymerization. It is obvious that the capacitive component played a prominent role in the modified electrode current. These electrodes were used as capacitive chemical sensors in the subsequent measurements.

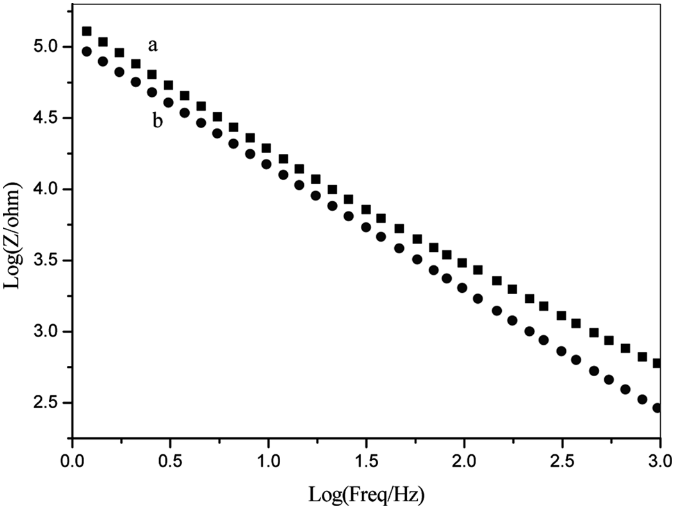

Figure 3. Impedance response in 0.05 mol/L borate solution when the measuring electrode was (a) imprinted polymer-coated electrode and (b) theophylline-removed polymer-coated electrode.

Theophylline Assay

The polymer-coated electrodes exhibited capacitive behavior, which enabled the initial capacitance of the electrode (C_0) to be calculated in the absence of the analyte. The imaginary impedance component (Z'') was used to calculate the electrode capacitance (C). No capacitance changes caused by the addition of theophylline were observed if the template was not removed from the imprinted polymer on the surface of the electrode. The capacitance of the imprinted electrode decreased on addition of theophylline to the sample solution. This might be related to surface effects and the analyte binding in the bulk polymer. The imaginary and real parts of the impedance were both increased, because of interaction with analyte. The response time was about 10 min, and a steady value was obtained after about 15 min. It was observed that the change of capacitance caused by the addition of theophylline was reversible and the sensor could be restored by immersion into an ethanol-water (5:1) solution after 10 min. The values of $(C_0–C_f)/C_0$ as a function of theophylline concentration are plotted in Figure 5. By using the ratio $(C_0–C_f)/C_0$, one could diminish the deviation caused by the variation in the measurement of capacitance. Using the data generated after 15 min incubation, a linear range up to 1.5×10^{-5} mol/L can be obtained with the detection limit of 1×10^{-6} mol/L. Comparable with previous

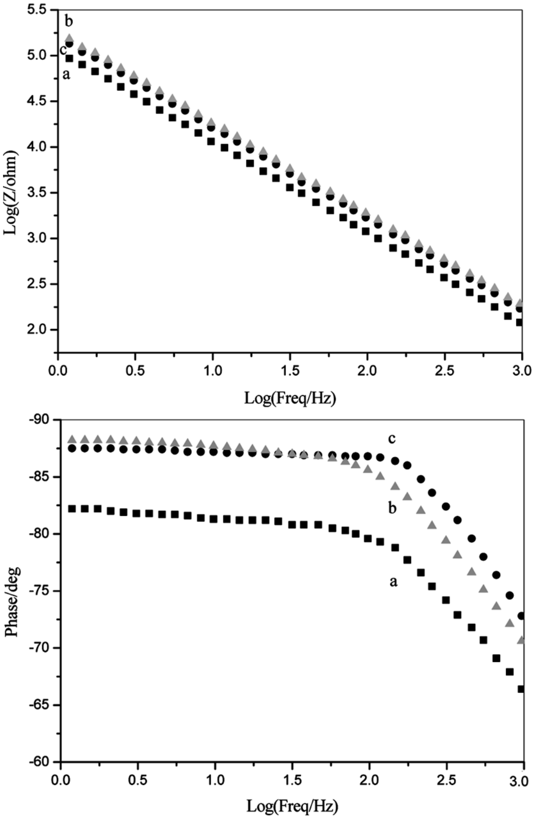

Figure 4. Bode plots for imprinted polymer-coated gold electrodes at different scan rates: (a) 0.01 V/s, (b) the electrode treated with alkanethiol, (c) 0.005 V/s.

methods for theophylline assay, the proposed method here has the advantages of easy preparation and shorter responding time. It was demonstrated that the sensors could retain 90% of their original response after 10 days' storage and that the reproducibility was good for preparation of different batches of sensors ($RSD \leq 10\%$).

Selectivity

To test the selectivity of the capacitive sensor for theophylline, the interference of the substances with structures similar to theophylline was investigated, and the results are shown in Figure 5. In the experiments, xanthine, 3,7-dimethyl xanthine, and trimethyl xanthine were selected to test the interference. As can be seen in Figure 5, no significant effects

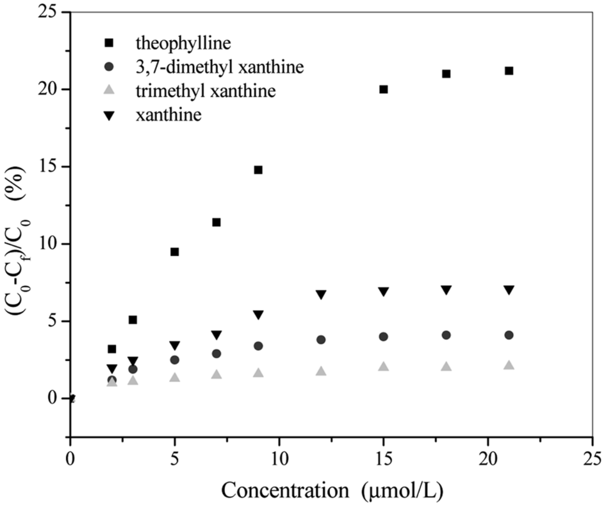

Figure 5. Effect of concentration of various substances on the change in C_0–C_f/C_0 ratio.

were observed upon addition of these interferents. The main interference was caused by xanthine. The sensor shows satisfactory selectivity.

CONCLUSIONS

We have developed an ultrathin capacitive chemical sensor for theophylline with electropolymerized molecularly imprinted polymer as receptor layer based on measurement of the change in electrode capacitance using the AC impedance technique. The proposed sensor provides a simple and selective alternative tool for the assay widely used in medicine.

REFERENCES

[1] Haupt, K. and K. Mosbach. (2000). Molecularly imprinted polymers and their use in biomimetic sensors. *Chem. Rev.* **100**, 2495.

[2] Haupt, K. (2001). Molecularly imprinted polymers in analytical chemistry. *Analyst* **126**, 747.

[3] Muldoon, M. T. and L. H. Stanker. (1997). Molecularly imprinted solid phase extraction of atrazine from beef liver extracts. *Anal. Chem.* **69**, 803.

[4] Zander, A., P. Findlay, T. Renner, B. Sellergren, and A. Swietlow. (1998). Analysis of nicotine and its oxidation products in nicotine chewing gum by a molecularly imprinted solid-phase extraction. *Anal. Chem.* **70**, 3304.

[5] Mullett, W. M., E. P. C. Lai, and B. Sellergren. (1999). Determination of nicotine in tobacco by molecularly imprinted solid phase extraction with differential pulsed elution. *Anal. Commun.* **36**, 217.

[6] Kala, R., J. M. Gladis, and T. P. Rao. (2004). Preconcentrative separation of erbium from Y, Dy, Ho, Tb and Tm by using ion imprinted polymer particles via solid phase extraction. *Anal. Chim. Acta* **518**, 143.

[7] Ensing, K. and T. de Boer. (1999). Tailor-made materials for tailor-made applications: Application of molecular imprints in chemical analysis. *Trends Anal. Chem.* **18**, 138.

[8] Owens, P. K., L. Karlsson, E. S. M. Lutz, and L. I. Andersson. (1999). Molecular imprinting for bio- and pharmaceutical analysis. *Trends Anal. Chem.* **18**, 146.

[9] Stevenson, D. (1999). Molecular imprinted polymers for solid-phase extraction. *Trends Anal. Chem.* **18**, 154.

[10] Masqué, N., R. M. Marcé, and F. Borrull. (2001). Molecularly imprinted polymers: New tailor-made materials for selective solid-phase extraction. *Trends Anal. Chem.* **20**, 477.

[11] Matsui, J., M. Higashi, and T. Takeuchi. (2000). Molecularly imprinted polymer as 9-ethyladenine receptor having a porphyrin-based recognition center. *J. Am. Chem. Soc.* **122**, 5218.

[12] Vallano, P. T. and V. T. Remcho. (2000). Affinity screening by packed capillary high-performance liquid chromatography using molecular imprinted sorbents: I. Demonstration of feasibility. *J. Chromatogr. A* **888**, 23.

[13] Vlatakis, G., L. I. Andersson, R. Muller, and K. Mosbach. (1993). Synthetic hosts by monomolecular imprinting inside dendrimers. *Nature* **361**, 645.

[14] Haupt, K., A. Dzgoev, and K. Mosbach. (1998). Assay system for the herbicide 2,4-dichlorophenoxyacetic acid using a molecularly imprinted polymer as an artificial recognition element. *Anal. Chem.* **70**, 628.

[15] Andersson, L. I. (2000). Molecular imprinting for drug bioanalysis: A review on the application of imprinted polymers to solid-phase extraction and binding assay. *J. Chromatogr. B* **739**, 163.

[16] Andersson, L. I. (2000). Molecular imprinting: Developments and applications in the analytical chemistry field. *J. Chromatogr. B* **745**, 3.

[17] Mullett, W. M. and E. P. C. Lai. (1998). Determination of theophylline in serum by molecularly imprinted solid-phase extraction with pulsed elution. *Anal. Chem.* **70**, 3636.

[18] Chen, W., F. Liu, Y. Xu, K. Li, and S. Tong. (2001). Molecular recognition of procainamide-imprinted polymer. *Anal. Chim. Acta* **432**, 277.

[19] Kempe, M. and K. Mosbach. (1995). Molecular imprinting used for chiral separations. *J. Chromatogr. A* **694**, 3.

[20] Sallacan, N., M. Zayats, T. Bourenko, A. B. Kharitonov, and I. Willner. (2002). Imprinting of nucleotide and monosaccharide recognition sites in acrylamidephenylboronic acid-acrylamide copolymer membranes associated with electronic transducers. *Anal. Chem.* **74**, 702.

[21] Gabai, R., N. Sallacan, V. Chegel, T. Bourenko, E. Katz, and I. Willner. (2001). Characterization of theswelling of acrylamidophenylboronic acid-acrylamide hydrogels upon interaction with glucose by Faradaic impedance

spectroscopy, chronopotentiometry, quartz-crystal microbalance (QCM), and surface plasmon resonance (SPR) experiments. *J. Phys. Chem. B* **105**, 8196.

[22] Blanco-Lopez, M. C., M. J. Lobo-Castanon, A. J. Miranda-Ordieres, and P. Tunon-Blanco. (2003). Voltammetric sensor for vanillylmandelic acid based on molecularly imprinted polymer-modified electrodes. *Biosens Bioelectron*. **18**, 353.

[23] Cheng, Z. L., E. K. Wang, and X. R. Yang. (2001). Capacitive detection of glucose using molecularly imprinted polymers. *Biosens Bioelectron*. **16**, 179.

[24] Panasyuk-Delaney, T., V. M. Mirsky, M. Ulbritch, and O. S. Wolfbeis. (2001). Impedometric herbicide chemosensors based on molecularly imprinted polymers. *Anal. Chim. Acta* **435**, 157.

[25] Lahav, M., A. B. Kharitonov, O. Katz , T. Kunitake, and I. Willner. (2001). Tailored chemosensors for chloroaromatic acids using molecular imprinted TiO_2 thin films on ion-sensitive field-effect transistors. *Anal. Chem*. **73**, 720.

[26] Gutiérrez-Fernández, S., M. J. Lobo-Castañón, A. J. Miranda-Ordieres, P. Tuñón-Blanco, G. A. Carriedo, F. J. García-Alonso, and J. I. Fidalgo. (2001). Molecularly imprinted polyphosphazene films as recognition element in a voltammetric rifamycin SV sensor. *Electroanalysis* **13**, 1399.

[27] Kirsch, N., J. P. Hart, D. J. Bird, R. W. Luxton, and D. V. McCalley. (2001). Towards the development of molecularly imprinted polymer based screen-printed sensors for metabolites of PAHs. *Analyst* **126**, 1936.

[28] Sergeyeva, A. V., S. A. Piletsky, A. A. Brovko, E. A. Slichenko, L. M. Sergeeva, and A. V. El'skaya. (1999). Conductimetric sensor for atrazine detection based on molecularly imprinted polymer membranes. *Analyst* **124**, 331.

[29] Kroger, S., A. P. F. Turner, K. Mosbach, and K. Haupt. (1999). Imprinted polymer-based sensor system for herbicides using differential-pulse voltammetry on screen-printed electrodes. *Anal. Chem*. **71**, 3698.

[30] Pizzariello, A., M. Stred'ansky, S. Stred'anska, and S. Miertus. (2001). A solid binding matrix/molecularly imprinted polymer-based sensor system for the determination of clenbuterol in bovine liver using differential-pulse voltammetry. *Sens. Actuators B* **76**, 286.

[31] Panasyuk, T. L., V. M. Mirsky, S. A. Piletsky, and O. S. Wolfbeis. (1999). Electropolymerized molecularly imprinted polymers as receptor layers in capacitive chemical sensors. *Anal. Chem*. **71**, 4609.

[32] Weetall, H. H. and K. R. Rogers. (2004). Preparation and characterization of molecularly imprinted electropolymerized carbon electrodes. *Talanta* **62**, 329.

[33] Hilt, J. Z. and M. E. Byrne. (2004). Configurational biomimesis in drug delivery: Molecular imprinting of biologically significant molecules. *Adv. Drug Deliv. Rev*. **56** 1599.

[34] Tan, Y., J. Yin, C. Liang, H. Peng, L. Nie, and S. Yao. (2001). A study of a new TSM bio-mimetic sensor using a molecularly imprinted polymer coating and its application for the determination of nicotine in human serum and urine. *Bioelectrochemistry* **53**, 141.

[35] Feng, L., Y. Liu, Y. Tan, and J. Hu. (2004). Biosensor for the determination of sorbitol based on molecularly imprinted electrosynthesized polymers. *Biosens. Bioelectron*. **19**, 1513.

[36] Dockendorff, B., D. A. Holman, G. D. Christian, and J. Ruzicka. (1998). Automated solid phase extraction of theophylline by sequential injection on renewable column. *Anal. Commun.* **35**, 357.

[37] Mentz ,F., M. D. Mossalayi, F. Ouaaz, S. Baudet, F. Issaly, S. Ktorza, M. Semichon, J. L. Binet, and H. Merleberal. (1996). Theophylline synergizes with chlorambucil in inducing apoptosis of B-chronic lymphocytic leukemia cells. *Blood* **88**, 2172.

[38] Slamenova, D., A. Gabelova, A. Ondrejkova, L. Ruzekova, T. Farkasova, and A. Collins. (1998). Preculturing of Chinese hamster V79 cells with sub-lethal concentration of theophylline sensitizes cells to cytotoxic effects of MNNG. *Mutat. Res.–DNA Repair* **408**, 11.