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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 nano-scale 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 μ M 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 nano-scale ISEs were in the 10 – 20 sec timeframe. Electrical resistance measurements were in the G Ω 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 nano- scale apertures. These micro- and nano-scale ISEs are expected to have significant impact in the field of micro-scale analytical processes.

Keywords

Ion Selective Electrodes; Nanosensors; Microsensors; Chromate; Aliquat336; Microelectromechanical Systems

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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 ultramicroelectrodes 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 and more 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 especially useful in automated biomolecule processing [15]. For example, a combination of an electrokinetic trap and downstream isotachopheresis 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, 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 M Ω -cm in a Barnstead NanoPure Infinity Laboratory Water System (Dubuque, IA). Chemicals used for preparing membrane cocktails included 1450 MW avg. 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); FeCl₃/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 inch diameter, 18 AWG copper wire, 18 AWG silver wire of 99.99% purity, and waterproof epoxy adhesive used as described in 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 $\times$ 1.9 $\times$ 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 T Ω 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 G Ω [22]. Atomic force microscopy (AFM) was employed to obtain topographic images of nano- and microscale ISEs by using a PicoForce™ Scanning Probe Microscope equipped with a 259PF scanner (Veeco Inc., Santa Barbara, CA).

2.3 Procedures

HCrO₄⁻ 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 inch PVC rod in an equally spaced pattern near the circumference of the rod. Then 3 μ L 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 μ L 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 3M 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 μ m 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 (Si₃N₄, 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 μ m by 600 μ m 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 aperture opening through the 3 mm window of the mounting chip, on the chip-side of the film; applying only a portion of the entire 2.5 μL prevented bubbles from forming in the cast membrane. The membranes were dried overnight under vacuum.

All electrode testing was conducted at room temperature ($\approx 22.7^\circ\text{C}$). Coated wire electrode arrays were conditioned overnight in $10^{-1}\text{ M HCrO}_4^-$ (aq) solution before each experiment and were tested in a magnetically stirred 4 mL solution volume. Nano and microscale ISE test cells were set up in a format identical to what Choi and Moon describe [20] as reflected in Fig. 1b. Nano- and microscale ISE chips were affixed to the test chamber using waterproof epoxy to ensure good electrical sealing. A Ag/AgCl electrode was immersed in 4 mL of reference electrolyte, consisting of 10^{-4} M KCl combined with $10^{-4}\text{ M K}_2\text{Cr}_2\text{O}_7$, on the reference side of the test chamber. A reference electrode, consisting of a Ag/AgCl electrode immersed in 3 M KCl(aq) saturated with AgCl(aq) and separated from sample by a NRL reference membrane, was placed into the sample side of the test chamber. Nano- and microscale ISEs were tested such that the sample side of the testing chamber was magnetically stirred.

Linearity and limit of detection (LOD) studies were performed using the standard addition method over concentrations ranging from 10^{-8} M to 10^{-1} M . Electrode response slopes were determined by assuming an activity coefficient of unity for calibration species and fitting the modified Nicolsky-Eisenman model to calibration data using nonlinear regression [21]. The model used was:

$$E = E_0 + S \cdot \log_{10}(a + U), \quad (1)$$

where E is membrane voltage, E_0 is the cell constant, S is the ISE slope, a is the activity of the analyte, and U is the coefficient of unmodeled interferents [21, 23-25].

Lower LODs were taken according to the IUPAC recommended method as the activity of HCrO_4^- at the point of intersection between the linear region of the calibration curve and the final low concentration level [26]. Interferences were determined using the Separate Solution Method (SSM) at matched activities [27] as a check on membrane quality. Selectivity coefficients for SO_4^- , CH_3COO^- , Cl^- , F^- , SO_3^- , I^- , NO_3^- , H_2PO_4^- , and SCN^- were determined at an ion activity of $1.16 \times 10^{-2}\text{ M}$. Response times were determined as the time to 90% of the steady-state EMF.

Axopatch patch clamping was used to take current measurements as holding potentials were varied. Output from the Axopatch was passed through a 60 Hz noise filter built in house and recorded to a computer using a digital multimeter. Ag/AgCl electrodes were immersed in test electrolyte on either side of the sensor membrane; one electrode was connected to the positive terminal of the headstage, and the other was connected to its negative terminal. The cell current was recorded as the holding potential of the cell was varied. A digital multimeter

was used in resistance testing mode to obtain an order of magnitude estimate of the electrical resistances of the coated wire electrode arrays for comparison.

AFM was used in contact mode in air at room temperature to take images over different areas of each miniaturized ISE surface tested. Nano- and microscale ISEs were fixed to a magnetic holder using double sided adhesive tape. The scanning area was set to $60\text{ }\mu\text{m} \times 60\text{ }\mu\text{m}$ and the scan rate kept set to 1 Hz with a resolution of 256 samples per line. The AFM images presented in this study did not undergo any specific image processing except flattening, unless otherwise indicated. To determine more exact dimensions of sensor membranes, line profiles were selected to traverse the AFM images collected.

3. Results and Discussion

The strategy guiding the present study in producing micro and nanoscale ISE based on MEMS fabrication techniques such as UV lithography and FIB is to apply a membrane forming solution to a nano- or microscale aperture located in an impermeable surface. After curing a solvent polymeric membrane is expected about the diameter of the aperture, on the opposing surface. This membrane will function as a miniaturized ISE membrane when introduced to sample. The hypothesis guiding this study was that these ISEs will perform identically to macroscopic ISEs if the experimental electrochemical cell is properly configured since the phase boundary potential of an ISE is independent of the cross sectional area of the exposed membrane surface [28]. The resistance imposed by the membrane will increase as the aperture is made smaller, but will remain within the limits required for our intended use. Sensors performing according to these expectations will be amenable to integration in μ -TAS employing pre-separation and pre-concentration technology and therefore usable in-situ as a means to interrogate microscale sample separation bands for trace-level analysis of analytes.

The experimental results presented here test the rationale stated above and demonstrate the efficacy of the sensor fabrication technique. Results are presented for micro- and nanoscale apertures functionalized into ISEs, their electrical resistances and implications thereof, and confirmation of micro- and nano-aperture geometry.

3.1 Control Studies with Coated Wire Electrodes

The straightforward CWE configuration was followed for producing electrodes serving as positive controls. Though CWEs are a solid contact design, when properly conditioned such ISEs will perform comparably to the supported liquid membrane format. Table 1 compares the slopes and LODs obtained for the various membrane formulations tried to the results of Choi and Moon, and Table includes additional results at lower Aliquat336 concentrations in the 1 – 3% (w/w) range. The ISE formulations prepared in this study achieved LODs in the range $1.78 \times 10^{-6}\text{ M}$ through $5.71 \times 10^{-6}\text{ M}$, along with near-Nernstian slopes in the range of -49.5 ± 1.3 to $61.7 \pm 2.4\text{ mV decade}^{-1}$. Response times, determined as the time to reach 90% of a new steady state value after a concentration increase, were 20 seconds or less. The optimally performing CWE membrane recipe was 7.8:62.2:30.0 wt % Aliquat336:2-NPOE:PVC, which is similar in composition to the 8.8:61.6:29.6 wt % recipe reported as optimal by Choi & Moon. Furthermore, a mere seven percent difference was obtained when

comparing the slopes associated with these two optimal recipes, and the LODs fell within an order of magnitude. The results reported are consistent with the majority of alternative solvent polymeric membrane recipes targeting chromium ions (including Cr^{3+} , HCrO_4^- , and CrO_4^{2-} [29-33], as most of these consist of 1% ionophore recipes with the exception of that of Aradkani, et al. [34] and Singh, et al. [35] which call for a 7% and 10% ionophore recipe, respectively. It was desirable in this study, however, to use higher concentrations of ion exchanger to help reduce bulk resistance. Hence the membrane recipe of 7.8:62.2:30.0 wt % Aliquat336:2-NPOE:PVC was adopted for testing miniaturized ISEs, since in addition to its higher ion exchanger content it also gave the best performance.

Some important precautions were followed when characterizing chromate ISEs including: 1) *Control of pH* because of the pH dependent equilibrium between the chromium(VI) species HCrO_4^- , and dichromate or chromium(III) species $\text{Cr}_2\text{O}_7^{2-}$. Tandon, et al. [36] showed that for total chromium(VI) concentrations up to $10^{-3} \text{ mol L}^{-1}$, the pH must be kept in the 2.4 - 4.9 range to maintain at least 90 mol-% of total chromate as HCrO_4^- , the species for which Aliquat336 is selective. For example, when we prepared calibration solutions in nanopure water the pH ranged from 4.2 to 6.5 and averaged 5.3 ± 0.7 ; hence we prepared our calibration solutions in water buffered at pH 3.9 using 0.01 M acetate buffer, which kept the pH within the range of 3.6 to 4.0 with an average value of 3.8 ± 0.1 ; 2) *Understanding of interference issues*. Aliquat336 may facilitate interference from some buffer anions, so the influence on the LOD of background interfering ions imposed by buffer use must be recognized. Acetate was chosen due to its low potentiometric selectivity coefficient as reported for the first time in this study.

In addition to acetate, the interferences of other common anions were determined all by using the SSM at identical activities [27] – this provided an additional check of the consistency of the membrane preparation used in this study and that reported by Choi and Moon. Table 2 summarizes the experimentally-determined selectivity coefficients, which show that values for anions tested in common with those of Choi & Moon fell within an order of magnitude, though the values in this study were generally lower. The systematic difference in selectivity coefficients can be explained by the difference in pH control methods, which can give rise to differing background levels of interfering ions that would influence both the LOD and the selectivity coefficients. In the case of Choi and Moon the method of pH control was not specified. In this study the selectivity coefficients would suggest that the LOD was determined by the acetate background from the buffer used to control the pH, such that the LOD would be limited to no better than 10^{-6} M . Though this was acceptable for the purposes of this study, it should be noted that better performance from this membrane formulation could be possible. For example, if sulfuric acid were to be used to control the pH the static detection limit may improve by 1-2 orders of magnitude, and state of the art methods such as ion buffering of the inner filling electrolyte associated with the supported liquid membrane design could also be used.

3.2 Nano- and microscale ISE performance compared to CWEs

Fig. 1 shows the calibration curves of a nanoscale ISE formed on an aperture 100 nm in diameter and a microscale ISE formed on an aperture 30 μm in diameter, compared to a

macroscopic CWE. All three electrode configurations show similarly Nernstian concentration sensitivity, -61.7 ± 2.4 mV decade⁻¹ for the CWE, -58.6 ± 5.6 mV decade⁻¹ for the microscale ISE, and -65.2 ± 5.6 mV decade⁻¹ for the nanoscale ISE. Differences appear in the LOD values; for example, both the nano- and microscale ISEs give similar LODs with values of 1.8×10^{-5} M and 2.1×10^{-5} M, respectively, nearly an order of magnitude higher than the 3.0×10^{-6} M LOD of the CWE. These LOD values are at least comparable to those of Choi and Moon, and likely is a result of the differences between properties of the inner filling electrolyte and the hydrated layer known to form in CWEs [37]; however, the goal here was to determine first that the ISE cocktails prepared in this study work like those in the literature and then check the suitability of the micro- and nano-aperture supports. Though our CWE was a novel implementation of the membrane cocktail and as such showed slightly superior performance, a much better performing ISE is theoretically possible using cutting edge macroscale ISE fabrication techniques, such as those involving the use of metal ion chelators [7]. Hence the greatest contribution of this study focuses on the novel miniaturization technique.

The average response time, defined as the time for an ISE to achieve an EMF that is 90% of its steady state value after adding an aliquot of a solution containing a higher concentration of analyte and calculated as the average values ($n=6$) for $10^{-5.5}$ to 10^{-1} M additions for the data presented in Fig. 1, for the CWEs, nanoscale ISEs, and microscale ISEs were 23.5 ± 12.5 s, 28.8 ± 6.1 s, and 40.2 ± 25.4 s, respectively. The CWEs have response times in the typical range of 20 sec or less observed for chromium ISEs in the literature [29-32, 34, 35]. The average response time for the nanoscale ISE was 22.7% and for the microscale ISE 71.1% greater than that of the CWE. However, both the nano- and microscale ISEs response times showed significant variability with certain electrodes from each category with response times of less than 20 sec as illustrated in Fig. 2. Reasons for the variation between individual electrodes are not immediately apparent, but in part may be a function of varying transmembrane diffusion rates associated with membrane thickness, or due to the greater sensitivity of the nano- and microscale electrodes to electrical interference from equipment in the laboratory or other laboratories within the building.

Finally, the longevity of the solvent polymeric membrane for all three configurations was consistent with what was reported by Choi and Moon [20]. All three electrodes showed consistent response characteristics over a period of 61 days, with no appreciable loss in slope or LOD (data not shown). These results demonstrate that no significant performance loss occurs from the miniaturization of the ISEs.

3.3 Electrical resistance of sensors

The electrical resistances of fabricated apertures with and without membrane allowed confirmation of aperture size and determination of potentiometer specifications for sensor measurements. Fig. 3 shows electrical data from which resistances could be determined. Shown are data collected with an Axopatch 200 for an 80 μ m microscale aperture with and without a membrane and using nanopure water for the conducting fluid, and a 100 nm nanoscale aperture using 10^{-3} M KCl(aq) as the test electrolyte. Fig. 3 shows a linear relationship between holding potential and current, reflecting ohmic behavior, and therefore

allowing calculation of resistances from the slopes since the electrical resistance of the aperture should dominate the test cell. CWEs and microscale apertures filled with KCl had resistances too low to be measured with the Axopatch. Therefore, resistances for these setups were estimated with a digital voltmeter, though these estimates are subject to some uncertainty because of the possibility of electrode polarization.

The measured electrical resistances showed that the aperture sizes resulting from fabrication procedures were consistent with those anticipated from the mask sizes. The electrical resistance model for an empty cylindrical aperture is given in Eq. 2:

$$R_{sensor} = \frac{\rho \times L}{\pi \times r^2} + \frac{\rho}{4 \times r}, \quad (2)$$

where ρ is the resistivity of the conducting medium inside the aperture, L is its depth, and r its radius. The first term of Eq. 2 models the electrical resistance imposed by the medium filling the aperture. The second term is from the Hall model [38] and is used to model the access resistance, which must be accounted for in small pores. It arises as current paths from bulk electrolyte converge into the pore, and is most significant when pore geometry is relatively wide compared to pore depth [39]. Since the resistances are in series they can be summed. Using this model, a diameter of 103 nm was calculated for the nanoscale aperture based on its measured resistance indicated in Table 3, the resistivity for 10^{-3} M KCl electrolyte of 6.81 k Ω ·cm [40], and its depth of 0.2 μ m that resulted from the nanofabrication protocol. This differs by only 3% from the 100 nm aperture diameter pattern size used during the nanofabrication protocol.

By applying the resistance model in Eq. 2 to the microscale aperture immersed in the higher resistance nanopure water with a measured molar conductivity of 0.0394 m² S mol⁻¹, an aperture diameter of 94.2 μ m was determined which gives a discrepancy of only 18% when compared to the 80 μ m aperture diameter anticipated from the microfabrication protocol. A similar analysis performed for the nanoscale aperture immersed in nanopure water yielded only a 1% difference between the anticipated and actual aperture diameters. The large changes in resistances when adding a membrane, 130-fold and 2500-fold for the microscale aperture in nanopure and KCl(aq), respectively, and 3-fold and 17-fold for the nanoscale aperture and KCl, respectively (Table 3) confirm that a functional membrane has been formed in the aperture. When the membrane is present one notes a lower relative change in resistance when switching between nanopure water and KCl solutions; this is expected as the membrane, when present, will dictate the resistivity through the aperture. The resistance model in Eq. 2 was also used to estimate the resistivity of the solvent polymeric membrane with which apertures were functionalized. Membrane resistivity values of 43.7 M Ω ·cm and 52.2 M Ω ·cm were calculated based on the resistance of the 80 μ m microscale ISE when immersed in nanopure water and when immersed in 0.001 M KCl electrolyte, respectively. These values are consistent with previous reports. For example, PVC plasticized with 66 wt-% di-2-ethylhexyl phthalate can have a resistivity as low as 1.63 M Ω ·cm [41], but higher values are possible depending on the type of plasticizer and the membrane composition [42].

Finally, the resistance data assists in determining potentiometer specifications. A potentiometer must have an internal resistance greatly exceeding the load of the sensors to avoid problems in taking the measurement. The resistances of the microscale ISEs (3.6 G Ω - 4.3 G Ω) and those of the functionalized nanoscale aperture (around 35-40 G Ω) are in a range compatible with readily available potentiometers having a typical input impedance of about 10 T Ω ; for example, a simple voltage divider calculation suggests that a 1% error or less will result when testing the functionalized nanoscale apertures.

3.4 Nano- and microscale ISE morphology

The morphology of ISE membranes produced using the methods presented in this study is important for potential applications related to μ -TAS integration, such as interrogation of sample bands formed during isoelectric focusing. Such bands may be on the order of 200 μ m in width, requiring the sensor surface to be both narrower than the band and non-disruptive to the band. Furthermore, fabrication of micro- or nanoscale ISE membranes on the distal side of a substrate can be made to be simple and practical if a macroscale application method is possible, e.g., pipetting of membrane forming solution onto the proximal side of the substrate in the vicinity of the aperture region.

It was expected that solution would fill the aperture and even pool out slightly on the opposing side, so that the membrane surface area exposed to solution would exceed the aperture surface area to some extent. To determine the form of the membrane surface microscale ISEs were imaged using AFM, which does not require sample preparation and preserves the sample from disruption while surface character is being measured. Fig. 5 shows the topology of an ISE membrane cast onto a $\sim$ 30 μ m aperture by pipetting solution from the opposing side. Below the image are two AFM profile scans taken perpendicular to each other; the X's on the topology image correspond to the vertical dashed lines plotted on the profile scan images. The digitally added dark circle is scaled to correspond to a diameter of 30 μ m, the size of the hole anticipated from the 30 μ m mask used to etch the aperture. The circle is centered on the light circular area that represents the protrusion in the center of the membrane, as this protrusion obviously corresponds to the location of the actual aperture. The fact that the black circle is approximately the same size as the membrane protrusion confirms that the aperture size controls the diameter of the membrane formed when liquid is added to the opposing side. The figure also clearly shows that the membrane pools beyond the perimeter of the aperture to a final diameter of 50 μ m, 1.7 times larger than the aperture on which it was cast. This relatively small spread of the membrane material beyond the aperture confirms that a macroscale application of membrane forming solution can result in a microscale-sized ISE that will be exposed to passing analysis sample bands.

The AFM images reveal other important features of the membrane. First, in Fig. 5B the approximate height of the membrane directly over the central 30 μ m diameter aperture is 500 nm while the extreme outer edge of the membrane protrudes above the surface by a mere 100 nm. Also, the membrane is asymmetric, exhibiting an elliptical pattern as indicated by the relative light and dark areas surrounding the protrusion and the differences exhibited in scans 90 degrees apart from each other. Scan Profile B shows a more rapid decrease in membrane thickness beyond the 30 μ m diameter to a $\sim$ 200 nm height, relative to

an arbitrary zero point, levels out and then falls off to a -300 nm at the edge of the membrane. By contrast Scan Profile A shows a gentler decrease in thickness to $+100$ nm where measurements level off until near the edge of the membrane after which it falls to 0.0 nm; apparently this scan never reached the extreme ends of the membrane as the -300 nm depth is never seen. Further support for a thicker membrane in the vertical direction is offered by the slightly concave surface for the protrusion that is 50 nm higher at its outer edges in the vertical direction whereas the membrane is virtually flat in the horizontal direction. Based on the measurements made, less than a nanoliter volume of membrane protrudes out of the aperture face. Given these heights and amounts of solvent polymeric membrane it is expected that the membrane will have little consequence in terms of perturbing any separation bands that pass over an ISE embedded in the wall of a channel which itself is on the order of $10\text{ }\mu\text{m}$ in height, though when channels are on the submicron scale the perturbation of passing fluid is expected to become more important and careful studies will be needed on the impact of this perturbation on sample measurements when such ISEs are used for determining concentrations.

Unfortunately, the nanoscale ISEs could not be imaged using AFM, because the membrane could not be distinguished from the other surface features and dust particles on the silicon surface (data not shown). This problem will be addressed in future studies by addition of a set of crosshairs to the mask, facilitating location of the nanoscale apertures with AFM. Of course there is no doubt about the existence of the nanoscale ISEs given the sound fabrication techniques used, the unequivocal evidence of voltage response to chromate, and the resistance studies.

3.5 Impact of New Nano- and Microsensors

The impact of this work on analytical chemistry is quite significant. We have created robust nano- and microscale sensors that will easily fit within μ -TAS geometries. AFM analysis shows the microsensors are relatively flat and will not protrude into a passing analysis band to any appreciable extent and therefore will not disturb the flow profile. Example recent works that will benefit from availability of such sensors include the work of the groups of Ivory and Dutta where microscale isotachopheresis [43] and isoelectric focusing bands [44] are as small as $150\text{ }\mu\text{m}$ and therefore can be sensed by ISEs smaller than the band. The group shows isotachopheresis bands traveling at $15\text{--}20\text{ }\mu\text{m/sec}$ that can be slowed down by adjusting the electric field strength. Thus, a $150\text{ }\mu\text{m}$ band moving at $5\text{ }\mu\text{m/sec}$ will give a 30 sec residence time over our nano-scale ISEs; this will allow resulting analytical ISE signals to be processed within the 18 sec response time shown for our best nano-scale ISEs. Isoelectric focusing bands will not be a problem because they can be positioned to stop directly over an ISE. It is possible that even faster response times may result by using thinner membranes to enhance transmembrane diffusion rates – this could be accomplished by refined fabrication techniques such as using finely metered volumes of membrane cocktail so there is just enough membrane to cover the aperture.

4. Conclusions

Working nano- and microscale ISEs were fabricated by applying an existing formulation for HCrO_4^- selective solvent polymeric membrane to nano- and microscale apertures made using MEMS fabrication techniques. Electrical resistance measurements provided clear evidence of proper nano- and microscale aperture formation and complete filling of the apertures with solvent polymeric membrane. AFM imaging provided additional evidence of proper microscale aperture formation and its complete filling with membrane, and showed that the surface area of the cured membrane exposed to sample is effectively limited to be about the size of the aperture. These images indicate that microscale ISEs are ideal for integration into micro channels, as they are relatively thin and smooth, protruding into the sample by only 250 nm at most.

The nano- and microscale ISEs performed comparably to the CWE, giving response slopes of -65.2 ± 5.6 mV decade⁻¹ and -58.6 ± 5.6 mV decade⁻¹, respectively; and LODs of 1.8×10^{-5} M and 2.1×10^{-5} M, respectively. The nano- and microscale ISEs achieved response times of 28.8 ± 6.1 sec and 40.2 ± 25.4 sec, respectively, which are comparable to those for conventional CWEs and make them very promising for future application in lab-on-a-chip technologies.

The nano- and microscale ISE fabrication techniques presented in this study, in which macroscale pipetting of membrane solution to the distal side of an aperture leads to a nano- or microscale ISE on the proximal side, can produce practical miniaturized ISE technology. These sensors, when mated with other analytical methods such as capillary electrophoretic separation technology, offer the potential for novel integration of ISEs into μ -TAS platforms operating on the micron- to submicron scale.

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Fig. 1.

(A) Protocol developed for fabrication of nanoscale apertures. **(B)** Diagram of the apparatus used to test miniaturized ISEs.

Fig. 2.

Potentiometric calibration curves of a microscale ISE formed from a 30 μm microscale aperture ($\circ$) and a nanoscale ISE formed from a 100 nm nanoscale aperture (Δ), compared to a CWE ($\square$), and all with identical membrane compositions of 7.8:62.2:30.0% (w/w) Aliquat336:2-NPOE:PVC. The figure confirms all three electrodes behave similarly with LODs on the order of 10^{-5} M or better and a Nernstian response slope of -61.7 ± 2.4 mV decade $^{-1}$ ($\square$) for the CWE, -58.6 ± 5.6 ($\circ$) for the microscale ISE and a slightly super-Nernstian value of -65.2 ± 4.2 (Δ) for the nanoscale ISE. Values and standard errors for the cell constant parameter E_0 , in units of mV, were: -269.6 ± 16.2 ($\circ$) and -314.1 ± 12.8 (Δ). Values and standard errors for the detection limit parameter U , in units of mol L^{-1} , were: $2.09 \times 10^{-5} \pm 1.82 \times 10^{-5}$ ($\circ$) and $1.79 \times 10^{-5} \pm 5.7 \times 10^{-6}$ (Δ).

Fig. 3.

A comparison of the fastest recorded response times for the CWE, microscale ISE, and nanoscale ISE configurations. These response times, defined as the time it takes the EMF of electrochemical cell to reach 90% of its final steady state value, were 18 s for the CWE, 11 s for the microscale ISE and 18 s for the nanoscale ISE.

Fig. 4.

A graph of current readings at various holding potentials for an 80 μm microscale aperture without membrane ($\circ$) and tested in nanopure water, the same microscale aperture functionalized with 7.8:62.2:30.0% (w/w) Aliquat336:2-NPOE:PVC membrane ($\square$) and tested in nanopure water, and a 100nm nanoscale aperture ($\triangle$) tested in 10^{-3} M KCl (aq), in nanopure ($\diamond$), and the same nanoscale aperture functionalized and tested in nanopure water (∇). Though current is the dependent variable, it is plotted on the x-axis because the slope of a linear plot of voltage versus current is equivalent to the circuit resistance according to Ohm's law. Current values exhibited measurement errors of ± 0.01 nA ($\circ$) and ± 0.005 nA ($\square$, $\triangle$, $\diamond$, ∇) and are reflected in the error bars (for $\circ$ the error is shown in the inset).

Fig. 5.

Topology image and line profiles obtained by AFM, showing a microscale ISE membrane which was cast by applying roughly half of the contents of a pipette tip containing 2.5 μL of 7.8:62.2:30.0% (w/w) Aliquat336:2-NPOE:PVC membrane forming solution to a microscale aperture of 30 μm diameter in SU-8. Only a portion of the pipette contents is expelled to avoid the presence of air bubbles in the dispensed membrane forming solution. The faint dark ring centered inside the membrane was digitally added to indicate a rough outline of the microscale aperture, which is based on the size of the pattern used to etch it. The membrane is approximately 50 μm in diameter, which is 1.7 times larger than the diameter of the microscale aperture. The white lines were digitally added along with X's and correspond to the horizontal line Scan Profile (A) and the vertical line Scan Profile (B).

TABLE 1

Response slopes and detection limits obtained for the various membrane forming solutions, compared to the results reported by Choi and Moon [7].

Membrane Composition Aliquat336 : o-NPOE : PVC (in % w/w)		Slope (mV decade ⁻¹)	LOD (mol L ⁻¹)
WSU	1.0:66.0:33.0	-50.7 ± 5.8	4.34×10 ⁻⁶
	2.0:66.7:31.3	-49.5 ± 1.3	3.65×10 ⁻⁶
	3.0:65.4:31.6	-53.0 ± 1.0	1.78×10 ⁻⁶
	7.8:62.2:30.0	-61.7 ± 2.4	3.02×10 ⁻⁶
	9.0:60.7:30.3	-52.2 ± 0.7	5.71×10 ⁻⁶
	18.0:54.8:27.2	-50.1 ± 2.9	2.34×10 ⁻⁶
Choi and Moon	4.6:64.4:31.0 ^a	-95.5	1.09×10 ⁻⁵ ^b
	8.8:61.6:29.6	-57.6 ^c	3.85×10 ⁻⁵ ^c
	10.1:56.2:33.8	-61.2	2.70×10 ⁻⁵
	12.7:59.0:28.3	-57.3	1.55×10 ⁻⁵
	12.8:44.5:42.8	-60.2	2.70×10 ⁻⁵
	17.4:24.3:58.3	-74.6	3.37×10 ⁻⁵
	18.2:55.2:26.6	-62.9	2.45×10 ⁻⁵
	23.0:0.0:77.0	-	-

^aChoi and Moon reported their membrane composition data in mL Aliquat 336 : mL 2-NPOE : gm PVC. These values were converted to weight percents using density values at 25°C for Aliquat336 of 0.884 gm mL⁻¹ and for 2-NPOE of 1.04 gm mL⁻¹.

^bChoi and Moon reported limits of detection in ppm Cr(VI), and these values were converted into mol L⁻¹ by assuming a water density of 0.9970 gm mL⁻¹ (true at 25 °C). They did not report errors.

^cChoi and Moon provided original calibration data points for this membrane composition. Nonlinear regression was used to fit the extended Nicolsky-Eisenman model to their data as described in section 2.5. The model parameters and their standard error are reported in the table, versus the original values reported by Choi and Moon of -53.7 mV/decade for the slope and 2.01×10^{-5} mol L⁻¹ for the LOD.

TABLE 2

Selectivity coefficient values obtained by the Separate Solution Method.

Interfering Ion	$\text{Log}_{10}(K_{A,B})$	
	Against HCrO_4^- Calibration in 0.01 M Buffer ^a	Choi and Moon (no buffering) ^b
SCN^-	-1.9 ± 0.4	1.14 (0.69, FIM)
I^-	-2.8 ± 0.4	0.049 (-0.43 , FIM)
NO_3^-	-3.2 ± 0.4	-0.54
H_2PO_4^-	-3.4 ± 0.6	–
F^-	-3.4 ± 0.5	–
Cl^-	-3.5 ± 0.4	-1.68
CH_3COO^-	-3.5 ± 0.5	–
SO_4^{2-}	-4.0 ± 0.5	-3.00
SO_3^{2-}	-4.3 ± 0.5	–

^aThe selectivity coefficients in this column were calculated using a primary ion calibration curve obtained from calibrating a coated wire electrode in 0.01 M Acetate/Acetic Acid buffering system at a pH of 3.9. Error was determined from the standard errors of the parameter values resulting from nonlinear curve fitting.

^bChoi and Moon [7] indicate their primary ion calibration solutions were prepared in deionized water at pH ranging from 3.5-6.0. They did, however, use an inner filling electrolyte containing 10^{-4} M $\text{K}_2\text{Cr}_2\text{O}_7$, whereas coated wire electrodes avoid inner filling electrolytes altogether.

TABLE 3

Electrical resistance of different sensor configurations

Sensor description ^b	Resistances, GΩ ^a	
	Conducting Medium	
	Nanopure H ₂ O	1×10 ⁻³ M KCl (aq)
Coated wire sensor array	0.0043 ± 0.0018	0.0043 ± 0.0028
Microscale aperture (80 μm)	0.0271 ± 0.0001	0.0017 ± 0.0005
Nanoscale aperture (100 nm)	12.1 ± 1.9	2.0 ± 0.1
Functionalized Microscale aperture	3.6 ± 0.2	4.3 ± 0.7
Functionalized Nanoscale aperture	40.2 ± 5.4	34.9 ± 6.4

^a These resistance values were determined using an Axopatch 200 with a CV-201 headstage, except the resistances for the coated wire sensor array which were estimated using a digital multimeter.

^b Sensors described as micro- and nanoscale apertures were not functionalized with solvent polymeric membrane, whereas sensors described as functionalized micro- and nanoscale apertures were functionalized with 7.8: 62.2: 30.0% (w/w) Aliquat336:2-NPOE:PVC solvent polymeric membrane.