**Development and characterization of an electrochemical biosensor for creatinine
detection in human urine based on functional molecularly imprinted polymer**

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Abstract

In this work, sensor based on a new molecularly imprinted polymer (MIP) for creatinine (Cre) detection, using screen-printed gold electrodes (Au-SPE), was developed. A carboxylic polyvinyl chloride (PVC-COOH) layer was first deposited on Au-SPE surface. The creatinine molecules were attached to the surface of Au-SPE/PVC-COOH. Afterward, the polymerization of acrylamide and N, N' methylenebisacrylamid filled vacant spaces around them. The subsequent templates removal left binding sites within the polymer which are capable of selectively recognizing creatinine at different concentrations. To test the sensitivity of this Biosensor, the same procedure without creatinine was performed on a gold non-imprinted polymer (Au-SPE/NIP). Their retention and molecular-recognition properties were qualitatively investigated by means of three instrumental techniques: voltammetry (cyclic voltammetry (CV) and differential pulse voltammetry (DPV)), electrochemical impedance spectroscopy (EIS), and UV-Visible spectrophotometry (UV-Vis). The obtained results indicate that the MIP had a specific recognition ability for creatinine, while other structurally related compounds, such as urea or glucose, could not be recognized on the MIP. In addition, the biosensor was tested on volunteers with different creatinine urine levels and seemed a promising tool for screening creatinine in point-of-care. Moreover, Partial Least Squares (PLS) analysis was used to obtain a correlation between the predicted creatinine concentrations from voltammetric measurements and concentrations measured by Jaffe's reaction as a reference method. The EIS and DPV biosensor responses show a limit of detection of 0.016 ng/mL and 0.081 ng/mL, respectively, with a linear range from 0.1 ng/mL to 1 µg/mL. This study provides a promising strategy to fabricate sensor devices based on MIPs with highly selective recognition ability, simplicity of operation, small size and low cost.

Keywords: Molecular imprinted polymers (MIP), screen-printed gold electrodes, creatinine, biosensor, human urine.

1. Introduction

The analysis of biological fluids, as blood, skin emanations, feces, saliva or even urine is becoming a common practice to find out particular patient pathologies. One of the most used fluids is urine, a multi-component complex system, from which the chemical composition can be related to several pathologies [1,2]. One of the urine components in concern is creatinine molecule, chemically designated as 2-amino-1-methyl-2-imidazoline-4-one. It can be found in human urine as result of creatine or creatine phosphate metabolism, after muscle contraction. In mammals, creatinine is the end product of creatine metabolism in the skeletal muscles to the release of energy and is removed from the body by renal excretion at a relatively constant rate [3]. The normal levels for creatinine concentrations in urine and blood are ranging from 500 to 1500 $\mu\text{g/mL}$ and from 700 to 1200 $\mu\text{g/mL}$, respectively [4]. However, it can be increased in patients with chronic nephritis or renal dysfunction [5]. Therefore, the assessment of creatinine level at this concentration range with specific detection methods is also important. For this purpose, many methodologies have been developed to detect and estimate this important molecule [6]. In addition, numerous techniques have been addressed, most of them, based on Jaffe's reaction in which the active methylene group of creatinine reacts with alkaline sodium picrate to form a colored substance observed by spectrophotometric measurement [7]. However, this reaction is not straightforward as it depends on some extent of particular urine composition, and therefore lacks specificity [8,9]. An electrochemical sensor based on creatinine oxidation to detect electrons transferred from H_2O_2 [10-12] was used by Khan and Wernet [10]. But that biosensor had several disadvantages of stability, cross reactivity and sensitivity. Moreover, Lloyd's reagent (aluminum silicate clay) has been performed for a selective absorption of creatinine and to leave the no creatinine chromogens in solution [13]. Nevertheless, several other components were also absorbed while using this technique. Other creatinine detection methods include the kinetic method [4], coupled with enzyme assay, and

high performance liquid chromatography (HPLC). Thus, several enzymatic methods have been reported to improve creatinine specificity, but they are time-consuming and rather costly [14,15]. Recently, electrochemical methods based on a combination of enzymatic cascade reactions have been addressed. These methods are cost-effective, complex, time consuming and the enzymes are not always available [16].

To overcome these drawbacks, biological recognition agents, such as antibodies, enzymes, and other receptor Molecules have been widely employed, in analytical and diagnostic practices. Although, these agents are highly specific and sensitive, they are labile, expensive, and have low binding sites densities. Hence, there is a significant demand for robust and stable receptor that can mimic bio-recognition elements [17]. Over the last decade, MIP_s have attracted much attention and been introduced as recognition metabolic changes in human urine related to the development of urinary tract infections like bladder, diabetic, prostate and other cancers [18,19]. Therefore, diagnoses of these can be achieved through the measurement of creatinine contents in human urine [20]. These emerging MIP_s, initiated by Polyakov in the 1930s [21], are classified as highly promising artificial biomolecular recognition materials mainly thanks to their versatility and functionality to incorporate binding sites for various types of analytes [22-25]. Sensor devices based on MIP_s are known to present high selectivity and sensitivity, inherent stability under drastic conditions, long shelf life and low-cost. Over recent years, MIP_s have reflected the gradual maturation and have attracted from the scientific community [26-35]. They have been regarded as a promising alternative in various research areas, such as chromatography [36-38], biotechnology [39], environmental science [40], food safety [41,42], biomedical sciences [43,44] and sensors design [45].

With this maturation, the preparation of creatinine-imprinted polymer has been made not only through using hydrogen-bonding as the main interaction in the complex between functional monomer and creatinine [46], but also using hydrogen-bonding and covalent bonding

to form the complex [47]. Generally, molecular recognition bio-elements are coupled with physicochemical transducer devices, which transform the host-analyte interaction into a readable output signal. Among various transduction methodologies, electrochemical methods have been widely investigated because of their potential advantages such as low-cost and small size [48-51].

This work proposes a novel creatinine biosensor device based on a MIP_s functionalized electrode system directed to electrochemical transduction. After NIP and selectivity test, the performances of this creatinine-imprinted polymer sensor and its application in human urine for creatinine assessment were explored.

2. Experimental

2.1 Reagents and solutions

Creatinine (98 %) was purchased from Riedel-de Haen (Seelze, Germany); carboxylic polyvinyl chloride (PVC-COOH), glucose, ethanol, phosphate buffered saline (PBS 0.01M), sodium hydroxide (NaOH) (96 %), picric acid (99 %), 1,4 dioxane (99.8 %) and urea (99.5 %) were from Sigma Aldrich. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), ammonium persulphate (APS), N, N'-methylenebisacrylamid (NNMBA), acrylamide (AAM), N,N,N',N'-Tetramethylethylenediamine (TEMED), N-hydroxysuccinimide (NHS), potassium ferricyanide $K_3[Fe(CN)_6]$ and potassium ferrocyanide $K_4[Fe(CN)_6]$ were all obtained from Fluka. 2-amino-2-hydroxyméthyl-1,3-propanediol (TRIS 0.5 M) was from Panreac quimica. The buffer solutions used in this work were TRIS and PBS. From a stock standard solution of creatinine (1 mg in 1 mL of PBS (pH 7.4)), less concentrated solutions were prepared by accurate dilutions of the previous solution using the same buffer. To ensure the complete dissolution of creatinine in PBS, a vortex and a hotplate stirrer were used. All the less concentrated solutions of the adopted range between 0.1 ng/mL to 1 µg/mL were stocked at 4 °C. For real detection, the urine samples were collected from volunteers students of the

Faculty of Sciences-Meknes (Morocco). They were morning midstream urine samples and were collected in polystyrene urine collection bottles. The samples were directly used to

Fig. 1. Schematic representation of Au-SPE/MIP procedures.

Fig. 2. Polymerization reaction.

avoid their degradation and consequent change in properties. After utilization, they were stocked at -4 °C in a freezer.

2.2. Preparation of molecularly imprinted polymers (MIPs) and non-imprinted polymers (NIPs)

Before any deposit, the working area of the Au-SPE was washed for three times with ethanol. A layer of PVC-COOH (11 mg in 1.25 mL of 1,4 dioxane) was cast on the Au-SPE. To activate the COOH groups, this layer was incubated during one hour and half in an aqueous solution of 4 mg EDC and 1 mg NHS prepared in 1 mL of water. By thoroughly rinsing with distilled water, unreacted carbodiimide species were removed. Afterwards, 3.5 µL of a standard solution (1 mg creatinine in 1 mL PBS buffer at pH 7.4) was deposited on Au-SPE and the device was incubated for 4 h at 4 °C. The Au-SPE was then washed twice with PBS buffer solution at pH 7.4 to remove unbound creatinine. Then a TRIS solution was deposited for 30 minutes, to block any unbounded COOH groups left on the surface of the electrodes. Afterwards, distilled water was used to wash the Au-SPE for several times. The polymerization

was then carried out by adding 13 mg of APS in a solution containing 11 mg of AAM functional monomer [52] and 71 mg of NNMBA as cross linker [53] in 2 mL of PBS to create polymer matrix around the template [54]. To accelerate the polymerization, 20 μ L of TEMED solution was also added, and the new modified SPE was placed overnight at 4 °C. The analyte was then removed from the polymer layer by incubating in distilled water at room temperature for 15 minutes under intensive stirring and with ethanol for 10 minutes. The same procedure was carried out for the sensor regeneration. The Au-SPE/MIP was ready to use after washing it with distilled water. For control, a gold non-imprinted polymer electrode (Au-SPE/NIP) was prepared similarly but without creatinine. The whole preparation steps are shown in Fig. 1.

Fig. 3. Schematic of the spectrophotometer and the portable device system used for electrochemical measurements.

Fig. 4. Cyclic voltammetry of sequential immobilization steps onto screen-printed electrodes towards functional molecular imprinting sensor in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (a) Nyquist plots of sequential immobilization steps onto Au-SPE towards functional molecular imprinting sensor in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (b).

2.3 Polymerization reaction

Acrylamide as monomer was used because of its stability and can be made at a wide variety of concentrations. Moreover, it polymerizes under mild conditions and carry uncharged functions that establish hydrogen bonds and dipole–dipole interactions with creatinine. Indeed, the polymerization are starting from the liquid solutions consisting of a mixture of acrylamide and bis-acrylamide (N, N 'methylene-bis-acrylamide) which react together. Acrylamide polymerizes in the presence of free radicals typically supplied by ammonium persulfate. Then, in

Fig. 5. Differential pulse voltammograms of different concentrations of creatinine solutions (a) and Nyquist plots of different concentrations of creatinine solutions (b) in 5.0 mM [Fe (C N)₆]³⁻ and 5.0 mM [Fe (CN) ₆]⁴⁻ in PBS buffer at pH 7.4.

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174 **Fig. 6.** Calibration curves of DPV (a) and EIS (b) obtained in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-4-}$
 175 in PBS buffer at pH 7.4 with different concentrations of creatinine solutions.

176 the presence of TEMED as a catalyst, the added ammonium persulfate is converted into an ion
 177 Sulfate free radical (SO_4^-) which initiates the acrylamide polymerization. Furthermore,
 178 acrylamide has a double bond between two carbons, which constitutes a privileged target for
 179 the sulfate radical. The result is a free-radical acrylamide molecule. This latter attacks a second
 180 molecule of acrylamide, to give polymers of acrylamide or polyacrylamide, which is a linear
 181 chain. To obtain a reticulated and stable polymer matrix, it becomes necessary to use a cross-
 182 linker. The bis-acrylamide (equivalent to 2 methyl-bonded acrylamide monomers), possessing
 183 two radical attack sites (2 double bonds between carbon atoms), constitutes a crosslinking agent
 184 of choice [55]. In the absence of bis-acrylamide, the acrylamide would polymerize into long
 185 strands (linear polymer). Bis-acrylamide contains two double bonds, which allow the
 186 compound to act as a cross linker between acrylamide chains and this is what gives rise to the

formation of pores. Moreover, the functional groups of the monomer link covalently amine groups of added creatinine molecules. [Fig. 2](#) illustrates this polymerization reaction.

2.4. Instrumental analysis

2.4.1. UV-Vis spectrophotometry analysis

Absorbance measurements were performed using a UV–Vis spectrophotometer. The urine samples were analyzed using ANACHEM instruments UV220 spectrophotometer in the range of 200 nm to 700 nm by using a quartz cell (1 cm path length). For the creatinine measurement, the wavelength was calibrated at 520 nm. The collected human urine samples were chemically analyzed, in the first phase, by using Jaffe’s method following the procedure: first, a solution of sodium hydroxide (NaOH) (1.4 M) and picric acid (0.036 M) were prepared. A standard solution (S_1) was obtained by adding 1 mL of the urine sample in 79 mL of distilled water. A blank solution was prepared by mixing 0.5 mL and 1 mL of NaOH (1.4 M) and picric acid (0.036 M) with 3 mL of distilled water, respectively. It was used to calibrate the spectrophotometer. After 15 minutes, 0.5 mL S (NaOH) and 1 mL S (picric acid) are added in 3 mL of S_1 and placed in the spectrophotometer to determine the absorbance value. To get the level of creatinine sample, a linear equation ($Y = 0.3365x + 0.0482$) obtained after synthesized creatinine measurements was used. Thus, for each urine sample, measurements were performed 3 times.

2.4.2. Electrochemical measurements

An electrochemical transducer (Potentiostat) interfaced with a computer has been employed to monitor the electrochemical measurements involved in this study. [Fig. 3](#) illustrates an overall view of the system. CV and DPV measurements were performed in a 5 mM solution of $[Fe(CN)_6]^{3-/4-}$. For CV, a scan range from -0.4 V to 0.6 V was used with a scan rate of 30 mV/s. For DPV, a scan range from -0.1 V to 0.2 V was used with a scan rate of 10 mV/s.

The electrochemical measurement setup consisted of a three-electrode system, where a gold electrode (0.19 cm^2) was used as a working electrode, a saturated silver electrode as a reference electrode and a gold strip plate (0.54 cm^2) as a counter electrode. The impedance analysis was performed with the PalmSens³ impedance analyzer in the frequency range from 0.1 Hz to 50 kHz, under a dc potential value of $\pm 5\text{ V}$. The impedance of the electrode is determined by applying a sinusoidal potential of small (peak-to-peak) amplitude and measuring the resultant sinusoidal current. The EIS data can be represented by Bode and Nyquist plots [56]. The results of the electrochemical impedance measurements were analyzed based on the best-fitting equivalent circuit analysis using the implemented EIS spectrum analyzer software.

3. Results and discussion

3.1 Chemical assembly of the molecularly imprinted material

The results of MIP preparation using both methods are represented in Fig. 4. According to voltammetry's signals Fig. 4a, the deposit of creatinine generates a more important current peak relative to that of PVC-COOH. This is probably caused by Hydrogen movement to form NH_2 (tautomerism) in aqueous solution. After the deposition of the polymer, a decrease of current peak is observed again. But after extraction, it becomes more important than the previous. This is explained by the formation of free sites that allow the movement of electrons. The plots obtained by EIS method are shown in Fig. 3b in which a significant increase of charge-transfer resistances is remarked after the different stages of the elaboration of the MIP. This reveals some modifications in the surface of the electrode which is due to a filled increasing of the free sites. Consequently, subsequent immobilization led to a further resistance increase due to the lower conductivity of the resulting surface. But after extraction stage, this resistance decreases because of the creation of free sites.

Fig. 7. Differential pulse voltammetry of sequential immobilization of different concentrations towards non-imprinting polymer sensor in 5.0 mM [Fe (CN) 6]^{3 -/4 -} (a) Nyquist plots of sequential immobilization of different concentrations towards non-imprinting polymer sensor in 5.0 mM [Fe (CN) 6]^{3 -/4 -} (b).

3.2. MIP and NIP sensor responses

The binding of the template to the imprinted creatinine and non-imprinted polymers was studied employing DPV and EIS characterization techniques. The obtained results during the MIP test are shown in Fig. 5. According to differential pulse voltammograms of the Fig. 5a, the current peaks decrease gradually with an increase of creatinine concentrations. DPV curves indicate the movement of electrons through the polymer sites. The current peak decrease can be explained by an increasing filling of MIP free sites for each increase of creatinine concentration. In Fig. 5b, the Nyquist plots inform about the interfacial charge-transfer impedances (R_{ct}) of the electrode which were determined from the diameter of the semi-circle. It was found that the charge-transfer impedance of the electrode increased by the addition of creatinine and increased further with increasing concentrations. With higher concentrations of creatinine, surface modification electrodes becomes more important due to electron promotion.

Calibration curves were presented in Fig. 6a, as relative variation of current density (I_0-I)/ I_0 versus Log (concentration of creatinine in $\mu\text{g/mL}$). In the range of [0.1 ng/mL; 1 $\mu\text{g/mL}$], good linearity of the

Table 1. Comparison of performance of various electrochemical biosensor employing MIP with the current MIP creatinine sensor.

Detection methods	Linear range ($\mu\text{g/mL}$)	Limit of detection (ng/mL)	References
capacitive	0 ; 67.87	1130	[16]
DPCSV*	0.0025 ; 84.0	2.5	[56]
SqWV*	41.8 ; 407	972	[58]
EIS	0.020 ; 0.67	20	[20]
EIS	0.05 ; 2	40	[26]
DPV and EIS	10^{-5} ; 1	0.016	Current Work

DPCSV: Difference Pulse Cathodic Stripping Voltammetry
SqWV: Square Wave Voltammetry

calibration plot was obtained with a correlation coefficient of 0.98 for DPV. The same correlation coefficient value was obtained for EIS technique in Fig. 6b where $(R_0-R)/R_0$ versus Log (concentration of creatinine in $\mu\text{g/mL}$) was represented.

A non-imprinted polymer (NIP) test based Au-electrodes has been also studied for control experiments in the absence of creatinine. DPV technique of Fig. 7a shows overlay voltammograms for each characterization step due to a non-existence of binding sites to be occupied. Therefore, a same electronic flow is ascertainable for each concentration of the range. Whereas Nyquist plots of creatinine Fig. 7b show simply linear plot with no semicircular pattern, relevant to an interfacial charge-transfer resistance, and this observation suggest that only a diffusion-controlled process occurs at the electrode.

All tests were performed several times, for days, to check the repeatability and reproducibility of the biosensor. Then, an average standard

Fig. 8. Biosensor responses for interfering molecules.

Fig. 9. The effect of polymerization time on MIP sensor response to creatinine.

deviation of around 1 % and relative standard deviation (RSD) < 4 % in a linear range of [0.1 ng/mL to 1 µg/mL] was obtained, demonstrating an excellent accuracy of the electrochemical measurements and indicating that the proposed method for the sensor preparation was reliable and reproducible. Finally, results of the NIP test have well confirmed the MIP's responses. Further, the obtained results were found to be reproducible in three independent experiments and the magnitude of curves and R_{ct} changes are negligible. Thus, with DPV method, the biosensor displayed a limit of detection (LOD) of 0.081 ng/mL. In the case of EIS method, a LOD of 0.016 ng/mL was found. Indeed, the performed biosensor is easy to fabricate, reusable after 3 months and highly sensitive compared to those seen in related studies. Table 1 illustrates a comparison between its performances and some reported works'. The low detection limit reported in these investigations ranges from about 2 ng/mL to as high as 972 ng/mL. Compared with related works for creatinine detection [51, 57-64], the current biosensor displays a low and highly significant LOD.

3.3. Selectivity study

To investigate interferences, due to possible reactive compounds coexisting in physiological samples, urea and glucose were used to test the selectivity of the creatinine-imprinted polymer [65,48]. Fig. 8 indicates the differences observed for these three analytes using the same polymer. DPV curves present little variations of their amplitudes whereas Nyquist plots of the interferers show negligible change of transfer

293 **Fig. 10.** The effect of elution time on MIP sensor response to creatinine.

294 **Fig. 11.** The effect of incubation time on MIP sensor response to creatinine.

charge. It certainly means that the fabricated MIP_s sensor surface show no response to the presence of urea and glucose. All these observations suggest concluding clearly that the recognition (binding) sites of MIP_s are specific to creatinine.

3.4. Optimization of some experimental MIP sensor parameters

In the aim of improving the biosensor performances, some parameters have been optimized. The polymer solution was deposited onto the Au-SPE with an overnight incubation time. Firstly, this duration was optimized by using 3, 4, 5, 6 h as incubation times. The results in Fig. 9 showed that the optimal signal is obtained for 5 h. Less durations presented no greater current peaks.

In following, an elution stage started by using ethanol and distilled water. This deposit (washing) took first 45 minutes. Secondly, duration of the extraction was studied for 15, 20, 25, 30 and 35 minutes. An optimal extraction time was obtained for 25 minutes in which the signal remains constant while the increasing durations (Fig. 10).

Thirdly, by taking 10, 15, 25, 30, 40 and 45 minutes the incubation time of a creatinine concentration has been investigated. As seen in Fig. 11, an optimal time was found for 30 minutes.

3.5 Application of the MIP sensor for the determination of creatinine levels in human urine samples

Analytical applicability of the present method was assessed by applying the present biosensor to the determination of creatinine in human urine samples of a group of healthy people with different creatinine levels. Considering that, the clinical range in urine sample (500-1500 µg/mL) [66] and the determination range of the present biosensor (0.1 ng/mL- 1 µg/mL) the developed sensor could be applied in urine samples. Indeed, after the step of developing the creatinine sensor, its biological application was then explored. Different biological urine samples were collected in polystyren bottles, put in freezer, diluted in PBS and shake before

analysis. A classification study of the different samples was previously done using Jaffe's method by UV-Vis. Therefore, three types of samples were used: Two urine samples with low, medium and high creatinine levels were tested by using the developed biosensor in order to measure creatinine levels.

Fig. 12 shows the results obtained by voltammetry and electrochemical impedance methods measurement performed on one urine sample of volunteer (P122) having a high creatine level by taking into account the value obtained by UV-Vis. For each sample, the quantifying test is

Fig. 12. DPV of one urine creatinine sample (a) Nyquist plot of one urine creatinine sample (b).

done repeatedly to take the average of creatinine concentration value. Regarding the values obtained by EIS, a best fitting was necessary by using EIS spectrum analyzer software. Obtained R_{ct} values allowed determining the creatinine level. For DPV and EIS methods, the amounts of creatinine in the human urine samples were determined by the calibration curves of

the Fig. 6. Creatinine levels in urine samples found using DPV and EIS methods agree with those obtained by the spectrophotometer (Table 2). This presented method for creatinine human urine assessments described averages RSD of 5 % for DPV and 11 % for EIS techniques with a recovery limit of 96 % and 105 %, respectively. That revealed a satisfactory measurements precision and trueness.

Fig. 13 describes the results of partial least square (PLS) regression model for human urine creatinine concentrations prediction. In this

Table 2. MIP sensor results for reproducibility of creatinine urine measurements.

	High creatinine levels		Medium creatinine levels		Low creatinine levels	
Code	P88	P122	P128	P77	P99	P118
Spectrophotometer (10³ µg/mL)	2.703	2.975	1.074	1.662	0.780	0.599
	2.748	3.054	1.142	1.685	0.791	0.554
	2.760	3.099	1.346	1.7872	0.803	0.576
DPV (10³ µg /mL)	2.668	2.975	0.974	1.705	0.565	0.498
	2.670	2.990	1.030	1.800	0.650	0.510
	2.720	2.980	1.200	1.740	0.700	0.580
RSD (%) (n=3)	0.890	0.250	8.900	2.200	8.730	6.830
Recovery (%)	98.7	100.8	99.6	105.2	82.0	88.4
EIS (10³ µg/mL)	2.670	2.850	1.090	1.300	1.010	0.600
	2.700	2.910	1.730	1.900	0.590	0.540
	2.730	2.880	1.100	1.870	1.020	0.570
RSD (%) (n=3)	0.900	1.040	22.900	16.330	22.900	4.290
Recovery (%)	99.8	96.8	121.9	102.2	111.5	95.1

case, PLS model was built by using all data without separating it neither in training nor validation matrix. One latent variable and a leave one out cross-validation technique have been used to build this model. Fig. 13a-b illustrates DPV and EIS versus creatinine levels determined by Jaffe's method, respectively. A good linearity of the calibration plots was obtained in both cases with a correlation coefficient of 0.99. From the above reported results, the possibility of the biosensor to monitor urinary creatinine content seems to be the most remarkable result.

4. Conclusion

In this work, two main strategies regarding the development of synthetic receptors based on molecularly imprinted polymers (MIPs) and their applications for real creatinine detection in human urine samples were presented. The combination of three different instrumental techniques, cyclic voltammetry (CV), differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS) and UV-Visible spectrophotometry (UV-Vis), has been revealed as a useful tool for sequential characterization of creatinine immobilization steps onto screen printed gold electrodes towards functional molecular imprinting sensor. The MIPs demonstrated specific recognition and sensitive detection ability for molecular recognition of creatinine in human urine. In addition, urine creatinine levels deduced from the practicability of the developed biosensor were all proved satisfactory when compared to those measured by Jaffe's method, demonstrating the good accuracy and potential applicability of the developed Au-SPE/MIP. Moreover, the limit of detection was found to be 0.016 ng/mL and 0.081 ng/mL, measured respectively for DPV and EIS methods, with a linear range from 0.1 ng/mL to 1 µg/mL. This study has confirmed the proposed electrochemical MIP sensor as simple, robust, rapid, non-invasive, portable and low-cost alternative tool for clinical applications of urinary creatinine.

Fig. 13. Results of PLS prediction of human urine creatinine levels by means of DPV (a) and EIS (b) methods versus Jaffe's method.

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