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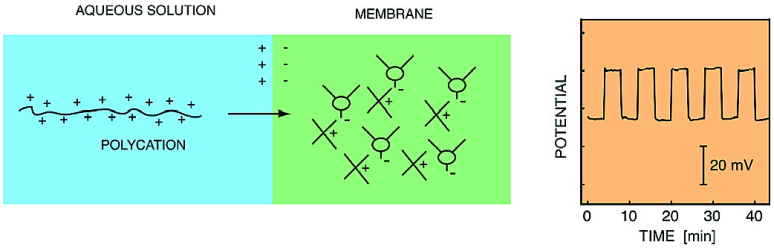

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Reversible Electrochemical Detection of Nonelectroactive Polyions

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We report here on the first reversible electrochemical detection principle for nonelectroactive polyions. Polyionic analytes, such as the widely used anticoagulant heparin (a polysaccharide with an average molecular mass of 15 kDa and an average charge of −70) or its antidote protamine (a polypeptide rich in arginine residues which has a charge of about +20, see Figure 1), may be selectively extracted from aqueous solution into hydrophobic polymeric solvents.[1−4] The driving force for this process is known to rely on a cooperative stabilization of the polyionic species in the organic phase by amphiphilic, electrically charged species[4] such as tridodecylmethylammonium cations (for recognizing heparin)[4] or dinonylnaphthalene sulfonate anions (for protamine).[5] The polyion extraction process is further dictated by the simultaneous ejection of a hydrophilic monovalent ion such as chloride (for heparin) or sodium (for protamine) from the membrane.[2] The selective extraction of such polyions with optimized membrane formulations has been demonstrated in undiluted whole blood samples, making this principle very promising in view of developing sensors for critical care applications.[3,5]

Unfortunately, despite the existence of a selective extraction principle, it has been impossible so far to design reversible polyion sensors. The reason for this lies in the high polyion charge, which renders the equilibrium extraction process effectively independent of the polyion sample concentration. Optical polyion sensors that were reported showed drifting signals and needed prolonged contact with concentrated salt solutions to strip the polyions from the sensing film before the next measurement.[6] Ion-selective electrodes were found to be very useful as endpoint indicators in heparin−protamine titrations, but suffered from the same limitation.[5] In an effort to improve the reversibility of the polyion sensor, a modified membrane composition was proposed to renew the membrane upon increasing the sample pH.[7] These electrodes do not operate under classical equilibrium conditions. Rather, the signal depends on the limited polyion mass transport rate from the sample bulk to the polymeric membrane.[2] This zero current polyion flux is a function of the polyion concentration in the sample and in the membrane, the electrode geometry, and the diffusion layer thicknesses in the aqueous and organic phases.[2] The latter increases with time upon continuous contact with polyion solution and results in significant signal drifts. This nonclassical response mode has also been used for ions of lower valency.[8−10] Note that polyion-selective electrodes cannot be used classically by obeying the Nernst equation, because the high polyion charge leads to an unacceptably small electrode slope. In fact, such systems were developed and proposed as novel reference electrodes.[11]

We report here on the first reversible protamine sensor by placing the two processes mentioned above (mass transport limited polyion extraction during measurement and subsequent back-extraction for reconditioning) under sequential instrumental control. The basic operation of this measuring principle was recently demonstrated with small monovalent ions.[12] Spontaneous polyion extraction is suppressed by working with membranes that possess no ion-exchanger properties. Here, the hydrophilic countercation of dinonylnaphthalene sulfonate was replaced by a tetradodecylammonium ion before membrane fabrication. This lipophilic counterion may no longer spontaneously exchange with protamine. Rather, a current pulse of fixed duration is imposed across the membrane, which results in a cation flux from the sample to the membrane. The resulting chronopotentiometric responses, recorded in stirred solutions, are given in Figure 2, bottom, for a 0.1 M NaCl sample with and without added protamine (PA). The potentials decrease with time, because of the steadily increasing diffusion layer thickness in the membrane. The mechanism for protamine response is thought to be as follows. The applied current pulse forces the extraction of a given amount of sodium ions, and the observed potential is a direct function of the sodium activity ratio on both sides of the sample−membrane interface. If protamine is present in the sample, it will compete with the sodium extraction process,

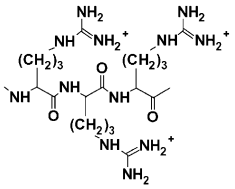

Figure 1. Typical subunit of protamine, a polycationic polypeptide with a charge of about +20. Protamine may be selectively extracted from aqueous solution into hydrophobic polymeric membranes doped with sodium dinonylnaphthalene sulfonate.

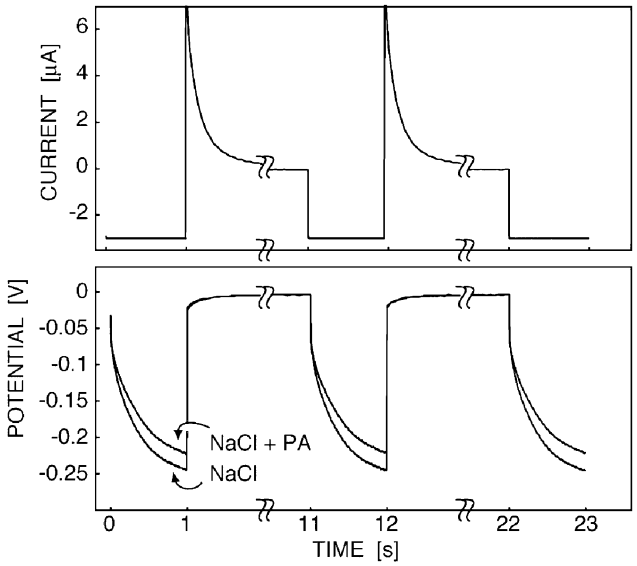

Figure 2. Current−time traces (top) and potential−time traces (bottom) for the pulsed galvanostatic measurement of 10 mg L^{-1} protamine. An applied cathodic current of −3 μA leads to extraction of protamine into the membrane, and the observed potential is significantly different for samples with and without protamine (bottom). The membrane is renewed potentiostatically at 0 V for 10 s before the next current pulse.

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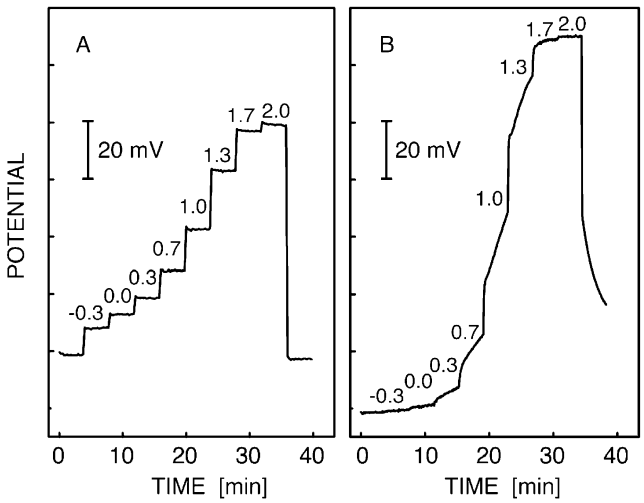

Figure 3. Calibration curves for protamine in 0.1 M NaCl with the technique proposed here (A) and with a traditional protamine ion-selective electrode (B). The strong potential drift observed in (B) originates from the poor control of the diffusion layer thickness on the membrane side. Logarithmic protamine concentrations (mg L^{-1}) are indicated on the traces (first and last samples are 0.1 M NaCl).

thus decreasing the sodium activity in the membrane. The resulting potential will therefore be higher. After the current pulse, polyions are removed from the membrane by imposing a baseline potential at 0 V. Figure 2 shows that the resulting current (top plot) decreases to zero within the potential pulse and, hence, that the previously extracted ions are effectively back-extracted from the membrane. In principle, valuable information about the kinetics of the back-extraction process could be obtained from this decay current, at least at high protamine concentrations. In the case shown in Figure 2, the current is largely dictated by the back-transfer of sodium ions, and no drastic difference was observed in the presence of protamine. Subsequent pulses showed highly repeatable response behavior.

Continuous, reversible detection of protamine becomes possible by repeatedly applying the pulse sequence shown in Figure 2 and by sampling the potential reading at the end of each current pulse. Figure 3 shows the time trace for the resulting protamine calibration curve in 0.1 M NaCl, obtained with this method (A) and with a traditional potentiometric protamine membrane electrode (B) containing sodium dinonylnaphthalene sulfonate. Because of the effective renewal of the electrode surface between measuring pulses, the polyion response in (A) is free of any potential drift, and the signal fully returns to baseline after the calibration run. In contrast, the zero current potentiometric protamine response in (B) shows very strong potential drifts.

Repeated exposure to 0 and 10 mg L^{-1} protamine in 0.1 M NaCl gave an excellent reproducibility of ± 1 mV (see Figure 5 in Supporting Information). The new protamine electrode was also

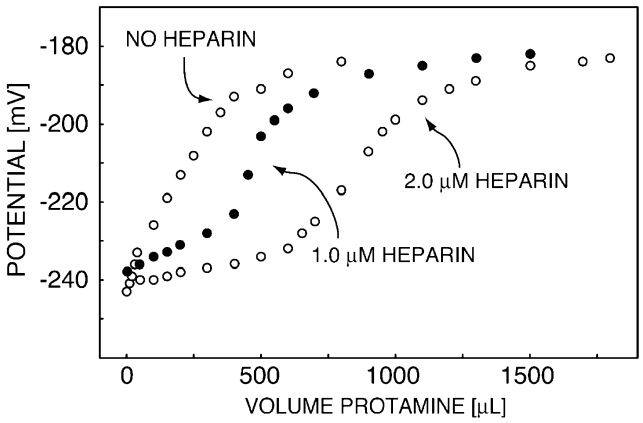

Figure 4. Monitoring the tiration of 100 mL solutions of 0.1 M NaCl containing indicated heparin concentrations with protamine by using the same protamine electrode. Increasing concentrations of heparin shift the endpoint to higher volumes, indicating that the sensor is capable of detecting the polyion binding event.

successfully used to monitor the titration of beef lung heparin with protamine, and the onset of protamine response was found to be proportional to the concentration of heparin, as with ion-selective electrodes (see Figure 4).[5] This is an important indication that the electrode does in fact respond to the polycationic species. Further studies on the mechanism of this new polyion detection principle and its application to whole blood measurements are in progress in our laboratory.

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Supporting Information Available: Additional experiments (reversibility study), membrane preparation, and instrumental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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