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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

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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

Abbreviations: MIP, molecularly imprinted polymers; NIP, non-imprinted Polymers; EVAL, poly(ethylene-co-vinyl ethylene); DMSO, dimethyl sulfoxide; TEGDMA, tetraethyleneglycol dimethacrylate; SOC, system on a chip.

* Corresponding author at: Department of Chemical and Materials Engineering, National University of Kaohsiung (NUK), 700, Kaohsiung University Road, Nan-Tzu District, Kaohsiung 811, Taiwan. Tel.: +886 7 591 9455; fax: +886 7 591 9277.
E-mail addresses: linhy@ntu.edu.tw, linhy@caa.columbia.edu (H.-Y. Lin).

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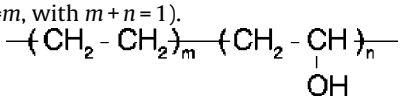
monomers that were used affected rebinding selectivity, likely by altering MIP hydrophobicity.

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 $m+n=1$).



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.

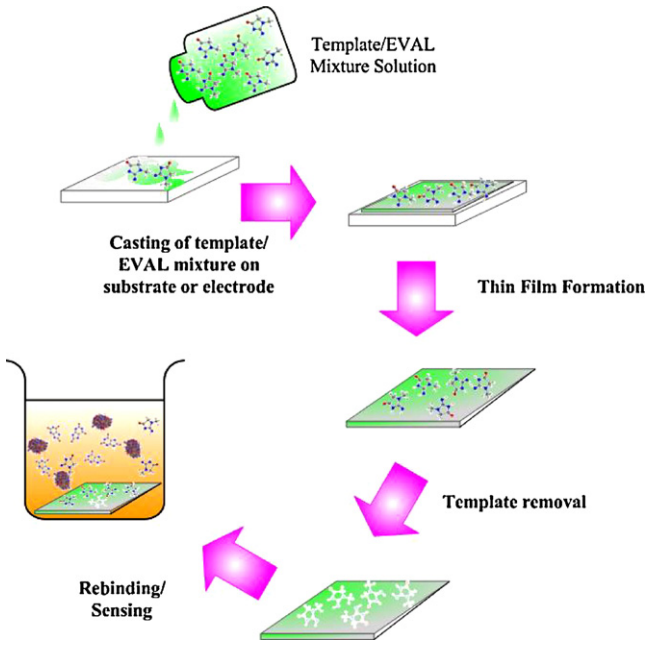
2. Experimental

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 0.1 wt% of template urea, creatinine and lysozyme molecules on a glass slide or on a gold electrode (2.5cm × 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 sulfer atom concentration using energy dispersive (X-ray)



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

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}) with current I_{pa}, and a cathodic peak potential