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Reversible Detection of Heparin and Other Polyanions by Pulsed Chronopotentiometric Polymer Membrane Electrode

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Abstract

The first fully reversible polymeric membrane-based sensor for the anticoagulant heparin and other polyanions using a pulsed chronopotentiometry (pulstrode) measurement mode is reported. Polymeric membranes containing a lipophilic inert salt of the form R^+R^- (where R^+ and R^- are tridodecylmethylammonium (TDMA⁺) and dinonylnaphthalene sulfonate (DNNS⁻), respectively) are used to suppress unwanted spontaneous ion extractions under zero-current equilibrium conditions. An anodic galvanostatic current pulse applied across the membrane perturbs the equilibrium lipophilic ion distribution within the membrane phase in such a way that concomitantly, anions/polyanions are extracted into the membrane from the sample. The membrane is then subjected to an open-circuit zero current state for a short period and finally a 0 V vs. reference electrode potentiostatic pulse is applied to restore the membrane to its initial full equilibrium condition. Potentials are sampled as average values during the last 10% of the 0.5-s open circuit phase of the measurement cycle. Fully reversible and reproducible EMF responses are observed for heparin, pentosan polysulfate (PPS), chondroitin sulfate (CS) and oversulfated chondroitin sulfate (OSCS), with the magnitude of the potentiometric response proportional to charge density of the polyanions. The sensor provides EMF response related to heparin concentrations in the range of 1–20 U/mL. The responses to variations in heparin levels and toward other polyanions of the pulstrode configuration are analogous to the already established single-use, non-reversible potentiometric polyion sensors based on membranes doped only with the lipophilic anion exchanger TDMA⁺.

Heparin, a highly sulfated anionic polysaccharide with an average molecular weight of ~ 15 kDa and a charge of ~ -70, is widely used as an anticoagulant/antithrombotic agent during clinical procedures such as cardiac/vascular surgery and kidney dialysis. Beyond the need for simple and ideally real-time continuous measurements of heparin levels in blood during such procedures, there is also a desire for detection methods that can monitor the levels of heparin within infusion solutions to avoid dangerous human errors in dosing, especially for pediatric patients. Further, simpler and reliable sensor-based methods that can detect the presence of high charge density polyanion contaminants in biomedical grade heparin preparations (e.g., OSCS) that have caused severe inflammatory responses in patients,¹ would obviate the need for costly NMR or CE screening methods, now mandated by FDA and other government agencies.¹

Potentiometric polymeric membrane-based polyion selective electrodes were introduced in the 1990s,^{2–6} and have been shown to function as irreversible single-use devices for either the direct or titrimetric detection of heparin in clinical samples, including undiluted whole blood.⁶ These sensors are based on selective extraction of the polyions into the organic membrane phase. In the direct detection of heparin and other polyanions, tridodecylmethylammonium chloride (TDMAC) has been used as the membrane active

species, while in the detection of heparin via titration with polycationic protamine, the concentration of the latter polyion is monitored using a membrane doped with dinonylnaphthalene sulfonate (DNNS). Further, it was demonstrated early on that TDMAC-doped polyanion sensing membranes yield EMF response in proportion to the charge density of given polyanions,⁷ and this property was employed recently to rapidly detect the presence of heparin preparations tainted with low levels of OSCS.⁸

Owing to the high charges of the polyions (~ -70 and $\sim +20$, for heparin and protamine, respectively), potentiometric polyion selective electrodes cannot be used in the classical equilibrium potentiometric mode. Rather, potentiometric polyion selective electrodes function under non-classical conditions, where a strong inward flux of the polyions is facilitated by ion-exchange processes at the sample/membrane interface of the polyions with the small hydrophilic ions initially present in the membranes as the counterions of the lipophilic ion-exchangers (i.e., TDMA^+ or DNNS^-). This non-equilibrium polyion extraction results in an analytically useful change in the phase boundary potential at the membrane/sample interface. However, the extraction of polyions into the membrane is an essentially irreversible process since the polyions are stabilized in the membrane phase via cooperative ion-pair formation with the lipophilic ion-exchangers. This leads to potential drift and insensitivity of the sensor response with prolonged contact with polyions due to the uncontrolled growth of the diffusion layer thickness within the membrane phase. This limits potentiometric polyion sensors to primarily single-use applications, unless the membranes are subjected to equilibration with high salt concentrations for extended time periods to force polyion dissociation from the ion-pairs and efflux from the membrane phase.

Bakker's group suggested the first fully reversible and stable pulsed galvanostatic polycation-selective electrode technology for detection of protamine.^{9,10} In that work, an ion-exchanger free membrane was used to suppress spontaneous extraction of cations or polycations. The membrane contained a lipophilic salt of the form R^+R^- , where R^+ and R^- are a lipophilic cation (tetradodecylammonium (TDDA^+)) and a lipophilic anion (DNNS^-), respectively. Under a cathodic current pulse for 1 s, these ions are redistributed in the membrane with the TDDA^+ migrating toward the inner interface in contact with a fixed internal salt solution, and the DNNS^- moving toward the outer portion of the membrane in contact with the sample solution. Hence, the outer surface essentially becomes a cation/polycation exchange interface and this results in concomitant cation/polycation extraction at the membrane/sample interface (and anion extraction at the inner solution/membrane interface). When the sample contains protamine, the measured EMF at the tail end of the current pulse was proportional to the concentration of protamine in the sample. This measured potential included the analytically useful membrane potential superimposed on a large background IR voltage drop, owing the high resistance of the organic membrane phase. When the working membrane electrode was subjected to potentiostatic control (0 V vs. reference) for 10-15 s, the original membrane composition was completely reestablished. Consecutive two pulse sequences of this type yielded remarkably stable and reproducible potentiometric responses to protamine.

While reversible detection of polycations with pulsed chronopotentiometric sensors has been successful for protamine, to our knowledge, a similar configuration has not been reported for detecting heparin or other polyanions. Reversing the direction of the current pulse for the membrane formulation used by Shvarev and Bakker^{9,10} for protamine would not function for heparin sensing owing to the inability of the TDDA^+ anion exchanger to form tight ion-pair complexes with polyanions.⁷ However, we have now found that if the plasticized poly(vinyl chloride) (PVC) membrane is prepared with the $\text{TDMA}^+/\text{DNNS}^-$ salt, very significant and fully reversible EMF response toward polyanions can be observed.

Figure 1A shows the observed calibration curve of heparin sodium salt from porcine intestinal mucosa in a background of 10 mM NaCl buffered at pH 7.4 with 10 mM phosphate buffer (phosphate buffered saline (PBS)). A lower concentration (10 mM NaCl) background was used since chloride has been found to be a greater interference on heparin detection in the present experimental setup compared to prior zero-current potentiometric measurements (see below). Figure 1B shows the potential-time trace for the calibration curve in Fig. 1A. The membrane composition employed for these measurements was 10 wt % TDMA⁺/DNNS⁻ in PVC:NPOE (1:2 by weight), with the membrane mounted in an electrode body (Oesch Sensor Technologies, Sargans, Switzerland) with an inner Ag/AgCl electrode and an inner solution of 10 mM NaCl in PBS, pH 7.4. A double-junction Ag/AgCl reference electrode (with saturated KCl as the inner solution and a 1 M lithium acetate bridge electrolyte) and coiled Pt wire (counter electrode) were placed in the test sample solution. Control and monitoring of the current/potential of the working polyanion sensor was accomplished via an AFCBP1 bipotentiostat (Pine Instruments, Grove City, PA), controlled by a potentiostatic/galvanostatic switch and Labview software, as described previously.¹¹ The pulse sequence used was a 1-s 20 μ A galvanostatic anodic current pulse, a 0.5-s zero current pulse (open circuit) and a 15-s potentiostatic potential pulse (0 V vs. reference). The average of EMF values of sampled potentials obtained during the last 10% of each 0.5-s zero current period were used to report the results shown in Figure 1. This triple pulse measurement mode was utilized by other researchers previously for the detections of small ions using the pulstrode technology.¹²⁻¹⁴ It should be noted that unlike the protamine pulstrode reported by Shvarev and Bakker,^{9,10} where voltages were recorded on top of the background IR drop during the galvanostatic pulse, in the present method for polyanion detection, it has been found that measurement of the cell voltages during the 0.5-s open circuit period yielded much more reproducible potentiometric behavior. The concentration of heparin in the test solution was varied by addition from a 2 mg/mL heparin stock solution (note: heparin preparation used had 180 U/mg of heparin). As clearly shown, a stable and reversible response toward heparin is obtained (Fig. 1B). The potential was measured in a background solution at the end of the calibration and the value returned to the initial baseline value, with an average change in the baseline potential of ≤ 2 mV in over an hour of measurements, confirming the reversibility of the heparin detection. The high sensitivity response region is in the vicinity of the therapeutic heparin concentration. Note that this high sensitivity response range can likely be adjusted by a careful control of the membrane composition (polymer: plasticizer ratio).¹⁵

One limitation of the classical single-use potentiometric polyanion sensors is that the potential drifts, especially in the lower concentrations of the polyanion, where, in theory, only a pseudo-steady state EMF responses can be realized when the rate of mass transfer of the polyion to the surface of the membrane is matched by the rate of diffusion of the ion-pairs into the bulk of the membrane. However, since diffusion layer thicknesses within the polymeric membrane cannot be controlled, the potential will always continuously drift slowly toward an equilibrium interfacial value, when all the chloride ions at the outermost edge of the TDMAC doped membrane are exchanged for polyanions. In contrast, for the pulstrode configuration reported here, the reproducibility of the potential measurement at lower concentrations of heparin (where membrane/sample phase boundary equilibrium potential responses are not expected) is quite good. Figure 2 shows the pulsed chronopotentiometric responses for alternate measurements of the background buffer solution (no polyanion) and solutions containing heparin at different levels (40 μ g/mL and 400 μ g/mL). Reproducible potentiometric responses were obtained in all the tested solutions including in the lower concentration of heparin (40 μ g/mL = 7.2 U/mL) with Δ EMF = -37.8 ± 0.8 mV for $n=5$ measurements, where drifting and non-reproducible EMF responses are expected under the conventional potentiometric measurement mode with membranes doped only with TDMAC.¹⁵ Note that the potential change observed for the 400 μ g/mL of heparin

($\Delta\text{EMF} = -79.9 \pm 1.1 \text{ mV}$; $n=5$) is an equilibrium potential change (very high heparin level; 72 U/mL), where the membrane/sample interface is fully exchanged with heparin as the counteranion to the TDMA^+ species. It is important to note that for the dynamic traces shown in Figure 2 actually include data points for the measurement of 11 separate voltages recorded (at the end of the 0.5 s open circuit) sequentially for eleven 16.5 s cycles of the pulse sequence when the sensor was exposed to either the given heparin sample or background buffer solution

We further examined whether the pulsed chronopotentiometric polyanion sensor also exhibits response to different polyanions based on their respective charge densities. Figure 3 shows the EMF responses obtained toward four different polyions of varying charge density: CS, heparin, OSCS and PPS, all measured at the same high concentration (400 $\mu\text{g/mL}$ in PBS), where a rapid establishment of equilibrium potential at the membrane/sample phase boundary should be achieved. Well-resolved equilibrium potential responses are found, with the largest response observed for PPS. This is completely consistent with observations made previously for TDMAC-based membranes used for irreversible polyanion sensing.⁷ This data also suggests that it should be possible to detect the presence of OSCS in heparin preparations, provided that the starting concentration of total polyanions in the test solution is high enough so that the response to the higher charge density OSCS impurity will govern the EMF response at the membrane/sample interface (see ref. 8 for details)

In summary, a very promising fully reversible pulsed chronopotentiometric heparin/polyanion sensor has been demonstrated. Highly reproducible EMF responses are observed not only for heparin, but for CS, OSCS and PPS as well. The only limitation of the currently tested membrane composition/pulse sequence combination is that the EMF responses toward heparin in the presence of 0.1 M chloride is limited to ca. -27 mV from baseline values (see Fig. S1 in Supplemental Information). This is approximately half of that of the equilibrium potential changes found for heparin when using the conventional TDMAC-based membrane and the conventional zero-current potentiometric measurement mode. Hence, it appears that chloride ions interfere to a greater extent when using the pulsed chronopotentiometric measurement mode. The origin of this difference is not yet clear. Further, optimization of the membrane composition and pulse parameters to obtain greater potential changes for heparin (and other polyanions) in the presence of 0.1 M chloride (desired for heparin measurement in undiluted blood), as well as demonstrating the detection of OSCS contaminants in heparin preparations, are currently underway in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Calibration curve toward heparin in 10 mM NaCl buffered at pH 7.4 with 10 mM PBS; each data point in the curve is an average of 16 potentials sampled at intervals of 16.5 s (i.e., full measurement cycle) when the sensor was exposed to the given buffer solution with given level of polyanion and (B) corresponding potential-time trace for the calibration curve shown in (A).

Figure 2.

Potential-time behavior of polyanion sensor EMF response obtained by alternating measurements in a background solution (without heparin) and in the indicated heparin solutions. Each discrete section in the curve is composed of 11 voltage data points sampled at intervals of 16.5 s.

Figure 3.

Potential-time trace of pulstrode polyanion sensor EMF response upon alternate measurements in a background solution and in 400 $\mu\text{g/mL}$ solutions of the indicated polyanions possessing different charge densities. Each discrete section (background solution and sample solution) in the dynamic response plot is composed of 16 voltage data points sampled at intervals of 16.5 s. Concentration used is at level where rapid full equilibrium potentiometric response at phase-boundary of membrane/sample is expected.