Urinalysis with molecularly imprinted poly(ethylene-co-vinyl alcohol) potentiostat sensors

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ABSTRACT

Among many important biomarkers excreted in urine are albumin, uric acid, glucose, urea, creatine and creatinine. In the growing elderly population, these biomarkers may be useful correlates with kidney dysfunction, infection and related problems such as glomerular, proximal, and distal convoluted tubule functions, diabetes, hypertension and proteinuria.

This study employed solvent evaporation processing of poly(ethylene-co-vinyl alcohol), (EVAL) to form molecularly imprinted polymers (MIPs) that recognize creatinine, urea, and lysozyme. The mole ratio of ethylene to vinyl alcohol affected the performance: 27 mol% ethylene gave the highest imprinting effectiveness for creatinine and urea, while 44 mol% gave the highest effectiveness for lysozyme. Electrochemical examination using a home made potentiostat and imprinted polymer electrode showed electrical signals responsive to the target molecules. Finally, an actual urine sample was tested using the electrode. The test results were compared with those of the commercial instrument ARCHITECT ci 8200 system to precisely determine the accuracy of the molecularly imprinted polymer electrode for urinalysis.

1. Introduction

The use of molecularly imprinted polymers (MIPs) as recognition elements in sensors has been reviewed in numerous articles (Haupt and Belmont, 2008; Lieberzeit and Dickert, 2008; Prasada Rao and Kala, 2008). Electrochemical, optical, mass sensitive thermometric and magnetometric transducers (Prasada Rao and Kala, 2008) have been designed to integrate with MIP thin films or micro/nanoparticles.

A severe challenge for MIP sensors is detection in chemically diverse environments, such as biological fluids (Gonzalez et al., 2008; Hugon-Chapuis et al., 2008; Lee et al., in press; Lee et al., 2008). For example, in addition to important biomarkers such as creatinine, urea, and lysozyme (Lee et al., 2008), urine contains non-protein nitrogen metabolites, carbohydrates, proteins and amino acids; detection of analytes must be made amid this complex chemical background.

One of the most useful analytical sensors is the electrochemical biosensor, specifically a potentiostat, because its reliability and ease of manufacture. A recent design using chip-type sensors has been used in preclinical trials (Bandyopadhyay et al., 2002; Frey et al., 2003; Kakerow et al., 1995; Linares-Barranco and Serrano-Gotarredona, 2003; Turner et al., 1987). Many researchers have attempted to develop a low cost, standalone and portable potentiostat which can be used in different sensors (Carullo et al., 1999; Huang et al., 2007; Liao et al., 2004; Rodríguez et al., 2003). Reviews of potentiometric sensors have been published by McCluskey et al. (2007) and Rao and Kala (Prasada Rao and Kala, 2008).

Several proteins of clinical interest (albumin, lysozyme, myoglobin and ribonuclease A) have been analyzed by surface micro-contact imprinting of poly(TEGDMA-co-styrene) in our previous work (Hsu et al., 2006a,b; Lin et al., 2006), which demonstrated the high imprinting effectiveness (high selectivity) of the material compared with similarly composed non-imprinted polymers. The polyethylene glycol lengths of the cross-linking
monomers that were used affected rebinding selectivity, likely by altering MIP hydrophobicity.

Poly(ethylene-co-vinyl alcohol), EVAL, has been used as an imprinting material for several proteins for filtration membranes. EVAL is commercially available with ethylene content of 27, 32, 38 or 44 mol\% (\(=m\), with \(m+n=1\)).

\[
\text{(CH}_2 - \text{CH}_2\}_m \text{(CH}_2 - \text{CH} = \text{OH)}
\]

Methods of forming EVAL membranes include precipitation by solvent evaporation and wet-phase inversion (Cheng et al., 1998; Young et al., 2000). Wet phase inversion has been adopted to prepare EVAL imprinted membrane matrices for the specific recognition of phospholipids (Pegoraro et al., 2008). With the high biocompatibility and easy manufacture of EVAL thin films, these membranes should be very suitable for use as sensing materials in biosensors. To reduce the time for synthesis, and to prevent possible covalent interactions between the monomer and functional groups in proteins (Hsu et al., 2006a,b; Lin et al., 2006), a new synthetic protocol using solvent evaporation without polymerization would be preferable (Lee et al., 2008).

This objective of this work was to develop a method of potentiostat-cyclic voltammetry using a low-cost, portable and standalone cyclic potentiostat to analyze binding to MIPs. The proposed cyclic voltammetry potentiostat, which is constructed from an SOC-based chip and off-the-shelf circuit components, has a wide range of operating currents and achieves consistent and high quality measurement. For system verification, a urinary biosensor was used to verify the performance of the home built cyclic voltammetry potentiostat. Analyses of random urine samples were compared between the new potentiostat, a commercial potentiostat (CHI 411A, CH Instrument, Inc., Austin, TX, USA) typically used for research, and the commercial instrument ARCHITECT ci 8200 system used in hospitals. The experimental results demonstrate that the accuracy of the new potentiostat is comparable to that of commercial potentiostats. Moreover, the new potentiostat could potentially be used outside the laboratory and applied in daily life.

2. Experimental

2.1. Preparation and characterization of molecularly imprinted polymeric thin films

Creatinine, urea and albumin were purchased from Acros Organics (Geel, Belgium). Poly(ethylene-co-vinyl alcohol), EVAL, with ethylene 27, 32, 38 and 44 mol\% (product nos. 414077, 414093, 414085, 414107) from Sigma–Aldrich (St. Louis, MO). Dimethyl sulfoxide (DMSO, product # 161954) was purchased from Panreac (Barcelona, Spain) and used as the solvent to dissolve EVAL polymer particles at a concentration of 12.5 wt\%. Absolute ethyl alcohol (ACS grade) was from J. T. Baker (NJ, USA).

The synthesis of urea-imprinted, creatinine-imprinted, lysozyme-imprinted and non-imprinted (NIP) EVAL thin film briefly included three steps (as shown in Scheme 1), (1) casting the EVAL solution (EVAL/DMSO = 0.78, 3.125 and 12.5 wt\%) mixed with and without 1, 0.5 and 1 wt\% of template urea, creatinine and lysozyme molecules on a glass slide or on a gold electrode (2.5 cm \(\times\) 2.5 cm); (2) solvent evaporation in an oven at 60°C for 3 h to completely remove DMSO; and then (3) removal of the template molecule by rinsing in 20 mL of ethanol (for urea and creatinine) or 1 wt\% SDS solution (for lysozyme) for 30 min and then deionized water for 10 min, repeated three times. All membranes were equilibrated with phosphate buffered saline (PBS) overnight before use.

The residual DMSO in the EVAL is less than 1%, determined from the sulfur atom concentration using energy dispersive (X-ray) spectrometry. EDS, (HORIBA, Ltd., Minami-ku, Japan) connected with scanning electron microscopy (Hitachi S4700, Hitachi High-Technologies Co., Tokyo, Japan).

The nitrogen atom% on the EVAL MIP thin films were also measured by EDS, before washing, after washing, and after rebinding were 15.51, 12.49, 14.15 for urea MIPs; 18.69, 14.19, 16.63 for creatinine MIPs; and 22.05, 10.81, 12.93 for lysozyme MIPs, respectively. The changes show that washing removes some, but not all, of the template molecules—presumably, only template molecules that are at or very near the surface can be removed by washing, while EDS detects nitrogen to a depth of several microns. We cannot rule out the possibility that some surface template molecules remain permanently bound; however, the possible permanently bound templates do not prevent the use of these films for amperometric sensing, as we show.

The preliminary binding measurements of target molecules to the molecularly imprinted or non-imprinted EVAL polymers were performed with 5 mL of 0.1 mg/mL target molecules (unless otherwise stated) for 30 min. A UV–vis spectrophotometer (Lambda 35, PerkinElmer, Wellesley MA) was then used to measure the concentration decrease in the stock solutions, determined by absorption at 235 nm for creatinine, 278.5 nm for lysozyme.

Atomic force microscopy scanning of the molecularly imprinted polymers coated on the sensor was performed before and after ethanol or SDS solution washing of template from EVAL thin film, on samples dried under nitrogen. AFM (model: NT-MDT Solver P47H-PRO, Moscow, Russia) images were made in air (room temperature ca. 27°C and 87% relative humidity) using the tapping mode with scan rate 0.75 Hz. The cantilever was a SiO\(_2\) probe (model: TGS1, NT-MDT, Moscow, Russia) with probe tip size and resonant frequency 2 nm and 144 kHz, respectively.

2.2. Cyclic voltammetry

Cyclic voltammetry applies a linear scanning potential for a stationary working electrode (in an unstirred solution) with a triangular waveform, and records the resulting current. Fig. 4 (vide infra) is a plot of current vs. potential, which is termed a cyclic voltammogram. In Fig. 4, the characteristic peaks, which include an anodic peak potential \(E_{pa}\) and a cathodic peak potential
\( E_{pc} \), with current \( I_{pc} \), are caused by the formation of the diffusion layer near the electrode surface. For a reversible couple, the peak current is given by the Randles–Sevcik equation (Wang, 2000):

\[
I_p = (2.69 \times 10^5) n^{3/2} A C D^{1/2} v^{1/2}
\]

where \( n \) is the number of electrons, \( A \) is the electrode area (in cm\(^2\)), \( c \) is the concentration (in mol cm\(^{-3}\)), \( D \) is the diffusion coefficient (in cm\(^2\) s\(^{-1}\)), and \( v \) is the scan rate (in V s\(^{-1}\)). Accordingly, the current is directly proportional to concentration and increases with the square root of the scan rate. The ratio of the anodic-to-cathodic peak currents, \( I_{pa}/I_{pc} \), is unity for a simple reversible couple. The number of electrons transferred.

2.2.1. Design of the home-built potentiostat

Fig. 1 shows the circuit diagram of the portable cyclic voltammetry potentiostat. A three-electrode system is combined with an operational amplifier and a negative feedback loop to accomplish the potentiostatic control. The potentiostat includes a mixed-signal microprocessor (C8051F020), an instrumental amplifier for current to voltage conversion, three voltage level shifters, an operational amplifier, and an RS232 serial data transfer interface. The size of the printed circuit board (PCB) implementation of the potentiostat is 7.5 cm \( \times \) 6 cm, and the fabrication cost is less than 35 USDs. The C8051F020 chip contains an 8051 based CPU, 12-bit digital-to-analog converter (DAC), programmable gain amplifier (PGA), 12-bit 8-channel analog-to-digital converter (ADC), and digital peripherals. The C8051F020 chip can only process positive potential, the level shifters are indispensable in the cyclic voltammetry potentiostat for processing the scan voltages from negative potential to positive potential. The level shifters are simply inverting adders, which give an output voltage

\[
V_o = \left( -\frac{R_3}{R_2} \right) V_{in} + \left( -\frac{R_3}{R_4} \right) V_{bias}
\]

In the potentiostat, a bias circuit is used to generate a potential \( (V_{bias} = -1.2 \text{ V}) \) for these level shifters. The first level shifter with gain of four is used to convert the voltage from a DAC, whose range is between 0 and 2.4 V, to the scan voltages of the potentiostat, whose range is between 4.8 and -4.8 V. The second level shifter with gain of 1/4 and third level shifter with gain of one are used to adjust the measured voltage of reference electrode, whose range is from -4.8 to 4.8 V, and the measured voltage from current to voltage converter, whose range is from -1.2 to 1.2 V to ADCs, respectively. When the measured voltage is adjusted by the two shifters, the output voltage range will be from 0 to 2.4 V. This range is suitable for ADC signal processing. A personal computer (PC) is used to control the experiment and to collect the data.

Control of the applied voltage and measurement is realized by a ch2-ADC, a ch7-ADC, and a 12-bit DAC in the C8051F020 microprocessor. The ch2-ADC is used to measure \( V_{WR} \) (the voltage difference between a reference electrode and a working electrode), and to monitor \( V_{WB} \). The microprocessor generates a triangular-type ramp signal to DAC according to the preset scan potential, which produces a triangle waveform voltage between 0 and 2.4 V. A voltage level shifter converts this to a voltage between -4.8 and 4.8 V to be applied to the non-inverting terminal of the operational amplifier. The output of the operational amplifier injects current through a current measurement resistor \( (R_x) \) into the counter electrode to maintain the desired potential. A negative feedback control loop is using an operational amplifier ensures that the potential of the reference electrode is at preset scan potential.

In operation, the sensor current is measured by the current to voltage converter which combines an instrumental amplifier with current measurement resistor \( (R_x) \). The voltage dropping across the resistor \( (R_x) \) represents a corresponding current from counter electrode to working electrode. The conversion ratio of the current-to-voltage converter can be expressed as

\[
V_a = \left( 1 + \frac{R_x}{R_5} \right) \left( \frac{R_6}{R_7} \right) R_{I_{sensor}}
\]

This conversion ratio is designed as \( V_a = 10 R_{I_{sensor}} \). The resistor \( (R_x) \) has four different values to be adjusted by a switch to convert the range of current from mA to nA. The maximum and minimum detectable sensor currents are in the range of 12 mA-5 nA, respectively. A voltage level shifter is used to adjust the output voltage levels of the current-to-voltage converter from ±1.2 V to 0-2.4 V, measured by ch7-ADC.

The experiment is controlled by a PC running LabVIEW 6.0 software, via an RS-232 interface. Before the LabVIEW program plots the cyclic voltammogram, a digital ‘weighted averaging filter’ (Ozkan et al., 1993) was used to smooth the measured current signal. The algorithm of weighted averaging filter is

\[
Y(t) = \frac{[(L-1) \times Y(t-1)] + X(t)}{L}
\]
where $X(t)$ is the value of new input sample, $Y(t-1)$ is the value of previous output, $Y(t)$ is the value of present output, and $L$ is the weighted factor of the filter setting.

### 2.3 The measurement of urinary samples with the potentiostat and molecularly imprinted polymeric electrodes

The amperometric responses of the imprinted polymeric sensing electrodes were measured with a commercial potentiostat (CHI 411A, CH Instrument, Inc., Austin, TX, USA) and with the home made potentiostat, using different concentrations of target molecules in PBS, and using a urine sample secreted by one of the authors 4 h before the test. The setup of the electrochemical reaction can be found elsewhere (Huang et al., 2007); briefly, the working, counter and Ag/AgCl reference electrodes were placed in a 50 mL 0.5 M KCl, 20 mM K$_4$Fe(CN)$_6$ and 20 mM K$_3$Fe(CN)$_6$ solution with the scan rate 0.1 V s$^{-1}$. Creatinine-imprinted and urea-imprinted sensors were incubated with $5 \times$ diluted urine for 30 min. One millilitre of the urine sample was also stored in an eppendorf microcentrifuge tube at 4$^\circ$C and analyzed with ARCHITECT ci8200 system (Abbott Laboratories. Abbott Park, Illinois, U.S.A.).

### 3. Results and discussion

#### 3.1 Preparation of molecularly imprinted polymeric thin films for the sensing of urinary components

Urinalysis is likely to be an important component of home-care in the very near future. Urinalysis usually includes examination of pH, specific gravity, protein, glucose, ketones, nitrite and leukocyte esterase. Urea, creatinine and lysozyme are particularly important urinary analytes.

When target molecules are poorly soluble in polymer or monomer solutions, microcontact imprinting may be used (Hsu et al., 2006a,b; Lin et al., 2006; Lee et al., in press; Lee et al., 2008), in which target molecules are first allowed to adsorb on a glass slide and then the polymer is cast (or polymerized) over the adsorbed templates. We found no solubility problems with the three targets studied in this work, and so targets were simply mixed into polymer solutions prior to film casting.

The imprinting effectiveness for a molecularly imprinted polymer is defined as the ratio of the readsorption on an imprinted polymer to that on a non-imprinted polymer (NIP) of the same composition. Fig. 2 and Table 1 show that the highest imprinting effectiveness for urea and creatinine was obtained with 27 mol% of ethylene, but effectiveness for lysozyme was optimized at 44 mol% of ethylene. The highest imprinting effectiveness for each target molecule was 2.4. Different concentrations of EVAL in DMSO were used to form different thicknesses of molecularly imprinted EVAL thin films; however, with concentrations exceeding 1 wt% no electrochemical signals could be detected.

Fig. 3 depicts the surface morphology, measured by AFM, of creatinine-imprinted and non-imprinted polymer before (Fig 3(a) and (c)) and after (Fig 3(b) and (d)) 10 min of ethanol washing. Clearly, the change in the surface morphology of the non-imprinted polymer on washing (Fig 3(c) vs. (d)), is much less dramatic than the change in the imprinted film (Fig 3(a) vs. (b)). The atomic force microscopy images also showed that the surface of the imprinted polymer has larger cavities and larger surface area than non-imprinted films, as expected. However, the AFM images did not unambiguously show cavities that could be associated with single template molecules, and for this reason no quantitative analysis was performed. Creatinine-imprinted films which were usable as electrodes were about 200 nm thick (image not shown); thicker films were unusable.

![Fig. 2. The imprinting effectiveness of (a) urea (b) creatinine and (c) lysozyme in poly(ethylene vinyl-co-ethylene alcohol) (EVAL) films. The imprinting effectiveness was defined as the ratio of the adsorbed amount on the imprinted polymer to that on the non-imprinted polymer of the same composition.](image)

#### 3.2 Urinary samples measurements with molecularly imprinted polymeric electrodes and potentiostat

Fig. 4(a) shows the typical cyclic voltammetry of the molecularly imprinted polymeric electrode measured by a commercial potentiostat; the current peak occurred when 0.29 V was applied.
Table 1
Adsorption of target molecules to imprinted and non-imprinted polymers. Bold numbers indicate the highest imprinting effectiveness achieved.

<table>
<thead>
<tr>
<th>EVALs (ethylene mole (%))</th>
<th>Urea (µg/cm²) MIPs</th>
<th>Creatinine (µg/cm²) MIPs</th>
<th>Lysozyme (µg/cm²) MIPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIPs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>233.7 ± 50.7</td>
<td>12.83 ± 0.56</td>
<td>7.9 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>124.3 ± 72.5</td>
<td>7.74 ± 0.96</td>
<td>8.9 ± 0.7</td>
</tr>
<tr>
<td>32</td>
<td>189.4 ± 29.0</td>
<td>8.65 ± 1.36</td>
<td>13.6 ± 2.5</td>
</tr>
<tr>
<td>38</td>
<td>47.3 ± 0.1</td>
<td>1.96 ± 0.15</td>
<td>17.7 ± 0.7</td>
</tr>
<tr>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Comparison of the home-built potentiostat with molecularly imprinted EVAL polymeric electrode by amperometry and commercial ARCHITECT ci 8200 system.

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Samples</th>
<th>Home-built potentiostat sensor</th>
<th>ARCHITECT ci 8200 (mg/mL)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ΔCurrent (µA)</td>
<td>Convert concentration (mg/mL)</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>I</td>
<td>27.70 ± 0.22</td>
<td>10.10 ± 0.08</td>
<td>11.86</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>31.30 ± 0.11</td>
<td>11.00 ± 0.04</td>
<td>13.24</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>17.20 ± 0.05</td>
<td>5.24 ± 0.02</td>
<td>6.62</td>
</tr>
<tr>
<td>Creatinine</td>
<td>I</td>
<td>0.88 ± 0.10</td>
<td>0.74 ± 0.09</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2.40 ± 0.15</td>
<td>1.56 ± 0.10</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1.12 ± 0.06</td>
<td>0.90 ± 0.05</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Fig. 3. The surface morphology of creatinine-imprinted and non-imprinted polymers prepared using 27 mol% of ethylene EVAL. Figures (a) and (c) are creatinine-imprinted and non-imprinted polymers after solvent evaporation. Figures (b) and (d) are creatinine-imprinted and non-imprinted polymers after ethanol washing. The field of view is 5.0 microns square; the topography axis is 100 nm.

In contrast, the non-imprinted polymeric electrode exhibited much less change after adding creatinine concentrations varying from 0~1.6 mg/mL (Fig. 4(b)). The cyclic voltammetry of the MIP electrode measured by the home-built potentiostat, shown in Fig. 4(c), is the same as obtained with the commercial potentiostat. Fig. 4 was re-plotted into Fig. 5(a) to depict the relationship between the target concentration and the current difference at the current peak compared with buffer alone. The current responses for creatinine-imprinted and non-imprinted polymer both monotonically increased from 0.12 ± 0.07 µA to as high as 4.06 ± 0.24 and 1.56 ± 0.05 µA, respectively. The current increase was exponential from a creatinine concentration of 0.01 mg/mL to a reference concentration of 0.5–1.0 mg/mL in urine. Conversely, the current difference for non-imprinted polymeric electrode for creatinine remained at 0.4–0.5 µA when the concentration was lower than 1 mg/mL; this current may have been due to the non-specific adsorption of creatinine to 27 mol% ethylene EVAL.

In Fig. 5(b), the current changes were from 2.12 ± 0.87 to 10.9 ± 1.5 µA and from to 4.18 ± 0.65 µA for the urea-imprinted and non-imprinted polymeric electrodes, respectively, for urea concentrations from 0.05 to 6 mg/mL. The saturation concentrations of urea to the urea-imprinted and non-imprinted electrodes were approximately 2 and 0.4 mg/mL, respectively. For the lysozyme-imprinted and non-imprinted polymeric electrodes, the current difference increased to 3.2 mA as concentration was increased from 10 to 100 ng/mL, but actually decreased at higher concentrations.
Fig. 4. Cyclic voltammetry of creatinine solutions measured using (a) creatinine-imprinted and (b) non-imprinted polymer coated on gold electrodes using a commercial potentiostat. (c) Measurement using creatinine MIP electrode and the home-built potentiostat.

(0.1 ∼ 4.0 μg/mL) from 3.2 to 1.0 mA, as Fig. 5(c) shows. The reason for the reduction in response at high concentration is not clear.

To investigate interference (chemical cross-talk) affecting electrochemical signaling, several creatinine and lysozyme concentrations were measured with a urea-imprinted electrode. Fig. 6. The current signal from lysozyme was approximately 5 μA for concentrations in the 10−2000 ng/mL range, which is the possible concentration range in urine. Creatinine (at physiologically relevant concentration, above ∼1 mg/mL) can cause a large amperometric response; this large response may be caused by the chemical/structural similarity between creatinine and urea. Because of cross-talk between analytes, a multi-sensing system may be optimal when different target molecules have similarities in chemical structure when using molecularly imprinted polymers as the sensing material.

Although the low cost and high stability of MIPs offer important advantages as sensing materials, the reliability of the sensor must be confirmed before they can be considered acceptable for daily.

Fig. 5. Current difference for the (a) creatinine-imprinted, (b) urea-imprinted and (c) lysozyme-imprinted electrode for different concentrations of target molecules when voltages of 0.26 ∼ 0.29 were applied. Filled and empty symbols indicate electrochemical response with imprinted and non-imprinted polymeric electrodes, respectively.
homecare use. In Table 2, three individual random urine samples from author and colleagues were measured with home built potentiostat and commercial ARCHITECT ci 8200 system. We found that the accuracy is on average 82.46% and 80.04% for urea and creatinine, respectively. The accuracy of one measurement was less than 60%, which might be due to permanent analyte adsorption and loss of recognition cavities after washing. The EVAL MIPs in this work could be helpful for patients and for home-care use. In Table 2, three individual random urine samples tested, cross-talk was not a problem because the high specificity of molecular recognition of the molecularly imprinted EVAL thin film and very low concentration of lysozyme exists in urine.

4. Conclusion

The utility of MIP-coated electrodes was demonstrated for measuring biomedically important analytes in urine. Although additional work is needed to account and correct for analyte “cross talk”, the EVAL-coated electrode and low-cost potentiostat system could be helpful for patients and for home-care use. This work demonstrated that EVAL polymeric thin films can be imprinted with several target molecules which are soluble in DMSO, simply by mixing prior to film casting. Moreover, the EVAL coating on the molecularly imprinted thin film can be removed from the electrode in one hour and a new coating can be applied to another imprinted thin film within an additional 2 h. Finally, the homemade potentiostat can be integrated with the molecularly imprinted polymer sensing system for actual urine sample analysis. In the actual

Fig. 6. Current difference when different concentrations of an interfering analyte (lysozyme: squares and creatinine: triangles) were measured with urea-imprinted EVAL polymeric electrode.

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