Supporting Information For:

Reversible Electrochemical Detection of Non-Electroactive Polyions

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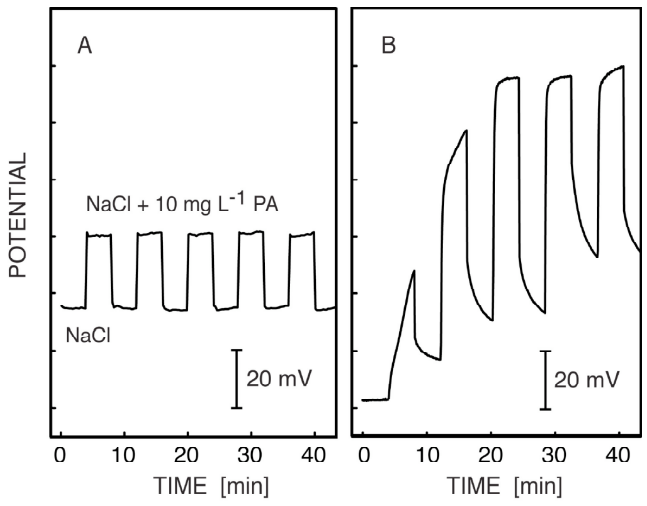

Figure 5. Electrode reproducibilities upon alternating between 0.1 M NaCl (lower potentials) and 0.1 M NaCl containing 10 mg L^{-1} protamine (higher potentials) for (A) the technique proposed here and (B) the protamine ion-selective electrode.

Experimental Details

Reagents. High molecular weight poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (NPOE), tetradodecylammonium chloride (TDDACl), dinonylnaphthalene sulfonic acid (DNNS) as 50% solution in heptane, tetrahydrofuran (THF), and sodium chloride were purchased from Fluka (Milwaukee, WI). Heparin, sodium salt (from bovine intestinal mucosa, 151 units/mg), Protamine sulfate (from herring) and organic solvents were purchased from Sigma (St.Louis, MO). Aqueous solutions were prepared with Nanopure-deionized water (18.2 MOhm cm).

The lipophilic salt DNNS–TDDA was prepared by metathesis of TDDACl and DNNS. In order to purify the salt, a solution of DNNS–TDDA in benzene was washed several times with distilled water. After evaporation of the benzene the product was dissolved in THF and used.

Membrane preparation. The membranes (ca. 200 μm thick) were prepared by solvent casting with THF as a solvent. Membranes prepared for chronopotentiometric experiments contained PVC and o-NPOE 1:2 by mass and 10 wt% of the lipophilic salt DNNS–TDDA. Membranes for the potentiometric experiments were formulated with PVC and NPOE (1:1 by mass) and DNNS (1 wt%) according to the reference.[5]

Electrodes. Circular membranes were cut with a cork borer (6-mm in diameter) from the parent membrane and incorporated into a Philips electrode body (IS-561, Glasbläserei Möller, Zürich, Switzerland). The exposed membrane area was 8 mm^2. The inner solution consisted of 0.1 M NaCl and was in contact with an internal Ag/AgCl electrode. The electrodes were conditioned before experiments in 0.1 M NaCl solution over night. The external reference electrode consisted of a double-junction Ag/AgCl electrode with a 1 M LiOAc bridge electrolyte.

Experimental setup. Voltammetric measurement were conducted in a three-electrode cell system, where the internal Ag/AgCl electrode acted as a working electrode, and the external reference electrode and counter electrode (large surface Pt-grid) were immersed into the sample. The chronopotentiometric experiments were performed with an AFCBP1 bipotentiostat (Pine Inst., Grove City, PA) controlled by a PCI-MIO-16E4 interface board and LabVIEW 5.0 data acquisition Software (National Instruments, Austin, TX) on a Macintosh computer. A solid state opto-isolated module (Crydom MODC5, distributed by Allied Electronics, Fort Worth, TX) was directly connected to the "Mode" button of the potentiostat and to a digital output of the interface board. This circuit creates rapid software-controlled switching between the potentiostatic and galvanostatic modes of the instrument. Sampled potentials were obtained as the average value during the last 10% of each 1 s uptake pulse. Baseline potentials were held at 0 V for 10 s.

Zero-current potentials were measured with a 16-channel EMF monitor (EMF16, Lawson Labs, Phoenixville Pike, PA) versus the same reference electrode.

Potentiometric experiments. The background solution in all experiments was 0.1 M NaCl. Titration of protamine was performed by addition of a 5 g/L stock solution. After the titration the electrodes were immersed into the new portion of background solution in order to return to the baseline potential. Reversibility tests (figure 4) were performed by immersing the electrodes in a 100 mL NaCl solution. After the baseline potential measurement (225 s), 10 mg/L (final concentration) of protamine was added to the solution and the potential was measured again. Subsequently, the electrodes were rinsed with distilled water for 20 s and re-immersed into fresh NaCl solution to restart the measurement cycle.

Chronopotentiometric experiments. Calibration and titration solutions were as described above. For calibration and titration curves, -3uA constant cathodic current pulses were applied. Potentials were found to be stable (standard deviations of potentials were less than 0.7 mV).

Titration of heparin with protamine. Either 0, 200 or 400 μL of Heparin stock solution (7.5 g/L, 5 x 10^{-4} M) was added to the 100 mL 0.1 M NaCl background before the titration. Heparin concentrations were calculated with a molecular mass of 15,000 daltons. Sampled potentials were obtained as the average of the 15 uptake pulses after each addition. Standard deviations of the potentials were less than 1.5 mV.