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Functionalization of micro- and nano-apertures with chromate-selective solvent polymeric membrane

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ABSTRACT

A new miniaturization approach to create micro- and nanoscale ion selective electrodes (ISEs) was demonstrated and the concept tested with an environmentally relevant chromate-selective membrane consisting of 7.7:62.2:31.1 wt % Aliquat336:2-NPOE:PVC. Apertures of 100 nM and 30 μM dimensions were made using MEMS fabrication techniques and functionalized through a macroscale application of solvent polymeric membrane. Performance studies for the microscale ISE showed a response slope of -58.6 ± 5.6 mV decade^{-1} and limit of detection (LOD) of $2.1 \times 10^{-5} \pm 1.1 \times 10^{-5}$ M, versus -65.2 ± 4.2 mV decade^{-1} and $1.8 \times 10^{-5} \pm 6 \times 10^{-6}$ M for the nanoscale ISE. This was consistent with control studies with carefully conditioned coated wire electrodes, which demonstrated a response slope of -61.7 ± 2.4 mV decade^{-1} and a LOD of $3.0 \times 10^{-6} \pm 1 \times 10^{-6}$ M. Response times for the best micro- and nanoscale ISEs were in the 10–20 s timeframe. Electrical resistance measurements were in the GΩ range for the microscale ISEs and nanoscale ISEs. Appropriate ISE geometry was confirmed through AFM measurements and calculations based on electrical properties for micro- and nanoscale apertures. These micro- and nanoscale ISEs are expected to have significant impact in the field of microscale analytical processes.

1. Introduction

The field of ion selective electrodes (ISEs) has expanded into new electroanalytical territory in recent decades, with impressive advances made in miniaturization of electrodes. The rationale behind miniaturization is centered on the development of microanalytical devices with applications in the life sciences. For example, the need for robust miniaturized potentiometric sensors has led to the coated wire electrode design, then methods to mediate a thermodynamically defined contact between the membrane and wire [1]. Studies based on these advances have given rise in recent years to solid-state potentiometric ion selective ultra-microelectrodes for flow-cell analysis [2] and scanning electrochemical microscopy (SECM) [3], and ion selective microelectrode arrays for monitoring extracellular ionic activities [4].

In parallel to the miniaturization effort there has been an emphasis on enhancing ISE sensitivity through elimination of bias. Though ISEs were once thought fundamentally inadequate for trace-level analysis, it was recognized in the last decade that without proper care zero-current measurements with ISEs result in undesirable ion fluxes through the membrane that lead to a bias in the detection limit and selectivity coefficients of several orders of magnitude [5]. To control this bias researchers have used techniques such as ion buffering to force an extremely low concentration in the membrane boundary layer and it has been demonstrated that ISEs can be designed to achieve trace-level capability as low as 100 ppt for Ag^+ [6] for example, proving that the previous limitations were more a consequence of accepted practice in experimental design. The benefit of this work has been further exemplified by ISEs with LODs ranging from 10^{-8} to 10^{-11} M for over 10 analytes [7]. Continued efforts to design improved selective carrier molecules also bear mentioning [8,9], and the ISEs produced in such studies would be compatible with elimination of bias techniques.

With these recent advances ISEs are now miniaturizable and useful at ultra-trace levels [10] suggesting applicability to remote sensing [11] and miniaturized total analysis systems (μ-TAS)[12,13]. However, very few applications in μ-TAS have been reported [12–14]. An attractive step forward in ISE detection in μ-TAS would be to integrate miniaturized ISEs into micro- and nanochannels of electrophoretic separation technologies which are

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especially useful in automated biomolecule processing [15]. For example, a combination of an electrokinetic trap and downstream isotachophoresis could serve the purpose of a preconcentrator and separator for a μ-TAS targeting low abundance proteins using immunosensing ISEs for detection [16,17]. Sensor geometry would be the central factor behind ISE design, as micro- and especially nanoscale electrophoretic separation quality is susceptible to deviations from a smooth separation channel [18]. To our knowledge, no ISE design has been proposed for this express purpose.

Working from a recently reported design using MEMS fabrication techniques for apertures used with artificial bilayer membranes (BLMs) [19], in this paper it is hypothesized that a simple adaptation will produce miniaturized ISEs of appropriate geometry and properties. Rather than coating such apertures with BLM forming solution, solvent polymeric membrane could be applied via a pipetting application technique. Using a well-characterized chromate-selective solvent polymeric membrane based on Aliquat336 ion exchanger [20], results are shown for ISE calibration, geometric analysis using atomic force microscopy (AFM), and assessment of sensor resistance using patch clamping techniques. In summary it is demonstrated that such a macrofabrication technique can lead to the desired miniaturized ISE configuration with micro- and nanoscale sensors that exhibit geometry and properties amenable to application as a detection element in combination with electrophoretic separations on a micro- or nanofluidics based lab-on-a-chip platform. Future work is expected with these micro- and nanoscale ISEs to couple the technology with ion buffering techniques to achieve the lower detection limits observed by others.

2. Experimental

2.1. Materials

All reagents were of analytical reagent grade and used without further purification. All aqueous solutions were prepared using water purified to a resistance greater than 18 MΩ cm in a Barnstead NanoPure Infinity Laboratory Water System (Dubuque, IA). Chemicals used for preparing membrane cocktails included 1450 MW average polyethylene glycol (Sigma–Aldrich, St. Louis, MO), high molecular weight polyvinyl chloride (PVC) of Selectophore grade (Fluka, Buchs, Switzerland), 2-nitrophenyl octyl ether (2-NPOE) of Selectophore grade (Fluka), Aliquat336 (Sigma–Aldrich), and tetrahydrofuran (THF) of 99+% purity (Sigma–Aldrich); $FeCl_3$/HCl PC-Board Etching solution to make Ag/AgCl electrodes (GC Thorson, Inc., Rockford, IL). Negative photoresist SU-8 2010 and its developer (MicroChem Corp., Newton, MA), and positive photoresist AZ5214 and its AZ400K developer (Clariant Corporation, Somerville, NJ) were used in microfabrication procedures. Materials for constructing coated wire electrode arrays included type II PVC rod of 0.5 in. diameter, 18 AWG copper wire, 18 AWG silver wire of 99.99% purity, and waterproof epoxy adhesive used as described by Plesha et al. [21].

N-type silicon wafers of 0.001–0.025 Ω cm resistivity and 0.4 mm thickness (University Wafer Inc., South Boston, MA) were used as a platform for nanofabrication. Photomasks used for fabricating microscale apertures were printed on transparent plastic film. Photolithography was performed using the contact method in a Model 500 mask aligner from Optical Associates, Inc. (San Jose, CA). Backing supports for processed SU-8 film were constructed using plastic from Petri dishes. Test chambers for the ISE chips were made out of 4.3 cm × 1.9 cm × 1.6 cm rectangular polystyrene boxes (Cargille Laboratories, Cedar Grove, NJ).

Ultrasharp silicon cantilevers (spring constant: 7.5 N m^{-1}) were used for experiments involving atomic force microscopy (AFM) (MikroMasch, Portland, OR).

2.2. Instrumentation

All sensors were calibrated with an inexpensive potentiometer designed at the U.S. Naval Research Laboratory (NRL) and having a 10 TΩ internal resistance, which is described more fully by Plesha et al. [21]. Approximate electrical resistances of nano- and microscale ISEs were determined using an Axopatch 200 (Molecular Devices, Sunnyvale, CA) operating with a CV-201 headstage in whole cell recording mode. Axopatch patch clamping devices are known for their high internal resistances and are capable of electrical measurements on systems imposing a load of up to 50 GΩ [22]. AFM was employed to obtain topographic images of nano- and microscale ISEs by using a PicoForce^{TM} Scanning Probe Microscope equipped with a 259PF scanner (Veeco Inc., Santa Barbara, CA).

2.3. Procedures

$HCrO_4^-$ selective membranes were prepared based on the method of Choi and Moon [20], but membrane cocktails were made with ionophore concentrations at 1, 2, 3, 7.8, 9, and 18 wt%, while holding the ratio of 2-NPOE to PVC fixed to 2:1, in order to explore additional compositions than originally reported. A reference membrane cocktail developed at the NRL was prepared as recommended by Plesha et al. [21]. For this, equal volumes of 100 mg mL^{-1} PVC in THF and 200 mg mL^{-1} polyethylene glycol in THF were mixed together.

Coated wire electrodes were constructed by carefully drilling seven holes through a 0.5 in. PVC rod in an equally spaced pattern near the circumference of the rod. Then 3 μL of membrane cocktail was carefully pipetted onto each polished copper wire surface and the membranes were dried overnight under vacuum. Reference electrodes designed at the NRL were integrated into the coated wire electrode arrays as described by Plesha et al. [21]. Briefly, a hole was drilled axially through the rod at the center of the cylindrical array and 25 μL of reference membrane cocktail was dispensed into the bottom of the hole from the polished surface, followed by drying under vacuum overnight. The center hole was backfilled with a solution of 3 M KCl(aq) and saturated AgCl(aq). Finally, a Ag/AgCl electrode was inserted into the hole and secured in place with epoxy adhesive.

The MEMS fabrication process for making our microscale apertures was completed in a cleanroom at WSU and is described in detail in a prior publication associated with this laboratory [19]. Briefly, spin coating was used to deposit a 10 μm thick SU-8 2010 layer on a sacrificial layer of positive photoresist AZ521. The coating was covered with a photomask for the desired aperture size and exposed to UV light followed by SU-8 developer. The SU-8 layer was freed using AZ400K developer to etch away the sacrificial AZ5214 layer; when freed it was glued onto a plastic chip for mounting.

The fabrication protocol for nanoscale apertures is displayed in Fig. 1A. A thin film of silicon nitride (Si_3N_4, thickness: 200 nm) was deposited on one side of the silicon wafer using plasma-enhanced CVD. Silicon dioxide was deposited on the other side of the wafer using thermal oxidation, and then spin-coated with a photoresist layer used for patterning. A 600 μm × 600 μm window was patterned on the photoresist using UV lithography and the exposed silicon dioxide etched using RIE. The exposed silicon surface was then wet etched using KOH. Hence after the main frame of the sensor was fabricated, the photoresist and silicon dioxide layers were removed, with the silicon nitride thin film acting both as an etch stopper and the impermeable membrane support for the sensor. A nanoscale aperture of 100 nm diameter was milled in the center of this impermeable membrane using a focused ion beam (FIB).

The micro- and nanoscale apertures were functionalized by pipetting a portion of 2.5 μL of membrane forming solution over the