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Response Mechanism of Polymer Membrane-Based Potentiometric Polyion Sensors

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The potentiometric response mechanism of a previously reported polymer membrane-based electrode sensitive to the polyanion heparin is established. Based on transport and extraction studies, the heparin response is attributed to a nonequilibrium change in the phase boundary potential at the sample/membrane interface. While true equilibrium polyion response, obtained for low heparin concentrations only after very long equilibration times (>20 h), yields the expected Nernstian response slope of <1 mV/decade, the observed large and reproducible EMF response to clinically relevant heparin concentrations ($\sim 10^{-7}$ M) during typical measurement periods (2–5 min) is ascribed to a steady-state kinetic process defined by the flux of the polyion both to the surface and into the bulk of the polymer membrane. A model describing this nonequilibrium response is presented. With this model, the uniqueness of the polymer membrane composition (e.g., very low plasticizer content, strictly controlled cationic site concentration, etc.) required to achieve analytically useful heparin response becomes clear. Practical working conditions and limitations of the sensor are discussed. To support the generality of the steady-state model proposed, corresponding EMF response data for a newly developed membrane electrode sensitive to a polycationic protein (protamine) are also presented. It is shown that the protamine-responsive membrane electrode appears to operate via the exact same kinetic mechanism as the heparin sensing system.

Although a large number of polymer membrane-based potentiometric sensors have been applied successfully in clinical chemistry laboratories for the measurement of small ions such as H^+, K^+, Na^+, Ca^{2+}, Cl^-, etc., in undiluted whole blood,[1,2] there are no analogous devices available for direct sensing of important macromolecular ionic species. Recently,[3,4] a specially formulated PVC membrane electrode has been proposed for the detection of heparin, a polyanion (average molecular weight 15 000) which is widely used as an anticoagulant during a variety of clinical procedures (e.g., open heart surgery, kidney dialysis, etc.). Initial studies have clearly demonstrated the utility of this sensor as an indicator electrode to probe the binding interaction of heparin with a variety of biologically important macromolecular species (e.g., low-density lipoprotein, protamine, DNase, etc.) via a classical potentiometric titration method.[5] Additional preliminary studies have suggested that the electrode may also serve as a simple analytical device to monitor relative heparin levels in undiluted whole blood.[4]

The optimum polymer membrane for sensing low levels of heparin is composed of 66 wt % polymer matrix (poly(vinyl chloride); PVC), 32.5 wt % plasticizer (dioctyl sebacate; DOS), and 1.5 wt % ion exchanger (tridodecylmethylammonium chloride; TDMACl), with $TDMA^+$ serving as anion-exchange sites within the organic membrane phase. This membrane composition is unique with respect to its low plasticizer content relative to conventional PVC-based ion-selective electrodes.[6] It has been found previously that this optimized membrane electrode exhibits >40 mV/decade potential change for heparin concentrations in the range of 0.04–0.4 μM (or 0.1–1.0 unit/mL; the conversion factor for heparin concentration is 1 unit/mL = 4×10^{-7} M, assuming an average MW = 15 000) in a background electrolyte solution of 0.12 M NaCl. For such experiments the response times to reach an "apparent" equilibrium EMF change range from 2 to 5 min, depending on the heparin concentration in the test solution (see Figure 4 in ref 3). It is known that lipophilic quaternary ammonium species can bind heparin tightly,[7] suggesting that the $TDMA^+$ in the membrane can selectively extract heparin from the aqueous sample into the organic membrane phase. However, simple *equilibrium* extraction of polyanionic heparin cannot account for the large potentiometric response slope observed. Indeed, the existing theory of ion-selective electrodes would predict <1-mV equilibrium potential change for such a concentration step, since the charge on heparin averages −70/molecule.[8] In addition, other response characteristics of the electrode are not typical for conventional polymer membrane-type ion-selective electrodes; e.g., cylindrical configuration coated wire electrodes exhibit a lower detection limit than planar electrode configurations, and stirring vs nonstirring of the sample solution has a profound influence on the sensor's heparin response (see below).

In this paper, the response mechanism of the heparin sensor is elucidated. It is anticipated that such mechanistic information will lay the foundation for the development of analogous membrane-based devices for sensing other important polyions, either by electrochemical or optical transduction. Indeed, an analogous potentiometric sensor has recently been proposed for sensing protamine,[9] a very important polycationic protein widely used in medicine to neutralize the anticoagulant effect

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(1) Yim, H.-S.; Kibbey, C. E.; Ma, S.-C.; Kliza, D. M.; Liu, D.; Park, S.-B.; Torre, C. E.; Meyerhoff, M. E. *Biosens. Bioelectron.* **1993**, *8*, 1.
(2) Oesch, U.; Ammann, D.; Simon, W. *Clin. Chem.* **1986**, *32*, 1448.
(3) Ma, S.-C.; Meyerhoff, M. E.; Yang, V. C. *Anal. Chem.* **1992**, *64*, 694.
(4) Ma, S.-C.; Yang, V. C., Fu, B.; Meyerhoff, M. E. *Anal. Chem.* **1993**, *65*, 2078.
(5) Jong, H. Y.; Ma, S.-C.; Yang, V. C.; Fu, B.; Meyerhoff, M. E. *Electroanalysis* **1993**, *5*, 719.
(6) Craggs, A.; Moody, G. J.; Thomas, J. D. J. *J. Chem. Educ.* **1974**, *51*, 541.
(7) Grant, D.; Long, W. F.; Williamson, F. B. *Biochem. J.* **1992**, *285*, 477.
(8) Casu, B. In *Heparin and Related Polysaccharides, Structure and Activities*; Annals of the New York Academy of Science 556; Ofosu, F. A., et. al., Eds.; The New York Academy of Sciences: New York, 1989.
(9) Jong, H. Y.; Yang, V. C.; Meyerhoff, M. E., *Anal. Biochem.*, submitted.

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of heparin (i.e., to reduce the risk of bleeding after heparin therapy). The protamine sensor is prepared with a polymer membrane quite similar in composition to the heparin sensing membrane (low plasticizer content), except that the anion exchanger, $TDMA^+$, is replaced with an appropriate lipophilic cation exchanger, specifically tetrakis(*p*-chlorophenyl)borate.[9]

In the case of the heparin sensing system, we have postulated previously that the observed potentiometric response is due to the interaction of negatively charged heparin with positively charged $TDMA^+$ species on the surface of the membrane. New experimental evidence reported herein now demonstrates clearly that heparin can actually be extracted into the bulk of the membrane and that the potentiometric response is due to a classical phase boundary charge separation that exists for any ion/polyion that can distribute between both the aqueous and organic phases. However, via long-term stability and bulk extraction experiments, it is found that the sensor's membrane is not at full equilibrium when the potentiometric response to heparin is typically recorded (after 2–5 min). Rather, because of the low concentrations of heparin and its slow diffusion in the aqueous test solution, a nonequilibrium steady-state potential develops at the membrane/sample interface during this time frame. An appropriate quasi-steady-state model is developed here that fully explains such a process. The resulting nonthermodynamic yet highly reproducible responses can be used for analytical detection of heparin in its clinically relevant concentration range. The thermodynamic response behavior of the electrode is also examined, both in theory and experimentally. In addition, corresponding kinetic and equilibrium EMF data for the recently developed protamine sensor are presented to demonstrate the general validity of the proposed mechanism for explaining potentiometric polyion response of all appropriately formulated ion-exchange-type polymeric membranes.

EXPERIMENTAL SECTION

Reagents. Tridodecylmethylammonium chloride (TDMAC), potassium tetrakis(*p*-chlorophenyl)borate (KTpClPB), dioctyl sebacate (DOS), *o*-nitrophenyl octyl ether (*o*-NPOE), high molecular weight poly(vinyl chloride) (PVC), and tetrahydrofuran (THF) were purchased from Fluka Chemika-Biochemika (Ronkonkoma, NY). Sodium heparin powder (from porcine intestine mucosa, 169 units/mg) was a product of Hepar Industries, Inc. (Franklin, OH). Fluorescein-labeled heparin was purchased from PolySciences, Inc. (Warrington, PA). Protamine sulfate salt and toluidine blue O dye were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade or better. Aqueous solutions were prepared with doubly deionized water.

Membranes Preparation and EMF Measurements. All experiments were performed at ambient temperature (22 °C). The heparin and protamine sensing membranes with different compositions were cast and assembled into a conventional electrode design as described elsewhere.[4,9] Unless otherwise specified, a solution of 15 mM NaCl was used as the internal filling solution for the assembled electrodes. The electrode potential was measured vs a double-junction Ag/AgCl reference electrode from Fisher Scientific (Itasca, IL). Potentials were measured via either a Fisher Scientific Accument pH meter (Model 25) or a Macintosh IIcx computer with a NB-MIO-16X analog/digital input/output board (National Instruments, Austin, TX) and a custom-built electrode interface module controlled by LabView 2 software (National Instruments) as described elsewhere.[10] To record the dynamic response profile of the electrodes, a Fisher Recordall Series 5000 chart recorder was connected to the pH meter. The test solution was constantly stirred with a small magnetic stirrer. To generate different concentrations of heparin or protamine, aliquots of concentrated heparin or protamine stock solution were added to proper background electrolyte (e.g., 0.12 M NaCl) solutions.

Preparation of Coated Wire Electrodes. Copper wires with a length of 6.0 cm and a diameter of 1.3 mm were used for preparing coated wire electrodes. Pretreatment of the wires involved washing with acetone, soaking in acid solution composed of concentrated nitric acid:water = 1:10 for 1 h, extensive rinsing with doubly deionized water, and drying at room temperature. The copper wires thus treated were dip-coated with the respective heparin and protamine membrane casting solutions (200 mg of total ingredients dissolved in 3.0 mL of THF solution). The length of the membrane casting was ~4.0 cm. After dip-coating 10 times, the electrodes were then kept in an oven at 45–50 °C for 6 h. This procedure helps improve the stability and reproducibility of the electrode potential.[11] The electrodes with polymer membrane coatings were soaked in doubly deionized water for ~5 min before use.

Transport Experiments. An in-house dialysis cell was used to carry out the dialysis experiment. A heparin sensing membrane with a thickness of 0.2 mm was placed between the two chambers of the dialysis cell. Two 1.5-mL solutions of 100 units/mL heparin and 2 M NaCl, respectively, were placed into the feed and recipient chambers of the cell. Small magnetic stir bars were used to mix the two solutions. During the dialysis process, fresh heparin was added to the feed solution chamber to compensate for the loss of heparin into the membrane and/or the recipient NaCl solution. After extensive dialysis, the recipient NaCl solution was placed in a dialysis tubing (from Spectrum Medical Industries, Inc., Houston, TX) with a molecular weight cutoff of 3500 and dialyzed against 15 mM NaCl solution for 24 h to remove the high concentration of NaCl electrolyte, which would otherwise interfere with the colorimetric measurement of heparin. The heparin level in the resulting solution was determined spectrophotometrically with toluidine blue O solution to ascertain whether heparin actually is transported through the membrane.[12] An analogous experiment was carried out with fluorescein-labeled heparin. To this end, the fluorescence of the recipient solution was detected to prove the transport of heparin. The fluorescein-labeled heparin was measured with a RF-5000 Schimadzu spectrofluorophotometer using excitation and emission wavelengths of 492 and 513 nm, respectively.

THEORETICAL CONSIDERATIONS

On the basis of preliminary experimental evidence suggesting that heparin may be extracted into the membrane

(10) Telting, M.; Collison, M. E.; Meyerhoff, M. E. *Anal. Chem.* **1994**, *66*, 576.
(11) Covington, A. K.; Whalley, P. D. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 1209.
(12) Jaques, L. B.; Bell, H. J. In *Determination of Heparin*; Glick, D., Ed.; Methods of Biochemical Analysis 7; Interscience Publishers, Inc.: New York, 1959.

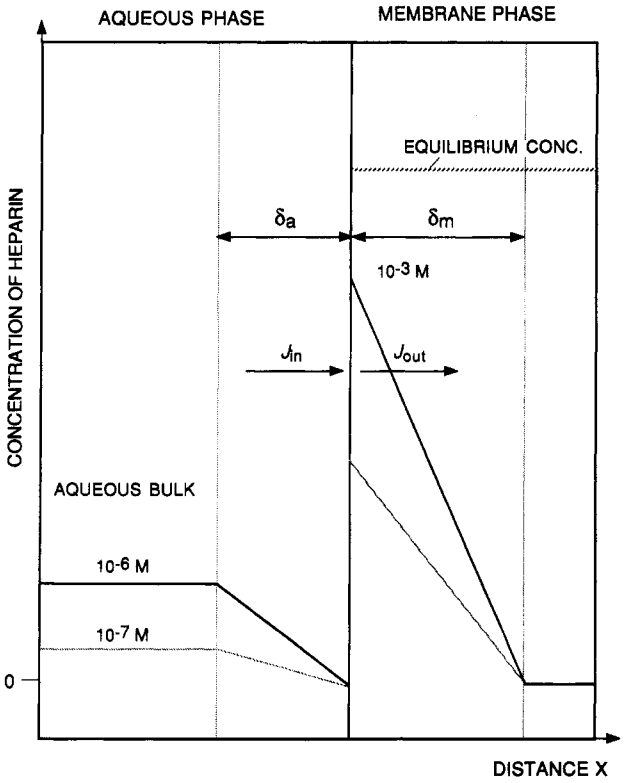

Figure 1. Schematic diagram of the quasi-steady-state model for the potentiometric response of polyanion heparin or polycation protamine sensors: δ_a, δ_m, diffusion layer thickness in the aqueous and membrane phases, respectively; J_{in}, J_{out}, heparin flux from the sample into the interface and from the interface into the bulk of the membrane, respectively.

phase by $TDMA^+$, the observed potentiometric response toward heparin can be ascribed to a change in the phase boundary potential at the membrane/sample interface. There are two possible cases to consider: (a) for high sample concentrations of heparin, or with long equilibrating/conditioning times in the presence of heparin, the initial chloride counterions of the lipophilic anion exchanger (TDMAC) in the membrane phase will be fully displaced by anionic heparin, and the electrode will respond, as classically expected, to heparin in the sample solution with a very low slope (so-called equilibrium or Nernstian response); or (b) for very low concentrations of heparin, and under the assumption that the membrane has been preconditioned in a solution containing only a chloride salt, heparin will not be able to fully displace the initial chloride counterions from the membrane boundary layer within the typical time frame used to measure the cell potentials (e.g., 2–5 min). Instead, the diffusion of heparin from the bulk of the sample solution into the surface membrane layer, and from there, with a much smaller diffusion coefficient, into the bulk of the polymer membrane phase, will lead to a quasi-steady-state nonequilibrium accumulation of heparin at the interface (see Figure 1). While the maximum signal of the sensor will be governed by the equilibrium response, the actual useful working range of the sensor may be quantified via this quasi-steady-state model. In this section, the EMF response function for these two possible cases will be derived mathematically, using heparin as the analyte anion and chloride as the original background counteranion in the sample solution and membrane phase. It should be noted that the equations derived below will also be valid for other important polyionic species (e.g., protamine) after appropriate modification of the respective charge number and sign of the detected polyion as well as the fixed ion-exchange sites in the membrane phase.

Equilibrium Response. To predict, theoretically, the thermodynamic response of the heparin sensor, the following assumptions are made:

(1) The membrane potential is described only by the phase boundary potentials at the inner and outer membrane/solution interfaces. However, since the inner phase boundary potential is fixed by the given internal electrolyte solution, this potential and the diffusion potential within the membrane phase are assumed to be constant.

(2) Activity coefficients in the membrane phase will be assumed to be unity for all ions; therefore, concentrations rather than activities will be used for all species considered to be in the organic membrane phase.

(3) The composition of the inner filling solution of the electrode is constant.

(4) Electroneutrality conditions hold for all phases.

(5) Heparin is assumed to be pure and to have a fixed number of charges.

Assumption 1 is a reasonable approximation and has been shown to hold for liquid membrane-type membrane electrodes.[13] The activity coefficients as mentioned in (2) may be influenced by the membrane composition change such as water uptake, but in a symmetrical arrangement (sensor with internal electrolyte), this effect should take place at the two interfaces equally and the overall influence should be negligible. Assumption 3 will hold under most measurement conditions specified in this paper. Although heparin will eventually migrate to the other side of the membrane, it will not be stripped out into the internal filling solution when 15 mM NaCl is used as the internal electrolyte (see below). Electroneutrality condition 4 is justified, in both aqueous and membrane phases. Assumption 5 is purely for simplicity and certainly not true since it is well-known that commercially available heparin is a highly polydisperse mixture.[8] However, all heparin structures present are polyions, and their general behavior is expected to be similar, albeit not identical.

For a membrane containing a dissociated anion exchanger such as TDMAC, the extraction of heparin into the membrane phase (assuming no strong ion pair formation) may be formulated by the following anion-exchange mechanism:

$$z\mathrm{Cl}_{org}^{-} + \mathrm{Hep}_{aq}^{z-} = z\mathrm{Cl}_{aq}^{-} + \mathrm{Hep}_{org}^{z-} \quad (1)$$

with the corresponding anion-exchange constant:

$$K_{exch} = \frac{[\mathrm{Hep}^{z-}]}{a_{Hep}}\left(\frac{a_{Cl}}{[Cl^-]}\right)^z = \frac{R_T - [Cl^-]}{z a_{Hep}}\left(\frac{a_{Cl}}{[Cl^-]}\right)^z \quad (2)$$

where, $a_{species}$ and brackets ([species]) refer to the activities and concentrations of the corresponding species in the aqueous sample solution and organic membrane phase, respectively; R_T represents the total concentration of ion-exchanger sites, $TDMA^+$, in the membrane phase; and z is the charge of heparin. While the actual ion-exchange process is, in practice, also influenced by ion pair formation in the membrane, this

(13) Cosofret, V. V.; Lindner, E.; Buck, R. P.; Kusy, R. P.; Whitley, J. Q. *Electroanalysis* **1993**, *5*, 725.

effect may shift the extraction equilibrium but does not change the general interpretation of the approach presented here.

Since z is high (on the average, $z = 70$),[8] according to eq 2, even a large heparin sample concentration change will have little influence on the equilibrium concentration of heparin in the membrane. However, a relatively small change in the background anion activity in solution, a_{Cl}, may have a very significant effect on the partitioning of heparin between the two phases. Accordingly, there will be a narrow activity range of chloride over which the concentration of heparin in the membrane changes dramatically. This effect is well-known and has been used previously in liquid/liquid extractions of polyelectrolytes.[14] In the current and previous work with the heparin sensor, this concept is utilized to recondition the electrode membrane after contact with heparinized samples (to return to baseline potential) and for interpreting results of the membrane transport experiments described in the Experimental Section.

The phase boundary potential of a polymer membrane electrode toward chloride as the analyte anion may be described as follows:[15]

$$E_{Cl} = E^0 - \frac{RT}{F} \ln \frac{a_{Cl}}{[Cl^-]} \quad (3)$$

By inserting eq 2 into eq 3, the phase boundary potential as a function of the heparin sample activity is obtained:

$$E_{Hep} = E^0 - \frac{RT}{zF} \ln K_{exch} - \frac{RT}{zF} \ln \frac{a_{Hep}}{[Hep^{z-}]} \quad (4)$$

According to eq 4, the slope of the electrode function will be near zero for a membrane fully saturated with heparin ($[Hep^{z-}] = R_T/z$). In practice, however, a potential change is measured after addition of heparin to a sample containing a constant chloride background (e.g., 0.12 M NaCl).[4,5] Under the assumption that a large amount of heparin is extracted, and chloride is completely displaced from the organic phase boundary layer, the expected equilibrium potential change may be formulated by combining eqs 3 and 4 after insertion of the respective electroneutrality conditions:

$$\Delta EMF = \frac{RT}{F} \ln \frac{a_{Cl}}{R_T} - \frac{RT}{zF} \ln \left(\frac{zK_{exch}}{R_T} a_{Hep} \right) \quad (5)$$

To measure this potential change, it is important that the membrane phase does not contain heparin prior to the experiment. Accordingly, a large and reproducible response toward heparin may be obtained in going from a sample containing the background electrolyte alone to one containing heparin. The magnitude of this response will be determined by the ion-exchange constant (eq 2), by the amount of lipophilic cationic sites in the membrane, and by the chloride activity in the sample. Because of its high valency, the actual amount of heparin in the sample is not expected to influence the final equilibrium response significantly. However, under the working conditions described previously[3-5] (potentials are recorded 2–5 min after samples are added and the heparin concentration is typically less than 4×10^{-7} M), heparin is not yet able to saturate the cationic sites in the organic membrane phase completely, and consequently, an apparent super-Nernstian response slope is obtained. This response is kinetic in nature and is modeled below.

Quasi-Steady-State Response. The quasi-steady-state model relies on the following assumptions:

(1) Under the conditions chosen, heparin completely displaces chloride from the membrane phase when equilibrium is reached.

(2) The rate of the ion-exchange reaction, which takes place in the phase boundary region, is rapid.

(3) Diffusion coefficients are constant for a given phase, and the diffusion coefficients for small ions are much larger than for polyions.

(4) The thickness of the aqueous stagnant layer adjacent to the membrane surface is uniform and constant for a given stirring rate.

Assumption 1 is justified since it has been found that the equilibrium potentials for all sample heparin concentrations of interest are nearly equal, indicating that the original chloride counterion is fully displaced by heparin (i.e., high equilibrium ion-exchange constant for heparin extraction into the membrane phase). For the present evaluation, assumptions 2 and 3 usually hold for ion-exchange-type electrodes.[16] Assumption 4 may be satisfactorily fulfilled in most cases, although the stirring rate and the sample flow were not carefully controlled under the experimental conditions used here. Nonetheless, on the basis of the reproducibility of EMF response data, small changes in convection do not appear to dramatically influence the diffusion layer thickness of the unstirred layer.

We consider a membrane containing an anion exchanger (TDMAC) imposed between a sample solution containing Hep^{z-} and an internal NaCl solution. The transport of ions into the ion-exchange membrane can be controlled or limited by either the membrane or the solution adjacent to the membrane (stagnant Nernst diffusion layer) (see Figure 1). It is well-known that the transport of co-ions into an ion-exchange membrane is almost always controlled by the membrane diffusion.[17] However, for the counterion transport, due to the high ion-exchange constant, the diffusion in the stagnant layer may become the rate-limiting step, depending not only on the membrane but also on conditions within the external solution (concentration, stirring rate, etc.). Helfferich[17] proposed the following equation to evaluate these two processes:

$$L = D_a c_a \delta_m / D_m c_m \delta_a \quad (6)$$

When $L < 2$, stagnant layer diffusion is the rate-limiting step. Here, δ_m and δ_a are the organic and the aqueous stagnant layer thicknesses, respectively, D_a and D_m are the diffusion coefficients in the solution and membrane phases, and c_a and c_m are the concentrations of the ion in the bulk solution and the outermost region of the membrane phase. To evaluate the rate-limiting step, the membrane thickness d and the limiting equilibrium concentration $c_{m,eq}$ are used here for δ_m and c_m, respectively. If the flux in the stagnant layer is indeed the rate-limiting step, a quasi-steady-state diffusion may be observed at the interface.[18]

(14) Chang, R. *Physical Chemistry with Applications to Biological Systems*; MacMillan: New York, 1981.
(15) Morf, W. E. *The Principles of Ion-Selective Electrode and of Membrane Transport*; Elsevier Science Publishing Co.: New York, 1981.
(16) Morf, W. E.; Lindner, E.; Simon, W. *Anal. Chem.* **1975**, *47*, 1596.
(17) Helfferich, F. *Ion Exchange*; McGraw-Hill Book Co., Inc.: New York, 1962.

The diffusion in a typical planar membrane electrode configuration may be formulated as one dimensional, and thus the flux is described as[19]

$$J = -D\frac{dc}{dx} \tag{7}$$

where x is the coordinate normal to the membrane surface, D is the diffusion coefficient, and c is the concentration. This flux equation holds for the diffusion processes in both the aqueous and membrane phases. For simplicity, we assume that the concentration profiles in each of the two segments are linear, and integration of the flux equations yields

$$J_{in} = D_a\frac{c_{Hep,bulk} - c_{Hep,pb}}{\delta_a} \tag{8}$$

and

$$J_{out} = D_m\frac{[Hep^{z-}] - [Hep^{z-}]_{bulk}}{\delta_m} \tag{9}$$

where J_{in} and J_{out} denote the flux into and out of the membrane surface layer, respectively; $c_{Hep,pb}$ and $[Hep^{z-}]_{bulk}$ represent the concentration of heparin in the boundary layer of the aqueous phase adjacent to the membrane and in the bulk of the membrane, respectively (the first value approaches zero for a high exchange constant); and δ_a and δ_m signify the respective diffusion layer thicknesses (see Figure 1). Equation 9 can be further simplified by assuming that the concentration of heparin in the bulk of the membrane is initially zero. Therefore, at quasi steady state, both fluxes are equal, and for $c_{Hep,pb} \ll c_{Hep,bulk}$ and $[Hep^{z-}]_{bulk} \ll [Hep^{z-}]$, the combination of eqs 8 and 9 gives

$$J_{in} = J_{out} = \frac{D_a c_{Hep,bulk}}{\delta_a} = \frac{D_m[Hep^{z-}]}{\delta_m} \tag{10}$$

and after rearranging

$$[Hep^{z-}] = \frac{D_a\delta_m}{D_m\delta_a}c_{Hep,bulk} \tag{11}$$

In accordance with electroneutrality conditions, the concentration of the original chloride counterion in the membrane phase boundary layer at steady state when heparin is present in the sample solution will be given by

$$[Cl^-] = R_T^+ - z\frac{D_a\delta_m}{D_m\delta_a}c_{Hep,bulk} \tag{12}$$

Interestingly, Hulanicki et al.[20] employed a similar model to quantify the steady-state response of ion-exchange-type membrane electrodes toward monovalent anions. However, they used the empirical Nicolsky–Eisenman equation, which has been shown to be invalid for ions of different valency.[15] The present approach therefore involves the more fundamental phase boundary potential. Accordingly, substitution of eq 12 into eq 3 yields the potential for a sample after contact with heparin:

$$E_{Cl} = E^0 - \frac{RT}{F}\ln\left(\frac{a_{Cl}}{R_T^+ - z(D_a\delta_m/D_m\delta_a)c_{Hep,bulk}}\right) \tag{13}$$

Again, the measured potential change in going from a sample containing chloride only to the same sample after the addition of heparin (at concentration $c_{Hep,bulk}$) may described by the combination of eqs 3 and 13:

$$\Delta EMF = \frac{RT}{F}\ln\left(1 - \frac{z}{R_T^+}\frac{D_a\delta_m}{D_m\delta_a}c_{Hep,bulk}\right) \tag{14}$$

This equation holds if the chloride activity in the aqueous phase boundary layer adjacent to the membrane is equal to the concentration in the bulk sample solution, the membrane does not contain any heparin prior to the measurement, and most importantly, heparin does not displace a large fraction of the total chloride from the outer boundary layer of the membrane phase. In the latter case, the bracketed term in eq 14 approaches 0, and the EMF response function is defined by eq 5, the equilibrium response.

The main factors influencing the quasi-steady-state response are the concentration of heparin in the sample, the site concentration in the membrane, the ratios of the two diffusion coefficients, and the thicknesses of the two stagnant layers. Many of these parameters may be controlled, e.g., by stirring the sample or changing the viscosity and composition of the membrane phase (e.g., via weight percent plasticizer, choice of polymer, etc.). However, the quasi-steady-state response gives no information about the overall maximum response of the sensor, and for this purpose, high concentrations of heparin must be measured for a sufficiently long time period in order to reach a true chemical equilibrium at the membrane/sample interface.

RESULTS AND DISCUSSION

Dynamic Response vs Equilibrium Response. We have reported previously that plasticized PVC membranes, doped with TDMAC, exhibit a large and reproducible potentiometric response toward clinically relevant heparin concentrations. However, the magnitude of the response, particularly toward low levels of heparin, is highly dependent on the polymer membrane composition, not only with respect to the specific quaternary ammonium ion exchanger, but also the fluidity of the polymer film. Specifically, only membranes prepared with 66 wt % PVC, 32.5 wt % DOS, and 1.5 wt % TDMAC exhibit a significant potentiometric response to submicromolar concentrations of heparin in both 0.12 M NaCl solutions and whole blood samples.[4]

To examine whether the observed potential changes for the optimal membrane composition depended on the measurement period, EMF values for the heparin sensor were taken after 5-min (as reported in refs 4 and 5) and after 24-h measurement periods for varying levels of heparin in a 0.12 M NaCl solution. As shown in Figure 2B, we repeatedly find that recording EMF values after 5 min (with stirring), where the potential exhibits an "apparent" equilibrium response, yields bioanalytically useful calibration curves with reproducible potentiometric heparin response over the range of 0.1–

(18) Crank, J.; Park, G. S. *Diffusion in Polymer*; Academic Press: New York, 1968.
(19) Crank, J. *The Mathematics of Diffusion*; Claredon Press: Oxford, England, 1975.
(20) Maj-Zurawska, M.; Sokalski, T.; Hulanicki, A. *Talanta* **1988**, *35*, 281.

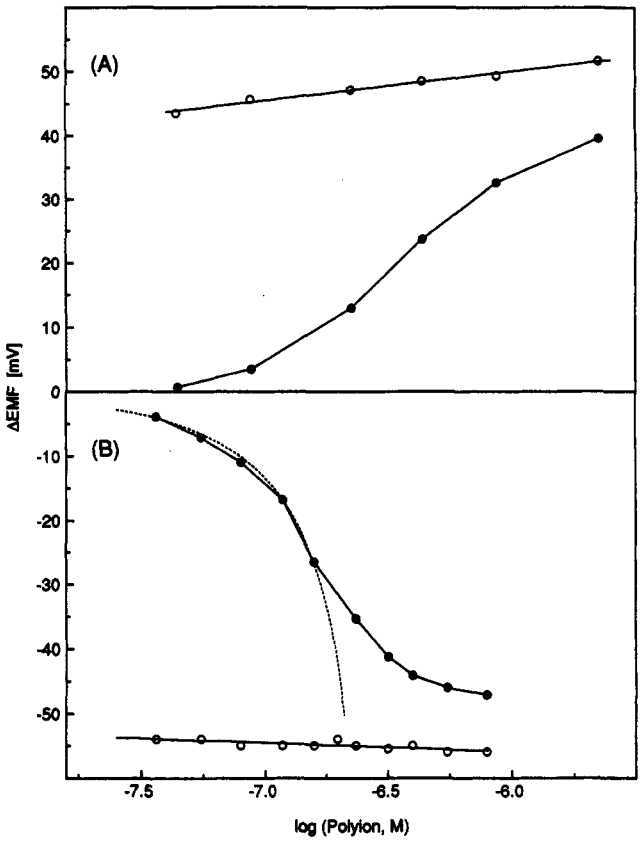

Figure 2. Potentiometric response of protamine (A) and heparin (B) polyion sensors in 0.12 M NaCl solution after different equilibration times: (●) 5 min; (○) 24 h. The membranes were composed of 66 wt % PVC, 32.5 wt % plasticizers, and 1.5 wt % ion exchangers. Dotted line, theoretical curve according to eq 14.

1.0 unit/mL (0.04–0.4 μM). However, if the EMF values are measured after equilibrating the electrode in the given concentrations of heparin for 24 h, little or no useful response to heparin is observed. Under such conditions, the initial chloride counterions of the ion exchanger in the membrane phase boundary layer are completely displaced by heparin and the interface between the organic membrane and the sample solution is at equilibrium. Therefore, according to the equilibrium theory presented above, the electrode should exhibit an ideal response toward heparin with a response slope of about −1 mV/decade. It should be pointed out, however, that the second interface between the membrane and the inner filling solution is assumed to remain unaffected by heparin in the membrane within the entire time frame of the equilibrium measurement. After much longer equilibration times (especially for the low sample heparin concentrations used in these experiments (<5 units/mL)), heparin may eventually reach this other interface and indeed change the overall membrane potential (see below and Figure 7). It can be seen from Figure 2B that the experimental data are in good agreement with that predicted by theory. Therefore, the apparent slope of the analytically useful calibration curve observed when the measurement times are short is obviously due to a kinetic effect. The prerequisite for the quasi-steady-state model is that diffusion in the stagnant layer be the rate-limiting step. In the case of heparin, estimation of L (see eq 6), using a value for D_{aq} of 5×10^{-7} cm^2/s,[21] $D_m = 10^{-9}$ cm^2/s (see below), $\delta_m = 0.003$ cm,[22] $d = 0.03$ cm, and $c_m = 4.5 \times 10^{-4}$ mol kg^{-1}, leads to a value of $L = 1.1 \times 10^7 c_{aq}$.

Obviously, if the concentration of heparin in the sample is less than 2×10^{-7} M, the diffusion in the aqueous stagnant layer will likely be the rate-limiting step during the shorter measurement period. Accordingly, a quasi-steady-state accumulation of heparin at the outer organic phase boundary region will occur under such conditions. To fit the experimental data with eq 14, the site ($TDMA^+$) concentration of 0.032 mol/kg, calculated from the membrane composition, and the ideal response slope of the sensor toward chloride (−59 mV/decade) is used. As shown in Figure 2B, the actual response function agrees reasonably well with the theoretically predicted function for low heparin concentrations. However, at higher heparin concentrations, the experimental points approach the equilibrium potential, indicating that the two models describe, in practice, completely divergent cases.

Figure 2A illustrates analogous EMF data for the newly developed protamine-sensitive membrane electrode.[9] This sensor is realized by incorporating potassium tetrakis(p-chlorophenyl)borate into a PVC membrane plasticized with 32.5 wt % *o*-NPOE. As shown, just as for the heparin sensing membrane, the EMF function of the protamine sensor exhibits an analytically useful response only when the measurement period is kept relatively short (so-called quasi-steady-state response). After 24 h, the membrane is at equilibrium with the sample solution and the response slope toward protamine is ~4 mV/decade. This thermodynamic response slope seems reasonable considering that the charge on protamine is approximately +20 (based on amino acid composition data determined at the University of Michigan Medical Center).

Effect of Membrane Plasticizer Content on the Sensor Response. The optimum compositions of membranes for both polyanionic heparin and polycationic protamine were selected from a large number of membrane formulations tested.[4,9] The criterion for choosing these compositions was to maximize the observed polyion sensitivity of the sensors. To this end, membranes with much lower plasticizer content, relative to normal ion-selective electrodes, were chosen for both heparin and protamine sensing. To display the effect of plasticizer content, the heparin responses of two different membrane formulations are shown in Figure 3B. As can be seen, nonequilibrium calibration data (measurement time of 5 min) is shifted to lower heparin concentrations when the plasticizer content is reduced (to 32.5 wt %) from that typical in most PVC-type ion-selective membrane electrodes (66 wt %). The exact same effect is seen with the newly developed protamine sensor (see Figure 3A). Qualitatively, the accumulation of heparin or protamine in the boundary layer of the organic membrane depends not only on the diffusion of the polyion in the aqueous Nernst layer but also on the diffusion away from the phase boundary into the bulk of the membrane. The faster heparin or protamine diffuses within the organic phase, the more difficult it is for these species to accumulate in the surface boundary layer of the membrane phase. It has been reported by Oesch and Simon[23] that the diffusion coefficient in the polymer membrane phase may be controlled by varying the relative amount of plasticizer. By decreasing the plasticizer

(21) Barlow, G. H.; Sanderson, N. D.; McNeill, P. D. *Arch. Biochem. Biophys.* **1961**, *94*, 518.
(22) Dinten, O.; Spichiger, U. E.; Chaniotakis, N.; Gehrig, P.; Rusterholz, B.; Morf, W. E.; Simon, W. *Anal. Chem.* **1991**, *63*, 596.
(23) Oesch, U.; Simon, W. *Anal. Chem.* **1980**, *52*, 692.

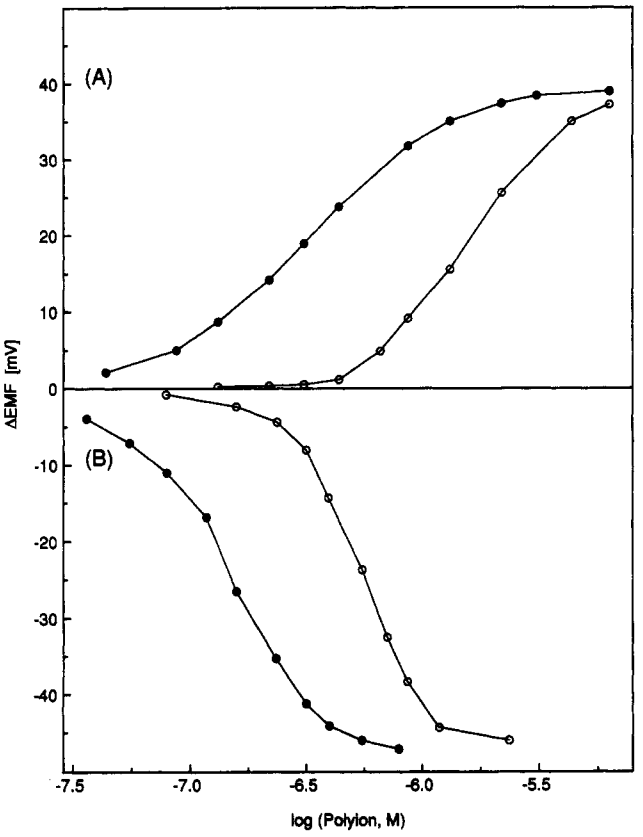

Figure 3. Effect of plasticizer content on the protamine (A) and heparin (B) polyion sensor response in background 0.12 M NaCl solution: (●) 32.5 wt % plasticizer; (○) 66 wt % plasticizer.

content in the membrane, the diffusion of heparin or protamine away from the boundary layer into the bulk of the membrane will be significantly reduced. Hence, a lower detection limit will be achieved when nonequilibrium measurements are made. This fact can also be explained by examining eq 14. For a larger D_m, only higher heparin (or protamine) sample concentrations will lead to the same potential change. Interestingly, the actual membrane selectivity (K_{exch} in eq 2) is not significantly altered by the plasticizer content of the membrane, since the maximum EMF change observed is about the same for the respective heparin and protamine membrane electrodes prepared with the two different weight percents of plasticizers (see Figure 3).

It should be noted that the observed super-Nernstian-type response seen for both heparin and protamine at low concentrations and nonequilibrium measurement times resembles data reported by Hulanicki and Lewandowski[24] for the response of anion-exchanger-based membrane electrodes toward low levels of perchlorate ion. In these early experiments, Hulanicki found that, after conditioning the electrode only in chloride solutions, response to perchlorate and other anions which have high ion-exchange constants for extraction into the membrane was quite super-Nernstian over a very narrow and low concentration range and this unusual response was later modeled using a steady-state approach.[20] Indeed, partly on the basis of such early observations, most workers in this field have always recommended preconditioning of polymer membrane-based electrodes in a solution containing high concentrations of the target analyte ion to obtain Nernstian response over a wide concentration range. On the basis of results for the heparin and protamine sensors, and the theoretical models presented above, one would expect that a similar nonequilibrium steady-state response can also be observed for smaller ions (e.g., perchlorate, etc.) when, at low concentration of such ions, the flux from the sample solution equals the flux into the membrane, a situation that will occur when the electrode is preconditioned in a solution containing relatively high concentrations of an ion that is not the target analyte ion.

Role of Electrode Geometry on Polyion Response Behavior. If the response of the heparin and protamine polyion sensors during practical measurement periods is truly dependent on reaching a quasi steady state of polyion flux up to and into the bulk of the organic membrane phase, then changes in electrode geometry should also influence the EMF response function of the sensors. To test this hypothesis, we examined the response of coated wire-type heparin- and protamine-sensitive electrodes prepared by dip-coating copper wires in the optimal polymer membrane casting solutions useful for detecting each of the polyions. Such coated wire electrodes[25] have been used in certain applications as substitutes for conventional polymer membrane electrode configurations due to their low cost and ease of fabrication. Response characteristics of the coated wire electrodes are usually very similar to the conventional electrode designs, although their long-term performance is inferior, mainly due to an ill-defined boundary potential at the metal/membrane interface.[26]

As shown in parts A and B of Figure 4, both the coated wire heparin and protamine sensors have significantly lower detection limits toward the respective polyions than the corresponding conventional planar membrane electrode designs prepared with the exact same membrane formulations. It is well-known from voltammetry that the mass transfer at a thin wire electrode is subject to cylindrical diffusion except in the vicinity of the tip.[27] Accordingly, in voltammetry, a larger analyte flux for thin cylindrical electrodes than for classical planar electrodes is usually observed.[28] Therefore, for a given sample, the steady-state accumulation of heparin or protamine in the surface layer of the membrane phase is expected to be larger for coated wire electrodes than for the normal planar electrode configuration. It is important, however, to recognize that the expected equilibrium potentiometric response, according to eq 5, does not depend on geometrical factors. That is, only under nonequilibrium or kinetic measurement conditions will the EMF response function be dependent on electrode geometry.

Effect of Ion-Exchanger Site Concentration. It is well-known that for conventional ion-selective membrane electrodes the ionophore concentration in membrane phase does not usually affect the membrane electrode response slope under equilibrium conditions.[15] In contrast, the observed EMF changes for useful polyion membrane electrodes are obtained at quasi steady state, and under such nonequilibrium conditions, the site concentration in the polymer membrane phase

(24) Hulanicki, A.; Lewandowski, R. *Chem. Anal.* **1974**, *19*, 53.

(25) Cattrall, R. W.; Freiser, H. *Anal. Chem.* **1971**, *43*, 1905.
(26) Miyabara, Y.; Simon, W. *Electroanalysis* **1991**, *3*, 287.
(27) Aoki, K.; Honda, K.; Masuda, H. J. *Electroanal. Chem. Interfacial Electrochem.* **1985** *182*, 267.
(28) Murphy, M. M.; O'Dea, J. J.; Osteryoung, J. *Anal. Chem.* **1991**, *63*, 2743.

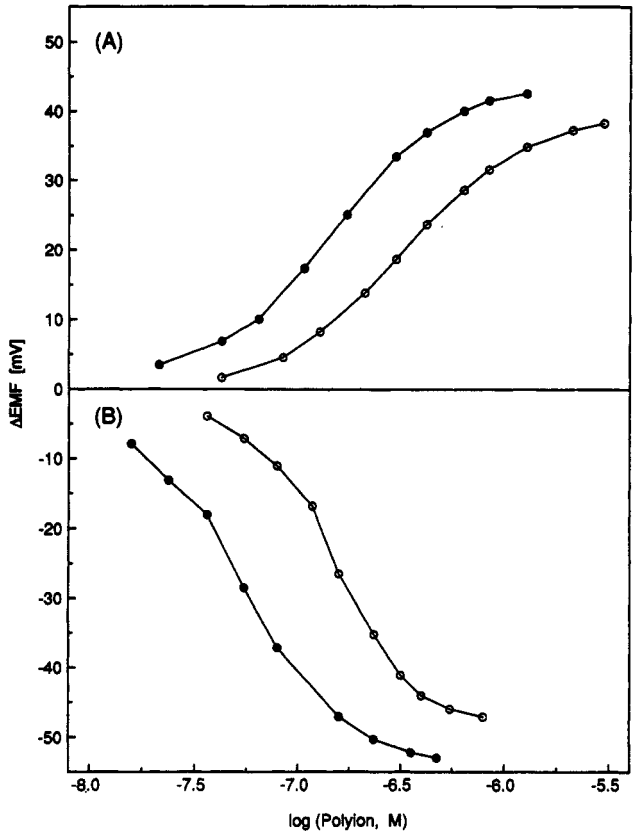

Figure 4. Effect of electrode geometry on the potentiometric protamine (A) and heparin (B) responses in 0.12 M NaCl solutions (membrane compositions were 66 wt % PVC, 32.5 wt % plasticizer, and 1.5 wt % ion exchanger): (●) cylindrically configured coated wire electrode and (○) normal electrode configuration.

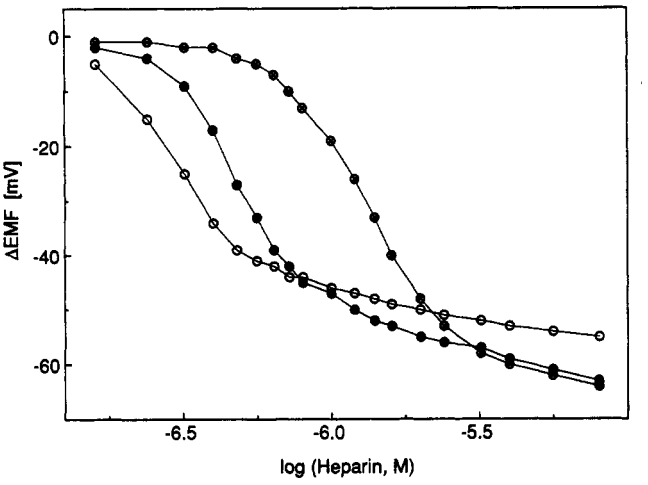

Figure 5. Effect of site concentration in the planar electrode membrane on the heparin response: (○) 2 wt % TDMAC; (●) 4 wt % TDMAC; (⊗) 10.0 wt % TDMAC.

does have a profound effect on the electrode response. Indeed, Figure 5 shows the heparin response of conventional planar membrane electrodes prepared with different membrane concentrations of TDMAC. As can be seen, lower site concentrations in the membrane yield a shift in the nonequilibrium heparin response toward a lower concentration range. This is in agreement with that predicted based on the quasi-steady-state model (eq 14); that is, an increase in the site concentration, R_T, requires a higher sample concentration of heparin to reach the same phase boundary potential (assuming that other parameters do not change). Consequently, the

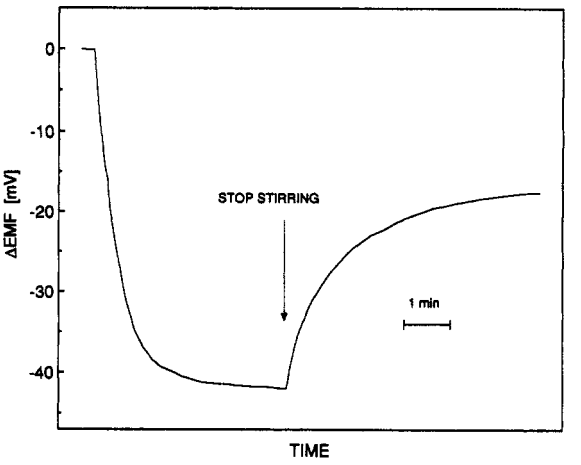

Figure 6. Effect of stirring on the planar heparin sensor's EMF response. The background electrolyte was 0.12 M NaCl; the electrode membrane was composed of 66 wt % PVC, 32.5 wt % DOS, and 1.5 wt % TDMAC; 1.0 units/mL heparin present.

calibration curves for nonequilibrium potentiometric measurements of polyions will shift to a higher concentration range with higher ion-exchanger site concentration in the membrane. The total potential change even under equilibrium conditions will also be affected by the site concentration (see eq 5), with lower R_T yielding less total potentiometric response.

Stirring Effect. It is well-known from fundamental hydrodynamics that convection has a profound influence on the effective thickness of the stagnant aqueous diffusion layer (δ_a).[29] For example, It was found[30] that the stagnant layer thickness at a planar surface is proportional to $V^{-1/3}$, where V (cm/s) is the maximum linear flow velocity. Therefore, it is expected that the stirring rate may affect the quasi-steady-state signal of polyion-sensitive membrane electrodes. As shown in Figure 6 for a conventional heparin-sensitive electrode, a sudden stoppage of sample stirring (via a magnetic stirring bar) causes the EMF signal to revert back and to approach a value closer to the baseline potential. This phenomenon is only observed for low concentrations of heparin. When the potential of the electrode is measured in a solution with a high sample heparin concentration (e.g., 100 units/mL), the electrode response toward heparin is fast and reproducible and is unaffected by the stirring rate of the solution (data not shown).

The observed effects of stirring vs nonstirring are now understood in light of the proposed response mechanism of the heparin- (and protamine-) sensitive electrodes. High heparin concentrations are expected to induce the equilibrium response of the electrode, and no stirring effect would be expected. A sensor in contact with low heparin concentrations will respond as a function of the quasi-steady-state accumulation of heparin in the membrane phase boundary region in contact with the sample solution. In this range, the flux of heparin into the membrane depends on the thickness of the stagnant aqueous layer. After stirring is stopped, the diffusion layer thickness will expand into the bulk of the sample solution, and the flux of polyion to the membrane will decrease. Consequently, this will lead to a smaller steady-state ac-

(29) Levich, V. G. *Physicochemical Hydrodynamics*; Prentice: Englewood Cliffs: NJ, 1962.
(30) Vielstich, W. *Elecktrochem.* **1951**, *57*, 646.

cumulation of heparin and partial return back to the original baseline potential (see eq 14).

Transport Studies. The validity of the quasi-steady-state model described above is highly dependent on the ability of the macromolecular polyions to actually diffuse into the bulk of the ion-exchanger doped polymeric membranes. Thus, one additional study required to further confirm the response mechanism suggested involves determining whether heparin (and/or protamine) can actually be extracted into and diffuse through the optimized membranes used to prepare heparin or protamine sensors. Such processes were investigated for the heparin sensing system via membrane dialysis (or transport) experiments. While, in earlier efforts to assess transport,[4] difficulties were encountered in detecting heparin within the recipient solution, we have now found that the NaCl concentration in that recipient phase has to be kept high, e.g., >1 M, in order to effectively strip the heparin into the aqueous phase (see theoretical section above). In addition, fresh concentrated heparin solution must be added to the feed solution periodically to compensate for the ion-exchange consumption of heparin into the membrane phase. In this way, a reasonable concentration gradient of heparin is maintained to facilitate the diffusion/transport process across the membrane. With these modifications of the experimental conditions, we are now able to detect heparin transported into the recipient solution, both directly by fluorescence (using fluorescein-labeled heparin) and colorimetrically by making use of the metachromatic interaction of heparin with a water-soluble dye, such as toluidine blue O.[12] Since the heparin used in this work is actually a mixture with different molecular weight fragments, explicit quantitative evaluation of the transport process is, however, difficult.

Another means to assess heparin transport through the polymer membrane involves monitoring, over a long time period, the EMF of a conventional electrode configuration in the presence of high sample concentrations of heparin. If heparin can actually diffuse through the membrane and reach the phase boundary between the membrane and inner electrolyte solution (containing 120 mM NaCl as well), then the EMF response of the sensor to heparin should actually begin to reverse in direction (i.e., analogous to adding heparin to the inner reference electrolyte solution). Since it is known that the heparin sensor only exhibits significant potentiometric response (under nonequilibrium conditions) to heparin fragments that are larger than 2500 Da, we can conclude that a significant reversal in the EMF response with time must mean that larger molecular weight fragments of heparin are actually transported through the membrane.

Figure 7 shows the potential vs time profiles for two heparin sensors, one prepared with low plasticizer content (32.5 wt %) and the other with high plasticizer levels (66 wt %). For the two electrodes, the response toward a sample containing 100 units/mL heparin in 0.12 M was monitored for extended time periods. As shown, for both sensors, a point in time occurs in which the EMF value begins to drift back toward the original starting baseline potential (potential without heparin added to sample solution). It is assumed that the potential turning point may be used as an indication of heparin reaching the inner phase boundary of the membrane. If the concentration of heparin in the sample solution is very high (compared to

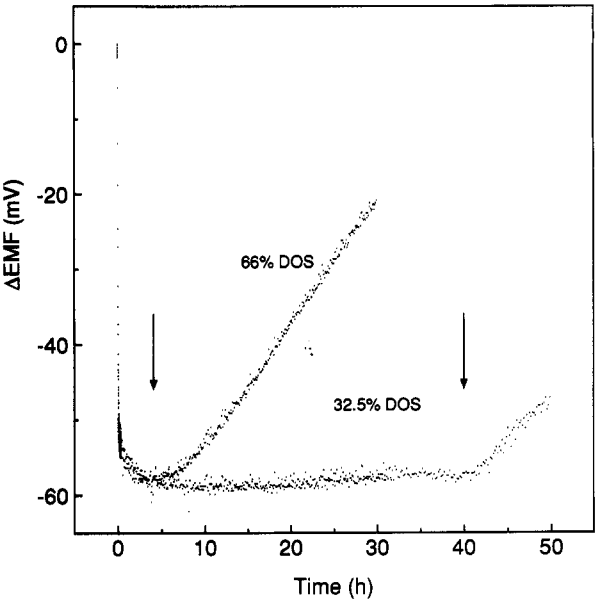

Figure 7. Long-term response profiles of two heparin electrode membranes containing 66 and 32.5 wt % DOS, respectively. The data were collected with a computer-controlled work station (see Experimental Section), which was somewhat noisier than the chart recorder (see Figure 6). The arrows indicate where the electrode potential starts to return back to baseline due to heparin reaching the phase boundary at the membrane/inner filling solution interface.

that used to obtain data in Figure 2) and the outer boundary layer (at sample/membrane interface) is always saturated with heparin, then the time (t) required for heparin to diffuse across the membrane may be used to estimate the diffusion coefficient:[19]

$$d^2 \approx 2D_m t$$

where D_m is the diffusion coefficient of heparin in the membrane phase, t is the time when heparin begins to reach the other side of the membrane, and d is the membrane thickness. Thus, from such experiments, the diffusion coefficients for heparin in 32.5 and 66 wt % DOS plasticized PVC membranes are calculated as 1×10^{-9} and 1.3×10^{-8} cm^2/s, respectively. If all other parameters remain constant, the kinetic EMF response function according to eq 14 is expected to be shifted along the concentration axis by ~1 order of magnitude for the membrane containing the higher levels of DOS plasticizer. This is in good agreement with the actual experimental data shown in Figure 3B.

CONCLUSIONS

We have shown that the observed potentiometric response of ion-exchanger-based polymer membrane electrode toward polyions may be explained via the development of a non-equilibrium, quasi-steady-state change in the phase boundary potential at the membrane/sample interface. Transport experiments clearly indicate that heparin may be extracted into and transported through the polymer membrane, proving that the relatively insensitive equilibrium polyion response can be treated by classical ion-selective membrane theory. Indeed, for long equilibrating times (>24 h for membranes with low plasticizer levels), the response slope toward heparin is found to be very small, i.e., ca. −1 mV/decade. However, for real samples and practical response times of 2–5 min, a

much larger apparent or super-Nernstian response slope for the electrode function is obtained. This behavior is now attributed to a quasi-steady-state accumulation of heparin in the boundary layer of the membrane phase and is akin to the behavior reported by Hulanicki for the low-level perchlorate response of anion-exchanger-based polymer membrane electrodes previously conditioned in solutions containing only chloride ions.[24] Since the flux of heparin from the sample into the membrane phase is small, heparin does not completely displace chloride from the surface of the membrane within the practical time frame of the measurement process. Because this flux is also proportional to the concentration of heparin in the bulk of the sample, an apparent useful slope of the electrode EMF function is obtained. As a result, the influence of the amount of plasticizer, geometric configuration of the electrode, membrane concentration of lipophilic ion exchanger, and stirring rate of the sample solution on the EMF response behavior of the sensor can now be explained semiquantitatively via the proposed steady-state model.

It should be noted that during potentiometric measurement with the polyion sensing membranes, the composition of the sample will be perturbed since the analyte is actually consumed by the membrane. Hence, either a large sample volume or a small membrane surface area must be employed in order to ensure an acceptably small perturbation of the sample composition during the measurement process. Also, since the electrode function relies on an effect which is kinetic in nature, the sensor is expected to measure polyion concentrations rather than activities. However, the assessment of binding constants of polyions to macromolecules as described earlier[5] should be possible if the exchange kinetics of the decomplexation step between the polyion and the other macromolecule are slow. The proposed kinetic model also fully explains the response function of a novel protamine-selective sensor based on a tetraphenylborate-type cation exchanger, indicating that the theory presented is versatile and can be used for understanding the response mechanism of all ion-exchanger-based polymer membranes toward polyions.

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