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Urinalysis with Molecularly Imprinted Poly(ethylene-co-vinyl alcohol) Potentiostat Sensors

Chun-Yueh Huang[a], Tain-Chin Tsai[b], James L. Thomas[c], Mei-Hwa Lee[d], Bin-Da Liu[e,*], Hung-Yin Lin[b,f]

[a] Department of Electrical Engineering, National University of Tainan, Tainan 700, Taiwan
[b] Institute of Biotechnology, National University of Kaohsiung, Kaohsiung 811, Taiwan
[c] Department of Physics and Astronomy, University of New Mexico, Albuquerque, NM 87131, USA
[d] Department of Materials Science and Engineering, I-Shou University, Kaohsiung 840, Taiwan
[e] Department of Electrical Engineering, National Cheng Kung University, Tainan 701, Taiwan
[f] Department of Chemical and Materials Engineering, National University of Kaohsiung, Kaohsiung 811, Taiwan

bdliu@mail.ncku.edu.tw

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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.

The use of molecularly imprinted polymers (MIPs) as recognition elements in sensors has been reviewed in numerous articles. Electrochemical, optical, mass sensitive thermometric and magnetometric transducers have been designed to integrate with MIP thin films or micro/nanoparticles. One of the most useful analytical sensors is the electrochemical biosensor because of its reliability and ease of manufacture. A recent design using chip-type sensors has been used in preclinical trials. Many researchers have attempted to develop a low cost, standalone and portable potentiostat which can be used in different sensors. 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).

The objective of this group research 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.

The synthesis of urea-imprinted, creatinine-imprinted, lysozyme-imprinted and non-imprinted (NIP) EVAL thin film briefly included three steps (as shown in Fig. 1): (1) casting the EVAL solution mixed with and without 1, 0.5 and 0.1 wt% of template urea, creatinine and lysozyme molecules on a glass slide or on a gold electrode ; (2) solvent evaporation in an oven for completely remove DMSO; and then (3) removal of the template molecule by rinsing 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.

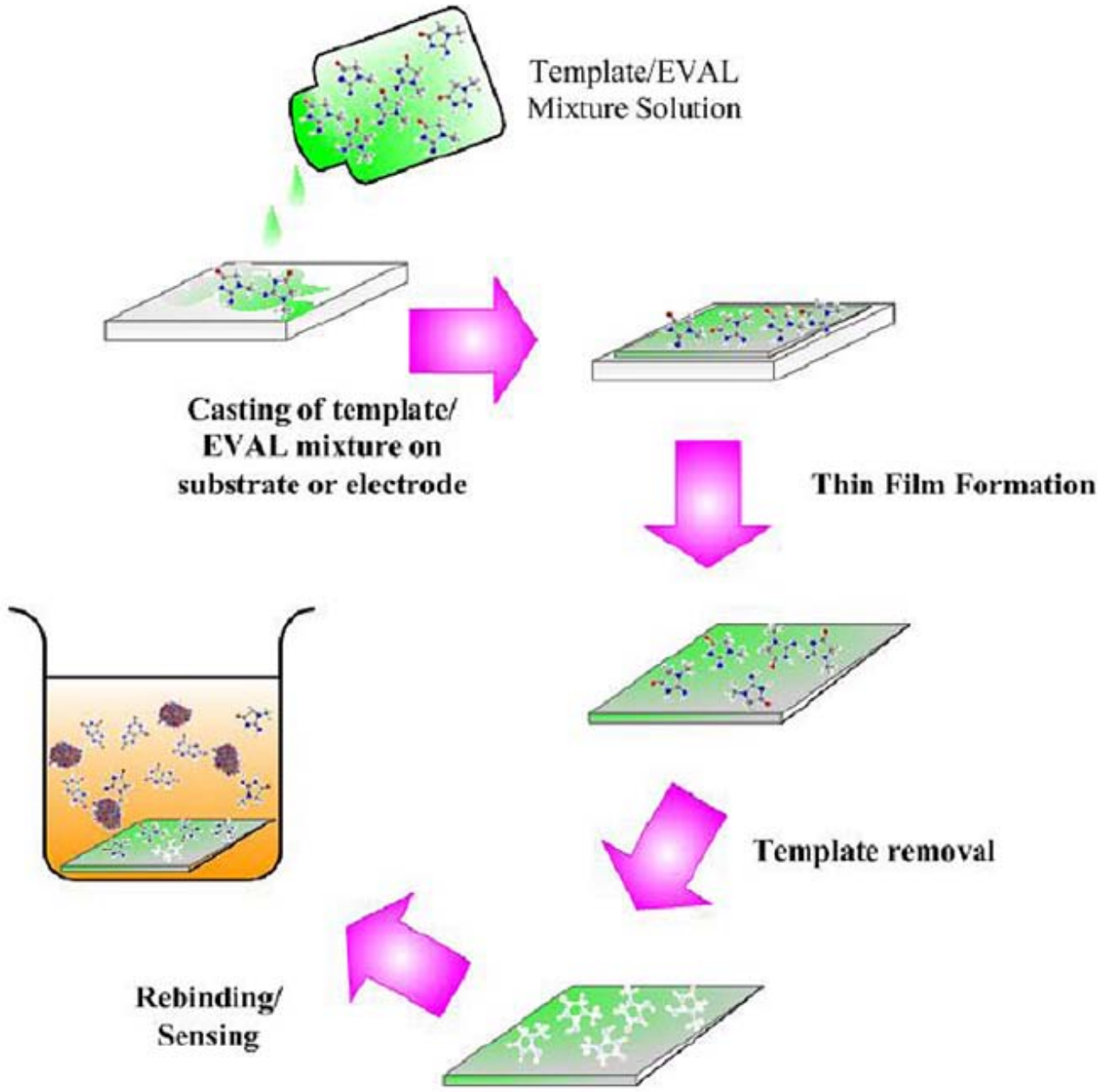


Fig.1 The preparation of the molecularly imprinted EVAL sensing electrode.

Fig. 2 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 amixed-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 printed circuit board (PCB) implementation of the potentiostat is 7.5 cm× 6 cm, and the fabrication cost is less than 35 USDs.

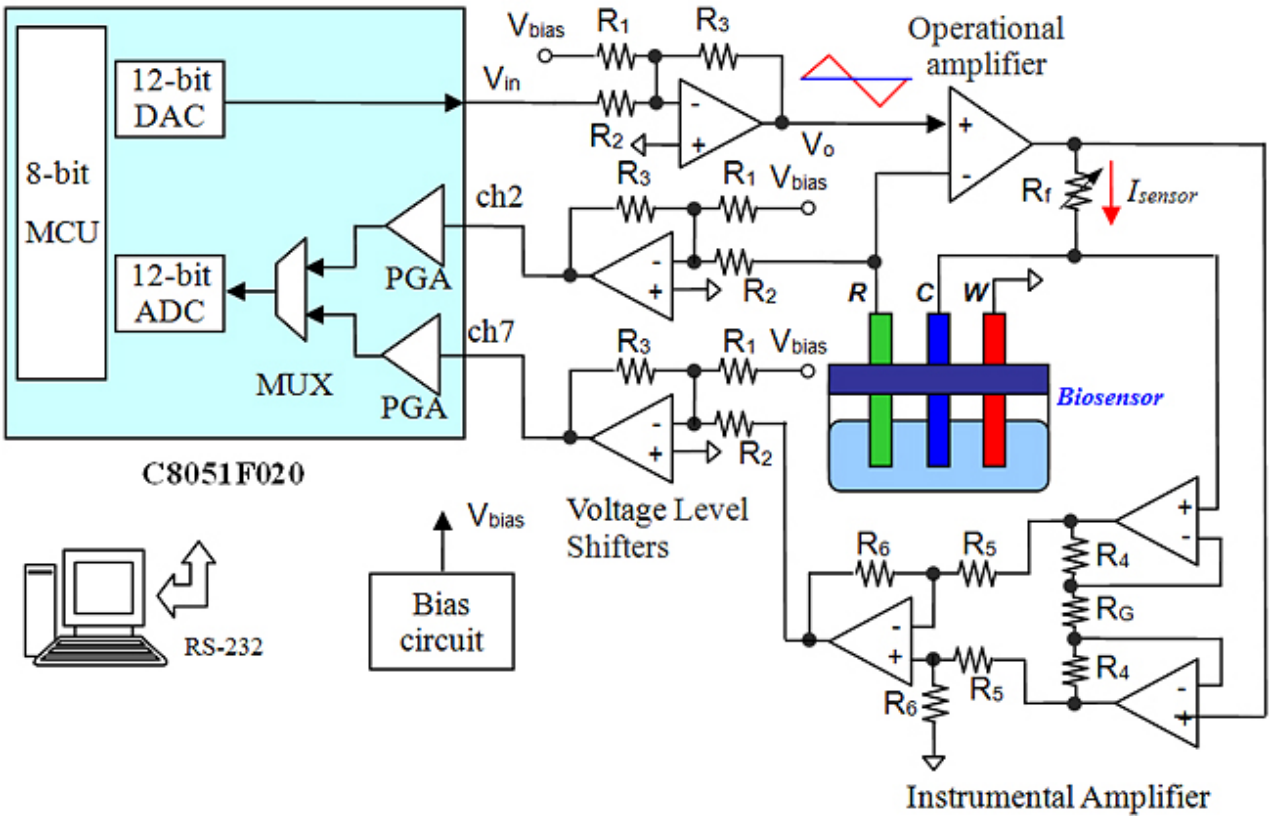


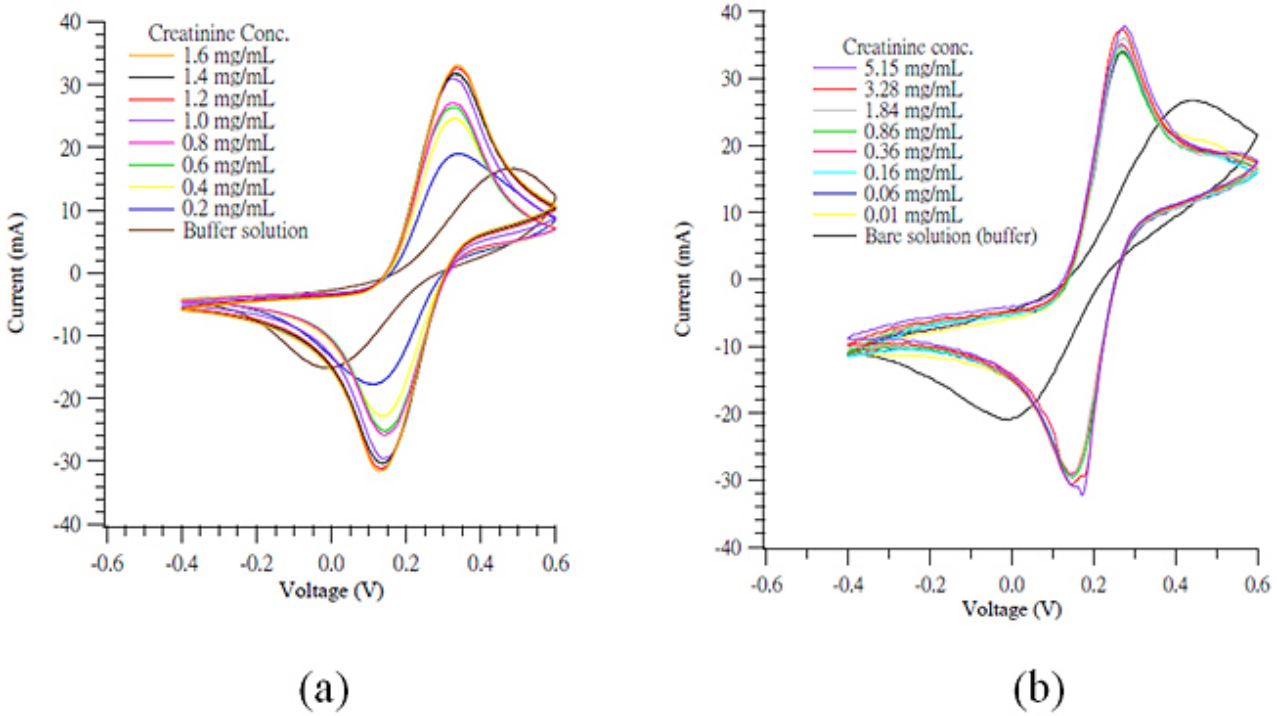
Fig. 2 The circuit diagram of a portable cyclic voltammetry potentiostat.

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. 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. 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.

Table 1 Adsorption of target molecules to imprinted and non-imprinted polymers. Bold numbers indicates the high imprinting effectiveness achieved.

EVALs	Urea (μg/cm²)		Creatinine (μg/cm²)		Lysozyme (μg/cm²)	
(ethylene mole %)	MIPs	NIPs	MIPs	NIPs	MIPs	NIPs
27	**233.7± 50.7**	**100.6± 4.5**	**12.83± 0.56**	**5.20± 1.30**	7.9± 1.6	5.1± 1.5
32	124.3± 72.5	82.5± 14.5	7.74± 0.96	5.45± 0.95	8.9± 0.7	8.3± 0.2
38	189.4± 29.0	109.5± 7.2	8.65± 1.36	5.58± 0.11	13.6± 2.5	9.1± 1.1
44	47.3± 0.1	207.1± 0.1	1.96± 0.15	1.52± 0.05	**17.7± 0.7**	**7.3± 0.2**

Fig. 3 shows the typical cyclic voltammetry of the molecularly imprinted polymeric electrode measured by a commercial potentiostat and by the home-built potentiostat; the current peak occurred when 0.29 V was applied. In Fig. 4, the current responses for creatinine-imprinted and urea-imprinted monotonically increased from 0.12 ± 0.07 μA to as high as 4.06 ± 0.24 μ and 2.12 ± 0.87 μA to 10.9 ± 1.5 μA, 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(0.1～4.0 mg/mL)



(a) (b)

Fig. 3. (a)Cyclic voltammetry of creatinine solutions measured using creatinine-imprinted by a commercial potentiostat and (b) Measurement using creatinine MIP electrode by the home-built potentiostat.

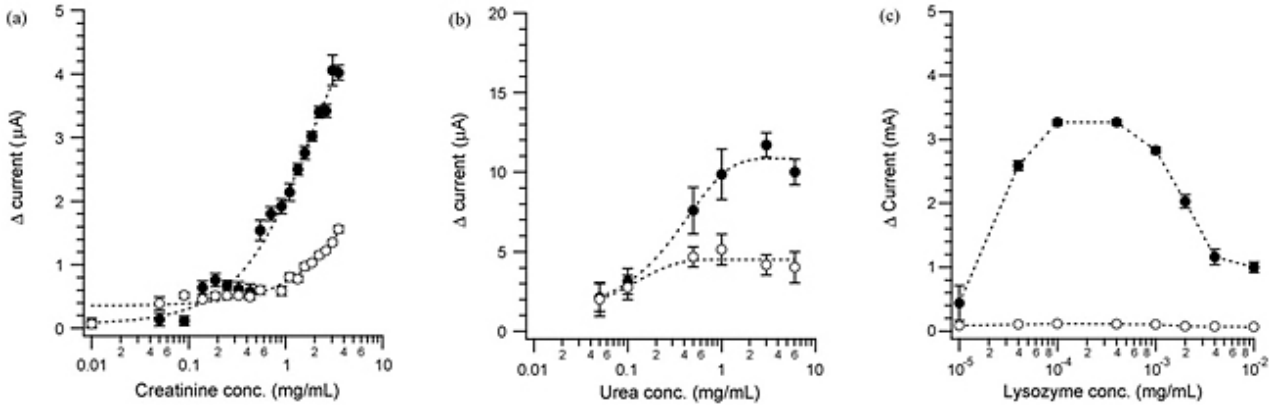


Fig. 4. Current difference for the (a) creatinine-imprinted, (b) urea-imprinted and (c) lysozyme-imprinted electrode for different concentrations of target molecules when voltages of 0.26～0.29 V were applied. Filled and empty symbols indicate electrochemical response with imprinted and non-imprinted polymeric electrodes, respectively.

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 were used six times; even if the electrode film fouls, a new film can be easily formed after the old film is dissolved with DMSO.

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

Molecules	Sample	Home-built potentiostat sensor		ARCHITECT ci	Accuracy
		Δ current(μA)	Convert concentration (mg/mL)	8200 (mg/mL)	(%)
Urea	I	27.70± 10.49	10.10± 3.25	11.86	85.16
	II	31.30± 8.75	11.00± 2.98	13.24	83.08
	III	17.20± 5.66	5.24± 1.98	6.62	79.15
Creatinine	I	0.88± 0.44	0.74± 0.28	0.61	82.43
	II	2.40± 0.24	1.56± 0.91	1.56	100.00
	III	1.12± 0.21	0.90± 0.11	1.56	57.69