Localized pH Measurements with Scanning Ion-Conductance Microscopy

by Celeste A. Morris

Scanning ion-conductance microscopy (SICM) utilizes a nanopipette to raster-scan a surface with tens-of-nanometer resolution and is best suited for operation in physiological environments. The working principle of SICM relies on the ion current through the nanopipette tip. A nanopipette, with a tip tens to hundreds of nanometers in diameter is filled with an electrolyte solution and houses a Ag/AgCl electrode. When the nanopipette tip is submerged in the bath electrolyte of the sample, an ion current is generated due to a potential applied between the pipette electrode and a reference Ag/AgCl electrode. As the nanopipette is approached to the sample surface, the ion current decreases significantly once the probe-surface distance is on the order of the radius of the nanopipette. This ion current is utilized as the feedback signal to control the probe-surface distance of the nanopipette while the pipette scans the surface.

Our specific research goals are to enhance the chemical information recorded by the nanopipette probe—an effort driven by the desire to increase the auxiliary information and analytical applications of SICM. To accomplish this, we fabricated Au electrodes with polyaniline (PANi) films for H⁺-sensitive measurements (Fig. 1a). Initially, nanopipettes are fabricated by laser-pulling a quartz capillary to form two nanopipettes. A Au electrode is thermally deposited on the surface of the nanopipette. The nanopipette tip is then isolated in a polydimethylsiloxane mask and the remainder of the nanopipette/electrode is insulated with parylene C. When removed from the polydimethylsiloxane mask, the nanopipette contains a Au electrode localized at the tip. For studies of localized pH measurements, polyaniline was electrochemically deposited on the surface of the Au electrode (AuE) (Fig. 1b).

Electropolymerization of PANi films on the Au electrode surface was performed in 0.1 M aniline/1.0 M H₂SO₄ with a Ag/AgCl/3 M KCl reference electrode and Pt counter electrode (Fig. 2a). To calibrate the EMF response of the PANi film to changes in pH, a differential amplifier was utilized to record the potential difference between the PANi film and a Ag/AgCl/3 M KCl reference electrode. Near-Nernstian responses of -56.6 and -56.0 mV/pH were obtained for the microscale and commercial pH probes, respectively (Fig. 2b).

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To study localized differences in pH, we utilized polyimide membranes with 2.5-µm-diameter pores in a diffusion cell. In one experiment, 0.1 M KCl, pH 11.7 was placed in the top chamber while 0.1 M KCl, pH 2.6 was placed in the bottom chamber. As shown in Fig. 3a, localized pH differences were detected by measurement of ΔEMF of the PANi film vs. the reference electrode. When each chamber of the diffusion cell housed 0.1 M KCl, pH 2.6 there was no difference in EMF and thus no difference in pH- as expected (Fig. 3b). The vertical resolution of these probes was studied by repeatedly advancing the probe into pore 1 (Fig. 3a) while recording the ΔEMF. When a pH gradient was present (Fig. 3c) (□), the EMF increased due to the increasing change in pH in the vicinity of the pore and inside the pore. Without a pH gradient (Fig. 3c) (○) a slight decrease in EMF was observed. At present these probes are being applied to make ion-selective measurements of physiological systems.

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**References**