Revisiting the Response Mechanism of Polymeric Membrane Based Heparin Electrodes

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Abstract
Potentiometric membrane electrodes that respond to heparin and other polyanions were introduced in the early 1990s. Herein, the mechanism of polymer membrane electrode type heparin sensors is revisited. The extraction/diffusion of heparin is studied via both potentiometric and impedance spectroscopic techniques using a prefractionated heparin preparation that contains polyanionic species >10000 Daltons. The reversal in *EMF* response using this heparin preparation indicates diffusion of higher MW heparin fragments to the backside of the membrane. Diffusion coefficients are calculated using a novel formula derived from the phase boundary potential model and Fick's second law of diffusion. Impedance spectroscopy is also employed to show that high MW heparin species are extracted and diffuse across the PVC membranes.

Keywords: Diffusion coefficients, Heparin, Ion-selective membrane, Transport mechanism

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1 Introduction

A series of studies carried out by this research group nearly 2 decades ago focused on developing a simple potentiometric sensor to monitor levels of the anticoagulant heparin (a heterogeneous highly charged polyanion) in whole blood [1–4]. It was found that a plasticized polymer (PVC) membrane doped with tridodecylmethylammonium chloride (TDMACl) could yield significant and analytically useful *EMF* response to heparin. The heparin response was attributed to the ability of the $TDMA^+$ cationic sites in the membrane to form a strong cooperative ion pair with heparin, and extract heparin species into the membrane phase [4]. The overall theory for the observed potentiometric response was developed based on two different response regimes to heparin: 1) the equilibrium potentiometric response at high concentrations of heparin; and 2) the quasi non-equilibrium steady-state response that occurs only at low concentrations of the polyanion. The equilibrium response is induced using high concentrations of heparin (e.g., >1 mg/mL) and a rapid anion exchange takes place at the sample/membrane interface (Cl^- is exchanged for heparin). The surface of the membrane reaches equilibrium with the sample quickly and the response can be predicted by the Nernst equation as applied for a polyionic species with a large negative charge, e.g. −70 (slope of <−1 mV/decade). A large change in potentiometric signal (ca. −50 mV) is observed initially to any added heparin in this higher concentration region and is due to a voltage difference in phase boundary potential between the front and backside of the membrane. This voltage difference is caused by a change in charge separation that is induced when the highly negative charge of heparin (ca. −70) interacts with the positively charged $TDMA^+$ in the membrane phase with a very favorable free energy change of $-11.42\ kJ{\cdot}mol^{-1}\ z^{-1}$ (where z is the charge on the polyanion) [4].

The sensor can also detect heparin via a significant *EMF* vs. concentration dose-response at a lower, clinically relevant levels of $\sim 10^{-7}$ M [1,3] (e.g., μg/mL range). At these low concentrations, the membrane response is described by a quasi-steady state non-equilibrium mechanism [3]. Here, the flux of polyanion toward the surface of the membrane must be equal to the rate of heparin diffusing (as a complex with $TDMA^+$) into the bulk of the membrane away from the sample/membrane interface. This response cannot be explained by the Nernst equation, since it only occurs under non-equilibrium conditions. Indeed, changes in stirring rate [3] or preparing rotating electrode type devices [5] can be used to alter the concentration range of heparin that can be detected. However, no matter what experimental conditions are employed, after long equilibration times (~24 h), the expected full equilibrium response to heparin of ca. −1 mV/decade is observed even at these lower concentrations.

The quasi-steady state model is highly dependent on the extraction of heparin into the organic membrane phase. Therefore, experiments were conducted to determine if extraction of heparin into the membrane does indeed take place via fluorophore labeled heparin as well as long-term potentiometric measurements [3]. In one set of experiments, after the addition of high concentrations

of heparin and the initial large negative *EMF* response toward heparin is observed, after several hours, a reversal in the potentiometric signal occurs [3]. At the time of these experiments, this reversal in the signal provided evidence of heparin extraction into the polymer films, since such a result should only occur if heparin reaches the backside of the membrane and changes the phase boundary potential at that interface, canceling the voltage change on the frontside of the membrane. Further, using fluorescein labeled heparin and multilayers of TDMACl doped plasticized PVC membranes that can be pealed apart after long-term equilibration with solutions containing heparin, the fluorophore labeled species were found to be present in the middle layers. Together, the results of these experiments suggested that heparin is likely extracted into the bulk of the organic polymer membrane phase.

Recently, there have been other electrochemical studies that suggest that heparin does not diffuse into the bulk of organic phases containing lipophilic anion exchangers like $TDMA^+$, but is only adsorbed at the aqueous/organic interface [6–10]. These studies employ voltammetry/amperometry and examine a liquid/liquid interface of two immiscible electrolyte solutions (ITIES) [8–10], or use glassy carbon electrodes coated with a plasticized PVC membrane [6,7]. Samec and co-workers employed amperometry with a rotating glassy carbon electrode coated with an *o*-nitrophenyl octyl ether (*o*-NPOE) plasticized membranes doped with different anion-exchangers, including $TDMA^+$, and a negligible response to heparin was observed [6]. However, another study compared the response mechanism of membranes doped with hexadecyltrimethylammonium ($HTMA^+$) and $TDMA^+$ and reported that the amperometric response of the electrode coated with the membrane doped with $TDMA^+$ was governed by the transport of the heparin complex with the $TDMA^+$ inside the organic membrane phase, unlike the response of the membrane doped with $HTMA^+$, which was governed by adsorption of the heparin at the sample-membrane interface [7]. Amemiya and co-workers employed voltammetric methods utilizing ITIES to analyze the response to heparin [10]. It was concluded that unfractionated heparin could only be adsorbed to the surface of the organic phase at the sample/organic phase interface owing to the large and narrow current peak observed on the reverse sweep seen on the cyclic voltammograms. This was hypothesized to represent heparin being desorbed quickly at the liquid (aqueous)|liquid (organic) interface as the voltage was decreased. In another study conducted by Amemiya and co-workers, Arixtra, a heparin mimetic with a molecular weight of only 1.5 kDa, was examined using the same techniques [9]. Cyclic voltammograms showed a much smaller and broader current peak on the reverse sweep, which indicated that Arixtra was indeed extracted into the organic phase doped with TDMACl [9]. The authors further concluded that if any heparin were to be extracted and diffuse into organic phases, it would likely be the low-molecular weight species.

It is possible that the data reported by Amemyia et al. [8–10] as well as that originally reported by our group [1–4] may have been a result of only low-MW components of unfractionated heparin being extracted and transported. In fact, unfractionated heparin is a heterogeneous mixture of sulfonated/carboxylated polysaccharides, with an average MW of 15 kDa, but with some fraction of species <10 kDa [13,14]. Therefore, in this paper, results of experiments to determine whether extraction/diffusion into/through plasticized PVC membranes containing TDMACl occurs when using a fractionated heparin preparation that contains predominantly high MW fractions are reported. The extraction/diffusion of the fractionated heparin is determined by two independent methods; *EMF* reversal of the polymer membrane response and impedance spectroscopy of the heparin sensitive polymeric membrane used to prepare heparin/polyanion sensors.

2 Experimental

2.1 Membrane Preparation

Membranes were made using 32.5 % by weight poly(vinyl chloride) (PVC) (high molecular weight, Fluka), 66 % by weight dioctyl sebecate (DOS) (Fluka), and 1.5 % by weight TDMACl (26.2 mM) (Fluka). This mixture was dissolved in distilled tetrahydrofuran (THF) (Fisher) to yield 10 mg/mL mixture, at volumes of 750 μL or 1500 μL (yielding membranes of ca. 100 and 200 μm thick, respectively) that were cast into glass rings (25 mm diameter) and left to dry overnight. Once the films were dry, they were conditioned in 50 mM NaCl for 24 to 48 h.

2.2 Transport Cell Setup

A single membrane was placed between two glass cells as part of the transport cell set-up (see Figure 1). The donor solution contained 5 mg/mL heparin sodium salt (8.3×10^{-5} M using 15 kDa as average MW) in 50 mM NaCl background solution and the recipient cell also contained 50 mM NaCl. Both cells contained a stir bar and a total volume of 6 mL. A Ag/AgCl wire was placed inside each cell to monitor the transmembrane potential and connected to a Lawson Labs high impedance 8 channel voltmeter (Pennsylvania, USA). Each cell was stirred continuously throughout a 68 to 72 h data acquisition period.

The thicknesses of the membranes were measured using a gravimetric method [11] and these values were used in the final calculation of the heparin diffusion coefficients within the membrane phase.

2.3 Impedance Measurements

For impedance measurements, the same membrane formulation and transport cell setup was used. The impedance spectra were recorded (EG&G instruments, model 6310) (Tennessee, USA) in the frequency range of 100 kHz to 1 Hz using a sinusoidal excitation signal with

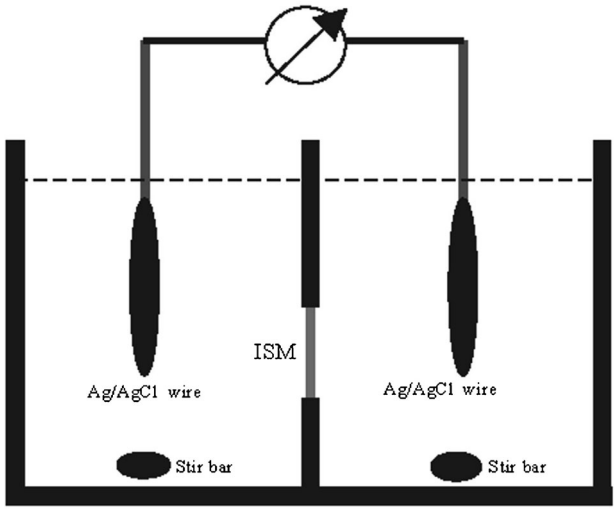

Fig. 1. Sketch of transport cell set-up. Ag/AgCl wires were used for reference and working electrodes and cells were stirred throughout the data acquisition period. The ISM is the ion-selective polymer membrane containing TDMACl that is sensitive to heparin.

amplitude of 5 mV and DC potential used was the open circuit potential of the system.

2.4 Sample Preparation

Fractionated heparin samples were prepared using 10 000 molecular weight cut-off tubes, centrifuged 3 times at 4000 rpm in a background solution of 50 mM NaCl, and rediluted to a final concentration of 30 mg/mL. The fractionation was confirmed by gel electrophoresis. Fifty mL of 20 % polyacrylamide gel (Fisher) was prepared with 300 μL of a 10 % filtered ammonium persulfate (Fisher) solution and 30 μL of tetramythylethylenediamine (TEMED) (BioRad). The gel was prerun at 16 W for 30 min using a 1×Tris/Borate/EDTA (TBE) (Fisher) as the running buffer (pH 8.0). Heparin and enoxaparin (LMW heparin preparation) samples for gel were prepared as above at concentrations of 5 mg/mL. A volume of 10 μL per lane for each sample was loaded onto the gel and was run at 16 W for 1.5 h. The gel was stained for 30 min with a 10×dilution of a mixture containing 0.1 % (w/v) toluidine blue-o, 50 % (v/v) methanol (Fisher), 10 % (w/v) acetic acid (Fisher), 0.25 % glycerol (Fisher), 4 mM Tris base (Fisher), and 0.1 mM EDTA (Fisher). All appropriate preparations were prepared using Milli-Q grade deionized water (18.2 MΩ, Millipore Corp., Billerica, MA).

3 Results and Discussion

3.1 Theory

The diffusion coefficient of ions in the polymer membrane can be assessed in a single ion-breakthrough experiment. The *EMF* of a primary ion-free membrane (Note: in this case heparin is considered as primary ion, but the theory is also valid for any ionic species) is measured in a symmetrical electrochemical cell. Initially there is a constant concentration of interfering ions on both sides of the membrane (i.e., chloride in the case of the TDMACl-based membrane). After a stable membrane potential is reached, a high concentration of heparin or primary ion is injected into the sample solution side of the membrane. By the application of the phase boundary potential model [15] it can be proven that the slope of the reverse potential response curve only depends on the diffusion coefficient in the membrane and the thickness of the membrane.

The *EMF* value for a symmetrical electrochemical cell with negligible diffusion potential according to the phase-boundary potential model [16,17] is given by the following expression:

$$EMF = E_f - E_b \tag{1}$$

where *EMF* is the electromotive force, E_f and E_b are the phase boundary potentials at the front and the backside of the membrane. Once the high concentration of heparin is added, the aqueous and outer (sample side) membrane phases equilibrate with the heparin via favorable anion exchange, with heparin displacing chloride in the outer region of the organic membrane phase. The phase boundary potential at the backside of the membrane is governed by the interfering ion (chloride) concentration until significant amounts of heparin reach the interface. The aqueous phase boundary concentration of chloride within the internal solution is assumed to be constant for the entire course of the experiment due to the high concentration of chloride. During the initial period of the experiment (first 1–2 h) the membrane phase boundary concentration of heparin on the backside is 0 until the first molecules of heparin reach that backside interface (when voltage starts reversal in Figure 2a). Of course, the time required for the backside of the organic membrane phase to reach full equilibrium (with all chloride replaced by heparin) and the cell potential to level off (long time period), depends on the thickness of the membrane employed. Further, given the favorable equilibrium constant for heparin ion-pairing with $TDMA^+$ in the membrane phase, even after long periods of time (i.e., 30 h) there is little or no heparin within the inner aqueous phase boundary layer ($c_J(aq) = 0$). Applying these considerations, an equation where the only time dependent term is the interfering ion concentration (Cl^- in this case) at the inner membrane boundary (backside) can be obtained, $c_J(org)$.

$$EMF = E_f - \frac{RT}{z_J F}\ln\frac{k_J c_J(aq)}{c_J(org)} = E^0 - \frac{RT}{z_J F}\ln\frac{1}{c_J(org)} \tag{2}$$

where k_J is a direct function of free energy of transfer of the interfering ion, $c_J(aq)$, $c_J(org)$ are the concentration of interfering ion in aqueous solution and the membrane, re-

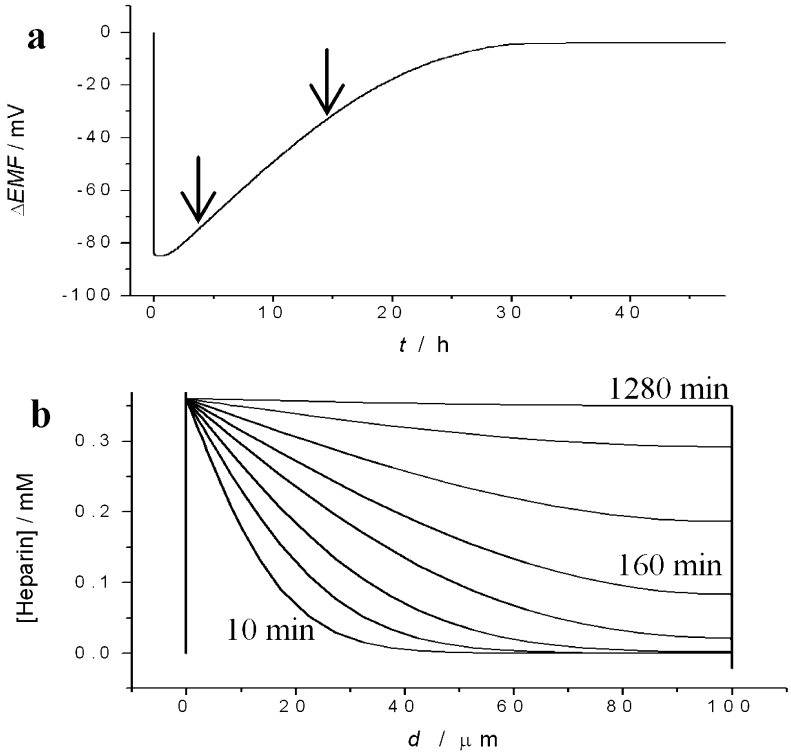

Fig. 2. a) Finite-element simulation of the potential response curve of a 100 μm membrane. The membrane is conditioned in 50 mM interfering ion (chloride) solution. At 0 h the primary ion (heparin) concentration is set to 5 mg/mL in the sample solution. Arrows show the range used for the linear regression. b) Concentration profiles of heparin in the membrane at 10, 20, 40, 80, 160, 320, 640, and 1280 min after the addition of 5 mg/L in the sample solution.

spectively. The z_J is the valence of the interfering ion. F, R, and T are the Faraday constant, the gas constant and the temperature, respectively. It is important to note that the Equation 2 is also valid when an ionophore is present in the ion-selective membrane (ISM), and the introduction of an equilibrium constant changes the E° but the only time dependent term remains the c_J(org) term.

Due to the diffusion of heparin through the membrane, when it arrives to the backside of the membrane it replaces the chloride. The total concentration of negatively charged polyanions in the membrane is determined by the concentration of the lipophilic cationic sites, $TDMA^+$ (R_T), in the membrane phase ($R_T = z_I c_I(\text{org}) + z_J c_J(\text{org})$).

By applying the classical solution of Fick's second law [18] to the ISMs, c_J(org) can be expressed.

$$c_J(\text{org}) = \frac{4R_T}{\pi z_J} \exp\left(\frac{-\pi^2 D_{org} t}{(2d_{org})^2}\right) \tag{3}$$

where D_{org} is the diffusion coefficient of the primary ion (heparin) in the membrane; d_{org} and t are the membrane thickness, and the time elapsed from the injection of the primary ion, respectively. Thus, by plugging Equation 3 into Equation 2, the EMF can be described by a first order equation as a function of time:

$$EMF = A + B \cdot t = \left[E^0 + \frac{RT}{z_J F}\ln\left(\frac{4}{\pi}\frac{R_T}{z_J}\right)\right] + \frac{RT}{z_J F}\ln\left(\frac{-\pi^2 D_{org}}{(2d_{org})^2}\right) t \tag{4}$$

The diffusion coefficient of the primary ion in the membrane (D_{org}) can be calculated from the slope of the line (B).

$$D_{org} = -\frac{4 \cdot B \cdot d_{org}^2 \cdot F \cdot z_J}{\pi^2 \cdot R \cdot T} \tag{5}$$

To validate this approach, we utilized a 1D finite-element method to solve a coupled Nernst–Planck–Poisson (NPP) differential equation system [19]. With this simulation technique, it is possible to assess the time-dependent behavior of ion-selective membranes in contact with aqueous sample solutions containing heparin in a background of chloride anions. The simulated system consisted of two aqueous diffusion layers and the ion-selective membrane. The simulation grid consisted of 600 elements. These elements were closely placed near the phase boundaries. The thicknesses of the diffusion layers and the membranes were considered to be 100 μm. Relative permittivities of water and the ISM were considered to be 80.1 and 10, respectively. The diffusion coefficient in the

aqueous phase and the membrane were $10^{-5}\,cm^2\,s^{-1}$ and $2\times10^{-9}\,cm^2\,s^{-1}$ (Note: the diffusion coefficient for heparin was an estimate made based on the experimental data shown in Figure 5a). The $TDMA^+$ concentration was 26.2 mM, and the charges of heparin and chloride were considered as −70 and −1, respectively. The forward heterogeneous rate constants across the interface for heparin and chloride were considered as $10^{-7}\,m\,s^{-1}$ and $2\times10^{-5}\,m\,s^{-1}$; the backward heterogeneous rate constants across the membrane/solution interface for heparin and chloride were $10^{-9}\,m\,s^{-1}$ and $10^{-3}\,m\,s^{-1}$.

The NPP simulations confirmed that the assumptions used in the derivation of the Equation 5 were correct. The backside phase boundary concentration of heparin in the membrane is zero during the initial portion ($t=0$–2 h) of the potentiometric curve (Figure 2a). Note that reversal in signal is observed after this initial time period since the diffusion layer inside the membrane continues to decrease in thickness and eventually heparin finally completely replaces chloride on the backside of the membrane phase, where the horizontal portion of the *EMF* response vs. time curve is observed ($t>30$ h). The E_f was constant during the entire course of the simulation. The slope of the linear range is marked in Figure 2a and that range was used to calculate the diffusivity of ions through the membrane. The slope obtained by linear regression was within 5% relative error of the value calculated with Equation 4.

3.2 Impedance Spectroscopy

The bulk resistance of an ion-selective membrane can be measured with electrochemical impedance spectroscopy experiments [20]. The potentiometric technique provides useful information on the backside phase boundary concentrations, and therefore the arrival of heparin to the backside can be observed with the reversal of the potentiometric signal. As a complementary method, impedance spectroscopy gives valuable information on the total concentration of heparin in the membrane.

Initially, the membrane is filled with chloride anion as the counter ion of $TDMA^+$. During the experiment, the negatively charged heparin replaces the chloride due to its higher preference by the ISM (formation of ion-pair with $TDMA^+$). The total bulk resistance of the membrane under steady-state conditions is given by the following formula [21]:

$$R_m = \frac{d_{org}RT}{AF^2}\sum_i \frac{1}{z_i D_i c_i(\mathrm{org})} \tag{6}$$

where A is the membrane area. From the classical solution of Fick's Law [18] it can be shown that the total resistance of the membrane changes in time according to the Equation 7:

$$\frac{R_m(t)-R_{min}}{R_{max}-R_{min}} = 1-\sum_{n=0}^{\infty}\frac{8}{(2n+1)^2\pi^2}\exp\left[\frac{-D_{org}(2n+1)^2\pi^2 t}{4d_{org}^2}\right] \tag{7}$$

where R_{min} is the initial resistance when the membrane only contains chloride as counteranions and R_{max} is the steady-state resistance when heparin exchanged the chloride in the entire membrane.

3.3 Fractionation of Heparin

Heparin was fractionated and gel electrophoresis was used to assess the success of the fractionation. Lanes of fractionated heparin were compared to lanes of enoxaparin and the original unfractionated heparin, which were used as the standards. It was hypothesized that the fractionated heparin should look similar to the unfractionated heparin on the gel based on the molecular weight distribution found in the literature [12] and the enoxaparin should represent a molecular weight distribution not contained in either the fractionated or unfractionated heparin samples. Gel electrophoresis (see Figure 3) demonstrated that the fractionated heparin preparation did not extend quite as far down in MW as the unfractionated sample but was more similar to the unfractionated heparin because it is known that the molecular weight distribution of unfractionated heparin does not extend much below 7 kDa and that which does is a very small percentage [12]. In contrast, the band for enoxaparin extended to a MW range far below that seen for the other two samples (Figure 3). These data suggest that heparin was successfully fractionated, and contained little or no low MW fragments.

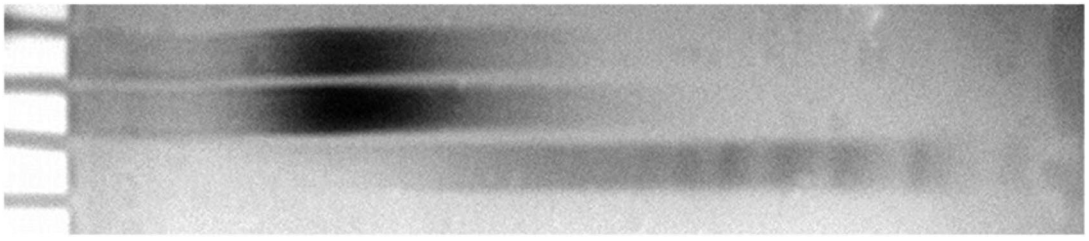

Fig. 3. a) Results of gel electrophoresis of various heparin preparations; upper lane contains fractionated heparin, center lane contains unfractionated heparin, and lower lane contains enoxaparin (low molecular weight heparin). Heparins were introduced onto the gel at the left side of the figure (left side negative (−), right side positive (+)). Gel was run at 16 W for 1.5 h.

3.4 Electrochemical Characterization and Determination of Diffusion Coefficients for Fractionated Heparin in Plasticized PVC Films Doped with TDMACl Using Classic Potentiometry and Impedance Spectroscopy

In this study, the fractionated heparin was used to study the extraction and diffusion of higher MW heparin species through polymeric plasticized membranes doped with TDMACl. The fractionated heparin sample prepared in this work was used to rule out diffusion of low molecular weight heparins present in the unfractionated sample (i.e., species < 8 kDa), unlike earlier studies that used unfractionated heparin. The potentiometric study was carried out using diffusion (transport) cells (see Figure 1) containing two solutions (50 mM NaCl and 5 mg/mL fractionated heparin in 50 mM NaCl) separated by the ion-selective polymer membrane. Membrane thicknesses of between 100 and 250 μm were used to study the diffusion of the fractionated heparin. Like earlier studies carried out during the development of the sensor, an initial drop in the potentiometric signal characteristic of heparin is observed and after several hours, the reversal in the potentiometric signal is also observed (see Figure 4) for every thickness of the membranes. However, the thinner membranes exhibit a much faster reversal in signal when compared to the thicker membranes, which is predicted by the theoretical model. Diffusion coefficients were calculated and an average value of ca. 2.7×10^{-9} $cm^2 s^{-1} \pm 0.27$ (*SD* for $n = 15$ measurements) was determined from all of the membranes tested. The clear reversal in *EMF* signal and relatively consistent diffusion coefficient values for the heparin-TDMA complex moving within the membrane, no matter what the thickness of the membrane, strongly supports the heparin extraction model and cannot be predicted to occur if only interfacial adsorption occurs.

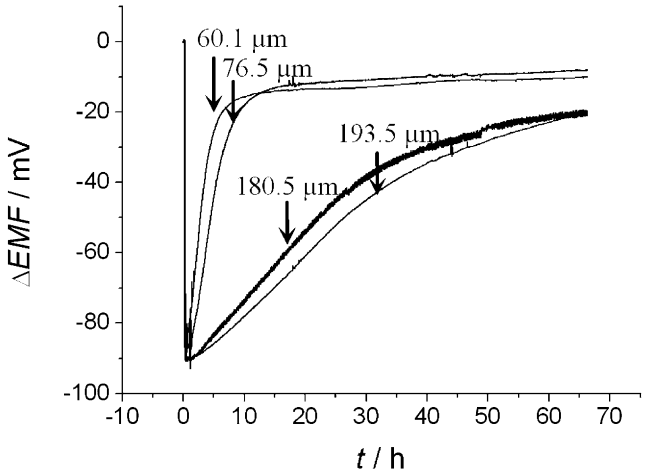

Fig. 4. Dynamic potentiometric response curves of ion-selective membranes containing TDMACl with thicknesses of 60.1 μm, 76.5 μm, 180.5 μm, and 193.5 μm, from left to right, respectively, after addition of 5 mg/mL of heparin to the frontside of the membrane.

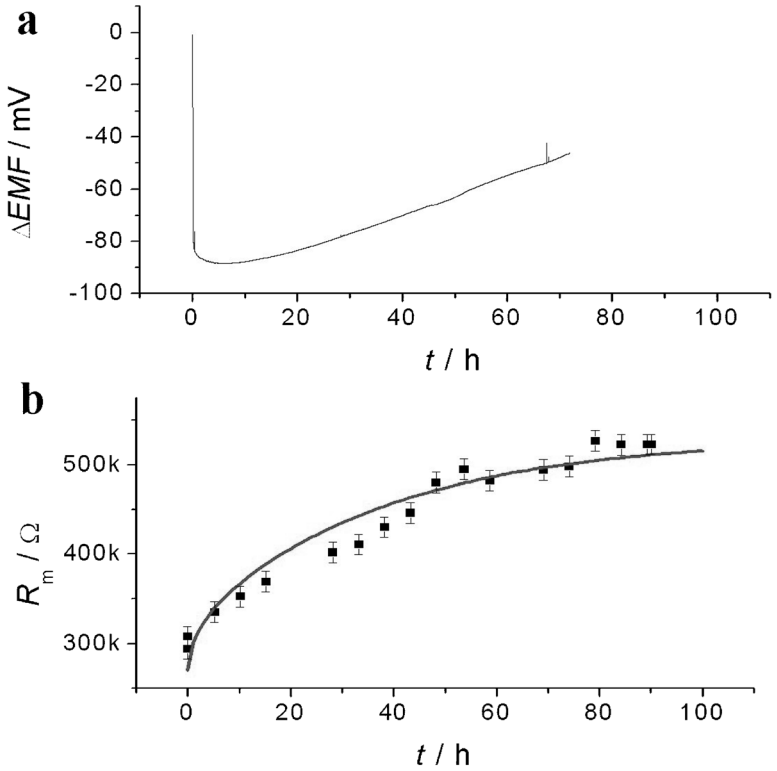

Fig. 5. a) Potentiometric response curve of a 300 μm ion-selective membrane. b) Bulk resistance of the ion-selective membrane, the dots are the experimental results, the solid line is the theoretical response curve based on Equation 7. Note: The potentiometric curve in Figure 5a was generated from the same membrane used to map resistance as function of time in Figure 5b. Potentiometric curves in Figure 4a were generated from different membranes.

Enoxaparin was also studied using the diffusion cell setup. The diffusion coefficient calculated for enoxaparin is expected to be larger than the fractionated heparin samples, and indeed, a diffusion coefficient of $3.2\times10^{-9}\pm 0.60\ cm^2 s^{-1}$ (for $n=9$ measurements) is found base on the rate in *EMF* reversal. Using a 95% confidence level, the diffusion coefficients calculated for heparin and enoxaparin are significantly different. The same initial potential change and reversal in signal is observed as the fractionated heparin samples. This may be further evidence that the fractionated heparin did not contain LMW heparin and that it is possible for larger molecular weight fragments of heparin to be extracted into the bulk of the organic membrane phase.

The diffusion coefficients determined here were calculated using the given membrane thickness (for an experiment with that membrane) and the slope of the linear region (Figure 4) in the reversal in *EMF* signal, *B*, by Equation 5. The experimental slopes for the thinner membranes are much steeper than the thicker membranes because heparin reached the backside of the membrane sooner, and hence the flux of heparin to the backside is greater for the thinner membranes. The calculated diffusion coefficients are very similar for all of the membrane thicknesses.

Electrochemical impedance spectroscopy was also employed to examine whether heparin is extracted and diffuses through the membrane as an ion-pair with $TDMA^+$. Impedance spectroscopy is a useful technique to determine the total bulk resistance of the membrane. This total resistance depends on the concentrations of the mobile ionic species in the membrane. It can be seen in Figure 5b that the theoretical curve (solid line) calculated with the diffusion coefficient from the slope of the reversed potentiometric signal (Figure 5a), fits the measured impedance data quite well. The bulk resistance was measured every 2 h over a ca. 80 h period using the same diffusion cell set-up as employed for the potentiometric experiments. As heparin diffuses into the membrane, the resistance of the membrane increases, due to the lower mobility of the heparin-$TDMA^+$ ion pair compared to the chloride ion.

4 Conclusions

We have provided new experimental results and corresponding theory concerning the extraction and diffusion of high charge density polyanionic heparin into and through plasticized PVC membranes doped with TDMACl, the same membranes used to prepare potentiometric heparin sensors. These results reaffirm earlier experiments [3,4] regarding the mechanism of potentiometric polyanion response, with fractionated heparin possessing only higher MW components still exhibiting the reversal in *EMF* response expected as it reaches the backside of the membrane. This reversal of potentiometric signal after several hours cannot be explained by a surface adsorption only process. Theory predicts this reversal, since, as heparin arrives to the backside of the membrane, the backside phase boundary potential starts to approach the phase boundary potential of the frontside, resulting in a smaller membrane potential (eventually approaching zero). It is important to emphasize that the ΔEMF depends almost entirely on the ion concentrations at the backside and the frontside of the membrane. Hence it is important that the extraction and diffusion of heparin is further verified independently via impedance spectroscopy, that provides information about the total concentration of heparin within the bulk of the membrane phase. Clearly, the impedance data presented here also supports the presence of the high MW fractionated heparin species within the polymeric membrane phase.

Acknowledgements

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