Extraction Thermodynamics of Polyanions into Plasticized Polymer Membranes Doped with Lipophilic Ion Exchangers: A Potentiometric Study

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ABSTRACT: The equilibrium potentiometric (EMF) response of a previously reported polymer membrane-based heparin sensitive electrode toward heparin and other macromolecular polyanions is examined in detail. The precise structure of the lipophilic tetraalkylammonium anion-exchange sites doped within the plasticized poly(vinyl chloride) membrane phase of the sensor is found to play a critical role in the extraction of the polyanions into such membranes. The influence of polyion charge density and molecular weight (chain length) for a series of structurally similar polyanions (sulfonated/carboxylated polysaccharides) on the membrane's EMF response is also studied. It is found that the membrane potential becomes more negative (for given mass/volume concentration of polyion) with an increase of polyion molecular weight up to 3800 for heparin-like structures. In addition, a greater overall equilibrium response is observed as the charge density of the polyanion increases. The observed role of polyanion structure on the membrane's equilibrium EMF response can be explained by a favorable ion-pair formation between the lipophilic anion-exchanger sites in the membrane and the extracted polyanions. The free energy change for such ion-pair formation can be estimated by comparing the membrane's equilibrium EMF response toward a given polyion to that of a second polymeric membrane doped with anion-exchange sites that do not form strong ion pairs with polyanionic macromolecules. The influence of the sample background electrolyte concentration on the extraction behavior of different polyions is also examined.

Introduction

Recently, it has been found that appropriately formulated polymeric membranes (e.g., poorly plasticized poly(vinyl chloride) (PVC)) containing lipophilic anion or cation exchangers (e.g., quaternary ammonium sites, tetraphenylborates, etc.) exhibit unexpectedly large and reproducible potentiometric responses toward specific polyions, including the anticoagulant heparin[1,2] and its polycationic antidote, protamine.[3] These observations have led to the development of simple polymer membrane-based electrochemical sensors that can be used to detect such polyions in samples as complex as whole blood.[2,4] Greater than theoretical EMF response slopes (i.e., the Nernst equation would predict a response slope of less than 1 mV/decade for heparin (with ca. 70 charges per molecule)) to low concentrations of polyions during short measurement periods (3–5 min) have been explained by a steady-state, nonequilibrium potentiometric response mechanism.[5] Accordingly, membrane compositions (i.e., much lower plasticizer content compared to typical ISE membranes) and experimental conditions (stirring the sample, using a tubular electrode configuration, etc.) can be optimized to achieve sensitive EMF responses toward low concentration ranges of the polyions.[5] Interestingly, regardless of the membrane's plasticizer content, all membranes are found to exhibit the same overall equilibrium EMF response toward a given polyion.[5] This observation suggests that the polymer membrane's equilibrium EMF response is determined by a thermodynamically defined extraction of the polyion from the sample solution into the organic membrane phase and is not influenced by the diffusional and other kinetic parameters that dictate short-term steady-state response. Consequently, to develop membrane formulations that will serve effectively for selective sensing of other polyions, equilibrium measurements should be employed to determine whether a favorable extraction exists for given polyion/membrane systems. Only membranes that exhibit large equilibrium EMF responses toward target polyions can be utilized under more practical shorter term, steady-state conditions for potentiometric measurements of polyions.

Herein, we report the equilibrium EMF responses observed for the heparin responsive membrane (dioctyl sebacate (DOS) plasticized PVC membrane doped with tridodecylmethylammonium chloride (TDMAC) or other lipophilic quaternary ammonium ion exchangers) toward various heparins and related sulfonated/carboxylated polysacchride structures (see Table 1). This study is intended to discern the relationship between the extractability of polyions into the membranes as a function of the specific structure (charge density (i.e., sulfonate content), molecular weight, etc.) of the polyanion and the precise structure of the anion exchanger doped within the membrane. Further, a theoretical model is developed that enables thermodynamic free energies for polyanion/anion-exchanger ion-pair formation within the membrane to be easily estimated on the basis of EMF measurements with two membranes containing different tetraalkylammonium species.

Experimental Section

Reagents. Tridodecylmethylammonium chloride (TDMAC), dioctyldimethylammonium bromide (DODMAB), dodecyltrimethylammonium bromide (DTMAB), tetradodecylammonium chloride (TDAC), potassium tetrakis(4-chlorophenyl)borate (KTpClPB), dioctyl sebacate (DOS), 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 Pharmacia Hepar, Inc. (Franklin, OH). Heparin powder from beef lung (142 units/mg), chondroitin sulfate A, dermatan sulfate, the disaccharide

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Table 1. Potentiometric Equilibrium Response of a Membrane Containing TDMAC toward Different Polyanions

polyanion	av MW	polyanion sulfur content[a] (wt %)	equilibrium response[b] (mV)	free energy change[c] (kJ mol^{-1} z^{-1})
beef lung heparin	10 000	13.2	−111.1 ± 2.1	−11.56
porcine heparin	15 000	11.0	−103.3 ± 1.7	−11.42
carrageenan, LC-5	270 000	10.9	−101.7 ± 1.8	−11.00
ι-carrageenan	250 000	10.5	−103 ± 2.1	−11.03
λ-carrageenan	300 000	8.7	−96.1 ± 1.3	−10.50
dermatan sulfate	35 000	6.9	−86.7 ± 1.9	−9.68
κ-carrageenan	154 000	6.5	−74.5 ± 1.8	−8.55
chondroitin sulfate A	25 000	5.2	−68.3 ± 1.4	−8.09
pectin (low methylated)	34 000	0	−28.3 ± 1.4	N/A

[a] The polyanion sulfur content was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). [b] The equilibrium potentials were measured with 100 μg/mL (final concentration) of the given polyanion in 15 mM NaCl. The membrane composition was 66 wt % PVC, 32.5 wt % DOS, and 1.5 wt % TDMAC. [c] The free energy change is normalized to per charge of the polyanion. The anionic site concentration in the polymer membrane is calculated by assuming that the PVC matrix used contains 60 mmol/kg of anionic impurities.[13]

α-2-sulfated uronic acid [1−4]-*N*,2-sulfate glucosamine (sodium salt), and pectin were obtained from Sigma Chemical Co. (St. Louis, MO). Small heparin fragments (tetra- and hexasaccharides), prepared by enzymatic digestion,[6] were kindly provided by Dr. Robert Linhardt's group in the College of Pharmacy, University of Iowa. Polydisperse low molecular weight heparins (average MW 2500, 3800, and 6400), obtained by nitrous acid hydrolysis, were generously provided by the Sanoti Research Center Choay (Gentilly, France). Carrageenans (ι, λ, and κ and one product designated as LC-5) and high- and low-methylated pectins were obtained as gifts from Hercules Inc. (Wilmington, DE). The polyanion stock solutions were prepared by dissolving appropriate amounts of the corresponding powders in doubly deionized water. To accelerate the dissolution process, sonication was used for certain species (e.g., carrageenan). Other chemicals were reagent grade and were used as obtained without further purification.

Membrane Preparations and Electrode Maintenance. The bromide salts of the quaternary ammonium ion-exchangers DODMAB and DTMAB were converted into their chloride forms by conventional two-phase extraction prior to their incorporation into the polymer membranes.[7] The polyanion responsive membranes with different lipophilic quaternary ammonium salts were cast using the method typically used to prepare membranes for fabricating ion-selective electrodes.[8] Generally, a 6 mL THF casting solution containing 800 mg of ingredients was poured into a glass ring (5 cm i.d.) affixed onto a glass slide. The solution was allowed to evaporate overnight and small disks (0.5 cm i.d.) cut from the parent membrane were incorporated into Philips electrode bodies (IS-561, Glasbläserei Möller, Zürich, Switzerland). A solution of 15 mM NaCl was used as the internal filling solution for the assembled electrodes. After each measurement, the electrode was exposed in a 2.0 M NaCl solution with constant stirring to strip out the polyanions from the membrane and regenerate the electrode. The electrodes were stored in a 15 mM NaCl solution while not in use.

Equilibrium EMF Measurements. All measurements were performed at ambient temperature (22 ± 1 °C). The membrane 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 Accumet pH meter (model 620) or a Macintosh IIcx computer equipped 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.[9] To record the time course of the membrane's polyanion response, an OmniScribe (Houston Instruments) chart recorder was connected to the pH meter. To generate different concentrations of polyions, aliquots of concentrated polyanion stock solution were added to the proper background electrolyte solutions. The choice of background electrolyte (NaCl) concentration is critical. For example, to obtain EMF responses toward polyanions with a membrane doped with TDAC, a low NaCl sample concentration (15 mM) had to be chosen; on the other hand a higher (e.g., 120 mM) concentration of NaCl can be used to enhance the EMF selectivity of membranes prepared with TDMAC for some polyanions over others (see also Results and Discussion). The test electrolyte solution was constantly stirred with a small magnetic stirrer.

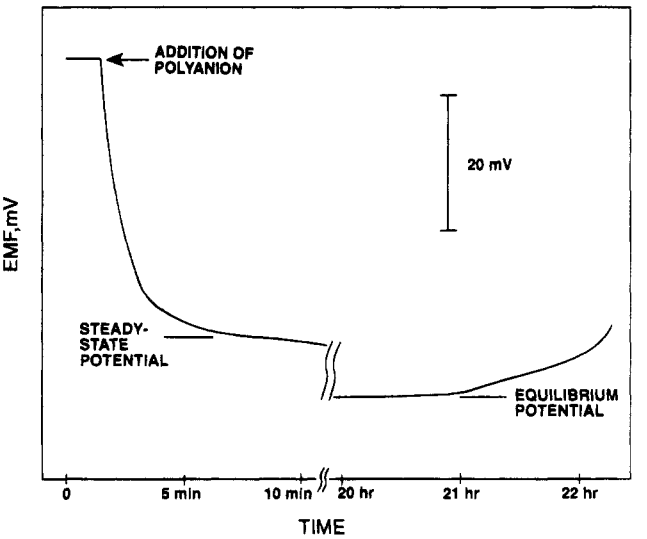

Figure 1. Time profile of polyanion heparin (final concentration of 1 unit/mL) EMF response for plasticized PVC membrane doped with TDMAC in 120 mM NaCl background electrolyte solution.

To determine the equilibrium EMF response, it is necessary to examine the membrane electrode's response profile. Typically, the potential of the electrode becomes more negative upon the addition of an aliquot of concentrated polyanion solution to the background electrolyte solution.[5] The measured potential reaches a steady-state value after about 5 min and then continues to slowly decrease to a minimum value over a much longer time period (10−20 h). At the end of this period the EMF begins to drift back to the original baseline potential value (see Figure 1 for typical response profile). This reversal in EMF response is due to the test polyanion eventually diffusing through the polymer membrane and reaching the inner interface at membrane/internal solution of the electrode, thereby changing the inner phase boundary potential of the membrane[5] (i.e., no polyanion is originally present within the membrane and internal solution). Depending on the diffusion coefficient of the polyanion within the membrane, the minimum potential is generally observed between 10 and 20 h after addition of the polyion to the background electrolyte solution. This most negative potential value is taken as the equilibrium EMF of the membrane electrode/reference electrode galvanic cell (i.e., equilibrium with respect to the membrane/sample interfacial potential).

Theoretical Background

While the theoretical treatment presented previously[5] successfully explained the polyion sensor's super-Nernstian response slope toward low concentrations of

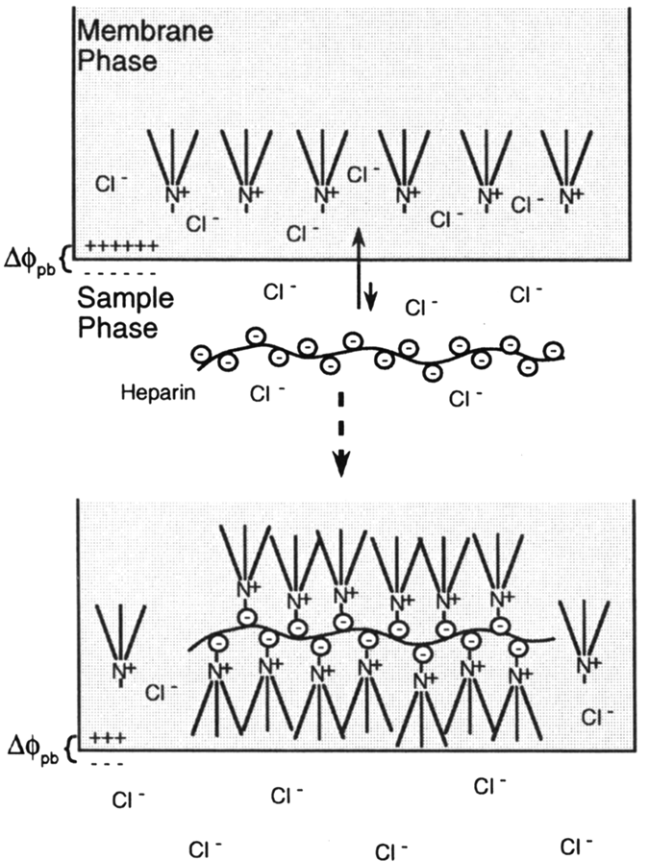

Figure 2. Schematic representation of how a polyanion may interact with the lipophilic quaternary ammonium groups and be extracted into the organic polymer membrane phase to yield a large potentiometric polyanion response. $\Delta\phi_{pb}$ refers to the phase-boundary potential.

heparin by neglecting ion-pair formation inside the membrane phase, a new more accurate treatment which takes into account ion-pair formation is presented here. Indeed, the formation of ion pairs inside plasticized polymeric membranes, especially with low dielectric constant plasticizers such as DOS, is documented in the literature.[10,11] It is demonstrated herein that such ion-pair formation between the polyanion and quaternary ammonium sites is expected to enhance the extractability of the polyion (see Figure 2) and hence to increase the magnitude of the membrane EMF response toward the specific polyionic analyte.

For a polymer membrane containing a charged anion carrier S^+ (e.g. $TDMA^+$) that can form ion pairs with a polyanion $Poly^{z-}$ (with charge z^-) in the membrane phase, the anion-exchange process of polyanions from the aqueous phase (aq) and chloride ions from the organic phase (org) may be formulated as

$$zS^+(org) + zCl^-(org) + Poly^{z-}(aq) = zCl^-(aq) + PolyS_z(org) \quad (1)$$

With the corresponding equilibrium constant:

$$K^{eq} = \frac{k_{Poly}}{(k_{Cl})^z}\beta = \frac{[PolyS_z]}{a_{Poly}[S^+]^z}\left(\frac{a_{Cl}}{[Cl^-]}\right)^z = \frac{[Poly^{z-}]\beta}{a_{Poly}}\left(\frac{a_{Cl}}{[Cl^-]}\right)^z \quad (2)$$

where K^{eq} is the overall ion-exchange equilibrium constant, $a_{species}$ refers to the activity of the species in the sample solution, square brackets [species] designate the concentration of species in the membrane phase (assuming that activity coefficients are constant in the membrane phase[12]), $k_{species}$ is the partition coefficient between membrane and aqueous phases which is governed by the lipophilicity of the species, and β is the overall ion-pair formation constant in the membrane phase, given as follows:

$$\beta = \frac{[PolyS_z]}{[Poly^{z-}][S^+]^z} \quad (3)$$

Since, in practice, the membrane potential is measured after an aliquot of concentrated polyanion stock solution is added into a solution containing a high concentration of chloride (see Experimental Section), the outer phase-boundary potential of the membrane can be described as a function of chloride activities in the membrane and the sample phase:

$$E_{Cl} = E^\circ - \frac{RT}{F}\ln\frac{a_{Cl}}{[Cl^-]} \quad (4)$$

Before the polyanion is added to the sample solution, the chloride concentration in the membrane phase is determined by the total concentration of the cationic ion-exchanger sites (S_T) and any negative sites R^- ($[Cl^-] = S_T - [R^-]$) that are either purposely added or present as endogenous anionic impurities within the PVC matrix.[13,14] After the polyanion solution is added to the background solution, the phase-boundary potential at the sample/membrane interface can equally be formulated by inserting eq 2 into eq 4:

$$E_{Poly} = E^\circ - \frac{RT}{zF}\ln\left(\frac{k_{Poly}}{(k_{Cl})^z}\frac{a_{Poly}}{[Poly^{z-}]}\right) \quad (5)$$

The potential change (ΔEMF) in going from a sample containing only chloride salt to one with added polyanion solution can be formulated by subtracting eq 4 (with $[Cl^-] = S_T - [R^-]$) from eq 5:

$$\Delta EMF = \frac{RT}{F}\ln\frac{a_{Cl}}{S_T - [R^-]} - \frac{RT}{zF}\ln\left(\frac{k_{Poly}}{(k_{Cl})^z}\frac{a_{Poly}}{[Poly^{z-}]}\right) \quad (6)$$

The maximum (i.e., equilibrium) potential change is observed when all chloride ions initially present at the outermost surface of the membrane are replaced by polyanions during the ion-exchange process. Accordingly, the free polyanion concentration $[Poly^{z-}]$ in eq 6 may be determined by combining eq 3 with the electroneutrality condition ($z[Poly^{z-}] + [R^-] = [S^+]$) and mass balance ($S_T = [S^+] + z[PolyS_z]$) inside the membrane phase. By assuming that the interaction between the polyanion extracted and $TDMA^+$ is strong (also see Results and Discussion), i.e., the residual free polyanion concentration $[Poly^{z-}]$ is very small compared to the anionic site concentration ($[S^+] = [R^-]$), the following relationship is obtained:

$$[Poly^{z-}] = \frac{S_T - [R^-]}{z[R^-]^z\beta} \quad (7)$$

Therefore, the expected maximum potential change can be quantified by combining eqs 6 and 7:

$$\Delta EMF = \frac{RT}{F}\ln\frac{a_{Cl}}{[R^-](S_T - [R^-])} - \frac{RT}{zF}\ln\left(\frac{za_{Poly}}{S_T - [R^-]}\frac{k_{Poly}}{(k_{Cl})^z}\beta\right) \quad (8)$$

According to eq 8, the magnitude of the overall potential change is determined by the total concentration of the lipophilic anion exchanger S_T and added/endogenous anionic sites $[R^-]$ in the membrane, the background chloride activity in the sample solution (a_{Cl}), the relative lipophilicity of the polyanionic analyte (k_{Poly}) and most importantly, the overall ion-pair stability constant β. It is obvious that the membrane's relative selectivity for heparin over other polyanions with comparable macromolecular structures results not from the lipophilicity of the polyanion but mainly from the formation of a highly stabilized and electrically shielded heparin–$TDMA^+$ complex (see Figure 2). As reported previously,[5] a membrane's maximum EMF response toward a polyanion is determined by the equilibrium EMF response, not by the operational factors such as the ratio of membrane plasticizer/PVC matrix, sample stirring rate, membrane geometry, etc. Therefore, for developing membrane formulations that will serve effectively for the selective nonequilibrium sensing of other polyions, equilibrium measurement conditions should be examined first to determine whether favorable thermodynamics exist for a given polyion and membrane system.

Since eq 8 above was developed for the membrane's equilibrium response, the free energy of the ion-pair formation in the membrane phase may be estimated directly from potentiometric measurements. To do this, however, it is necessary to independently evaluate the ion-exchange equilibrium of the polyanion and chloride between the aqueous and membrane phase in the absence of strong ion-pair formation. Experiments (see below) have shown that polymer membranes doped with tetradodecylammonium chloride (TDAC) in place of TDMAC exhibit very little overall response toward polyanions, suggesting that an essentially nonspecific ion-exchange process occurs when such a sterically hindered ion exchanger is doped within the membrane. Therefore, by comparing the EMF response of a membrane formulated with TDMAC to that of a membrane containing TDAC, it should be possible to estimate the magnitude of apparent ion-pair formation between polyanion and $TDMA^+$. By adopting the treatment of polyion response by a simple dissociated ion-exchange mechanism, as reported previously,[5] the phase boundary equilibrium potential change of such a TDAC based membrane can be formulated as

$$\Delta EMF(R^+) = \frac{RT}{F}\ln\frac{a_{Cl}}{[R^+] - [R^-]} - \frac{RT}{zF}\ln\left(\frac{za_{Poly}}{[R^+] - [R^-]}\frac{k_{Poly}}{(k_{Cl})^z}\right) \quad (9)$$

where R^+ represents the total concentration of TDA^+ inside the membrane. By subtracting eq 8 from eq 9, the potentiometric responses of both membranes can be compared for the same polyanion concentration under otherwise exactly the same experimental conditions ($[R^+] = S_T$):

$$\Delta EMF(R^+) - \Delta EMF = \frac{RT}{F}\ln[R^-] + \frac{RT}{zF}\ln\beta \quad (10)$$

The free energy contribution, Δg^0_{IP}, from the ion-pair formation per charge of the polyanion structure

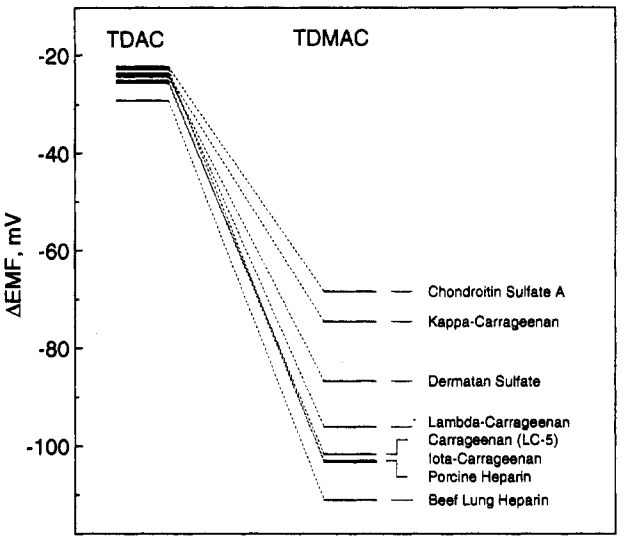

Figure 3. Potentiometric response of PVC membranes doped with TDMAC and TDAC toward various polyanions in a 15 mM NaCl background electrolyte solution. The membrane composition was 66 wt % PVC, 32.5 wt % DOS, and 1.5 wt % quaternary ammonium salt.

($z\Delta g^0_{IP} = \Delta G^0_{IP} = -RT\ln\beta$) can then be expressed as

$$\Delta g^0_{IP} = -\{\Delta EMF(R^+) - \Delta EMF\}F + RT\ln[R^-] \quad (11)$$

According to eq 11, the overall potential change for a polyanion is only dependent on the free energy change per charge of the polyanion, not on the total number of the charges. Therefore, it is expected that polyanions with identical substructures but different overall chain lengths will, above a given minimum molecular weight or chain length, induce the same overall potentiometric response. Consequently, the polyanion sensor's overall response should correlate to the structural parameters of the repeating units within the polyanion (e.g., the charge density) rather than equivalent parameters for the whole polyanion (e.g., total charge of the polyanion).

Results and Discussion

Influence of Anion-Exchanger Structure on the Membrane Response. As discussed above, the equilibrium EMF response of a polymer membrane toward a given polyanion is, under otherwise identical conditions, dictated by the lipophilicity of the polyanion (k_{poly}) and its interaction strength with the quaternary ammonium species doped within the membrane phase (β). Figure 3 summarizes the equilibrium EMF responses observed for PVC membranes formulated with TDMAC and TDAC (1.5 wt %) toward a wide range of sulfonated/carboxylated polysaccharide structures (see Table 1) at 100 μg/mL in a background electrolyte of 15 mM NaCl. As can be seen, membranes containing TDMAC exhibit a much more negative EMF response toward all the polyanions tested when compared to membranes containing TDAC. Moreover, while membranes doped with TDAC exhibit similar overall EMF responses toward all polyanions tested, the polyanion response of membranes containing TDMAC is quite variable, showing a very large dependency on the charge density/structure of the respective polyanions.

It should be noted that both TDAC and TDMAC ammonium salts have been used previously in the design of polymer membrane-based ion-selective electrodes (ISEs) for detecting small anions such as chloride and nitrate via a dissociated ion-exchange response

mechanism.[15,16] Since such response is governed exclusively by the free energies of hydration of the small anions,[17] the EMF response of both membranes toward small monovalent and divalent anions is essentially the same. In contrast, as shown in Figure 3, large differences in EMF responses toward various polyions have been observed for these two membranes. Since the polyanions' lipophilicity contribution to the overall EMF responses with the two membranes should be similar, the much larger and variable equilibrium EMF responses found for membranes doped with TDMAC must be due to a much stronger ion-pairing interaction taking place within the membranes phase. This can be explained with the scheme shown in Figure 2. When the ion-exchanger processes four long alkyl chains, the interaction between the polyanion and ammonium sites within the membrane is sterically hindered and considerably weaker.[18] On the other hand, the close proximity between the positively charged nitrogen in $TDMA^+$ and negatively charged sulfonate/carboxylated groups should lead to a much stronger electrostatic interaction between heparin and $TDMA^+$. In addition, considering that the EMF response of membranes containing TDAC is mainly controlled by the lipophilicity of the polyanions (i.e., k_{poly} in eq 8), the similar EMF responses exhibited by TDAC membranes to these structurally related polyanions suggest that these polyanions have similar free energies of solvation (in membrane) and hydration (in test solution) although their secondary and tertiary structures many differ considerably.

Furthermore, according to the scheme in Figure 2, if the quaternary ammonium exchanger possesses less than three long lipophilic alkyl chains, the ion-pair complex formed would be less electrostatically shielded from the nonpolar bulk of the membrane, and as a result, the extraction of polyanions would be less favorable. Indeed, membranes doped with dioctyldimethylammonium (DODMA) and dodecyltrimethylammonium (DTMA) sites yield equilibrium ΔEMF responses of -89.0 ± 2.0 mV ($n = 3$) and -65.7 ± 1.7 mV ($n = 3$), respectively, upon the addition of 100 μg/mL (final concentration) of porcine heparin to a 15 mM NaCl background electrolyte solution. These potential changes are much less negative than observed for the membranes containing TDMAC (-105.6 ± 2.1 mV, $n = 6$), but much more negative than the one doped with TDAC (-25.5 ± 1.6 mV, $n = 3$).

Evaluation of the Ion-Pair Formation in the Membrane. As suggested by eq 11 above, the free energy change for ion-pair formation between polyanions and $TDMA^+$ within the polymer membranes can be estimated on the basis of the equilibrium response data obtained with membranes containing TDMAC and TDAC. However, as described below (see Influence of Background Electrolyte Activity on Membrane Response), none of the TDAC membranes exhibit an EMF response toward any of the polyanions studied in 120 mM NaCl background solution. To obtain the corresponding EMF data for polyanions for TDAC membranes, a lower (15 mM) concentration of NaCl must be used as the background electrolyte. Further, the pH of the background solution on the polyanions' EMF response was also examined. It was found that the electrode's EMF responses toward beef lung and porcine heparin as well as LC-5 and ι-, λ-, and κ-carrageenan were virtually unchanged when the solution pH was altered from 5.0 to 9.1 (data not shown). To this end, the free energies of ion-pair formation for all test polyanion structures were calculated on the basis of the sensors' EMF responses in 15 mM NaCl background solution, and the results are summarized in Table 1. From the data, it is clear that the free energy of ion-pair formation per polyanion charge is relatively small, approximately in the range of the hydrogen bond energy. However, this contribution accumulates over the multiple charges of the polyanion to yield a favorable overall free energy change for the ion-pair interaction, and hence the overall polyanion extraction process. Similar calculations were made for the ion-pair formation between porcine heparin and $DODMA^+$ and $DTMA^+$ in plasticized PVC membranes. The free energy changes were calculated as -10.2 and -7.8 kJ/mol for $DODMA^+$ and $DTMA^+$ per anionic charge of heparin, somewhat less negative than for $TDMA^+$.

Previous two-phase partition experiments using water and pure organic solvent without any polymeric matrix PVC also demonstrated that TDMAC extracts heparin much more effectively than other lipophilic quaternary ammonium salts (e.g., TDAC).[19] These observations suggest that the driving force for the very hydrophilic polyanions heparin to be extracted into organic phase results from the interaction between heparin and $TMDA^+$ in the organic phase, not from any interaction between the PVC matrix and heparin. Further, the long lifetime (>3 months) of chloride membrane electrodes with TDMAC as the lipophilic ion-exchanger suggest that the partitioning of TDMAC from organic phase into the aqueous solution or blood sample is negligible.[20] In general, the results from membranes doped with different quaternary ammonium structures indicate that there is a hydrophobic interaction between the neighboring lipophilic alkyl chains in the ion-pair complex that further stabilize the ion-pair formation.

Influence of Polyanion Charge Density on Sensor Response. Since the interaction between the anion exchanger and the polyanion in the membrane phase is electrostatic in nature, it is expected that the charge distribution along the polyanion molecule may have a profound influence on the interaction strength and consequently determine the membrane's polyanion EMF response. To study the influence of polyion charge density on the EMF response, it would be ideal to know the precise charge density information (i.e., charges per unit length or charges per repeating saccharide unit). However, as pointed out previously,[21] this information is difficult to obtain due to the dispersity of the polyanion structures. Fortunately, the sulfur or sulfonate content in these polyanions is directly related to charge density, although these polysaccharides also contain other less ionizable groups such as carboxylic acid groups.[21,22] From Table 1, it is evident that the potentiometric response is directly related to the sulfur content (i.e., charge density) of the polyanionic molecules, with polyanions having a higher charge density, yielding a more negative equilibrium potentiometric membrane response. In a similar two-phase coextraction experiment,[22,23] Hurst and Sheng also found that there was a direct correlation between the charge density (sulfur content) of chondroitin sulfate samples and their tendency to be coextracted into the organic phase with a quaternary ammonium cation. More recently, Soedjak has reported[24] that the electrostatic interaction strength between a series of polyanions and the positively charged dye methylene blue correlates directly with polyanion charge densities.

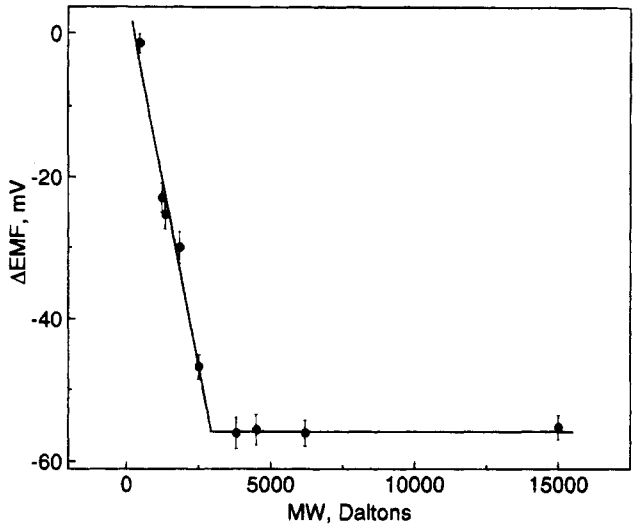

Figure 4. Equilibrium EMF response of membranes doped with TDMAC toward heparin fragments of different molecular weights at a final concentration of 100 μg/mL in 120 mM NaCl. The membrane composition was 66 wt % PVC, 32.5 wt % DOS, and 1.5 wt % TDMAC. The error bars represent the standard deviations of data from three different electrodes.

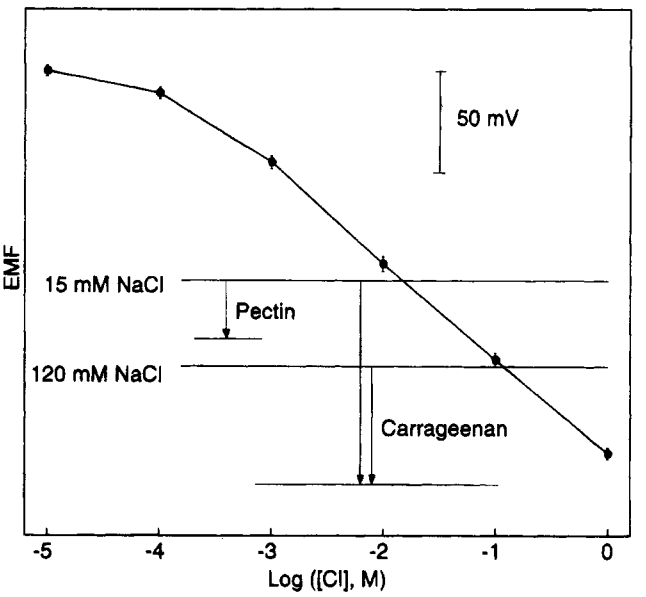

Figure 5. Typical example of how a background electrolyte concentration (Cl^-) may be chosen to discriminate one polyanion over another. The membrane composition was 66 wt % PVC, 32.5 wt % DOS, and 1.5 wt % TDMAC. The error bars represent the standard deviations of data from three different electrodes.

It should be re-emphasized that long-term equilibrium EMF response data should be used to obtain information about the thermodynamics of polyanion/ion-exchanger ion-pair formation because EMF responses observed over a short time period (i.e., steady-state response; see Figure 1) are highly dependent on polyion size. For example, at equilibrium, ι-carrageenan exhibits almost the same total EMF response as porcine heparin (see Table 1). However, during shorter measurement periods (e.g., 10 min), ι-carrageenan EMF response is much less (data not shown) than heparin owing to the fact that the average MW of ι-carrageenan is more than 10 times that of heparin (250 000 and 15 000, respectively). Thus, the diffusion coefficient of ι-carrageenan is much smaller than heparin, leading to a situation where higher concentrations of the carrageenan are required to achieve the same nonequilibrium steady-state response (see eq 14 in ref 5). Moreover, to study the charge density effect, high molecular weight preparations of polyanions must be used, since the equilibrium response is also molecular weight dependent (see below). It is expected that the diffusion of the mobile free ion-exchange site (e.g., $TDMA^+$) in the membrane phase is not a limiting factor compared to the diffusion of the ion-pair complex (e.g., heparin-TMDA) in this study. However, immobilization of the ion-exchange site onto the backbone of the polymer matrix molecule (e.g., PVC) on the membrane's polyion response is yet to be sorted out.

Influence of Polyanion Molecular Weight on Potentiometric Response. The effect of the polyanion molecular weight on potentiometric response was also examined. To this end, equilibrium EMF responses of membranes doped with TDMAC toward different MW porcine heparins at the same mass concentrations were obtained. The relationship between the MW and potentiometric response is plotted in Figure 4. It can be seen that the response to the heparins is essentially the same (i.e., −51 mV in a 120 mM NaCl background electrolyte solution), once the MW of the polysaccharide exceeds 3800. However, the membrane's EMF response toward small fragments of heparin is greatly reduced when the MW of the fraction is less than 3800. In the case of sulfonated disaccharide or monosaccharide structures, no EMF response is observed. This can be explained by the fact that the slope of the electrode function is strongly charge dependent, and hence, in dilute solutions, low polyion concentrations will induce a much smaller EMF response for low molecular weight fragments. By increasing the sample concentration dramatically, this effect might be reversed and small fragments could induce more negative potentials. This can be shown qualitatively by replacing β in eq 8 by Δg^0_{IP} ($\ln \beta = -z\Delta g^0_{IP}/RT$) and plotting ΔEMF vs z. However, in general, it may be incorrect to assume that Δg^0_{IP} values remain unchanged for low molecular weight fragments since long polyion chains may be more stabilized by simultaneous cooperative ion-pairing interactions with the lipophilic quaternary ammonium sites. This effect, as proposed by Hurst,[22] can either be due to the cooperative interaction among the long lipophilic alkyl chains of the quaternary ammonium salt or a conformational change of the polyanion polymer chain when the lipophilic exchanger condenses on the surface of the initially rodlike polyanion structure.

Influence of Background Electrolyte Activity on Membrane Response. As discussed in the theoretical section, due to the multiply charged nature of the polyanion, the background chloride (or other small anion) concentration has a profound influence on the partitioning of the polyanion between the membrane and the sample phases; i.e., small changes in background electrolyte concentration can completely shift the polyanion partitioning from one phase into the other. Since the membrane will not exhibit any EMF response to an ion which cannot be extracted, from an analytical standpoint, different background electrolyte concentrations may be chosen to prevent certain polyions from interfering in the detection of other polyions. One example is illustrated in Figure 5, where the electrode's equilibrium response toward pectin (low methylated) in a low concentration (15 mM) of NaCl background is found to be −28.4 mV. From the sensor's chloride response function, it is expected that when the NaCl

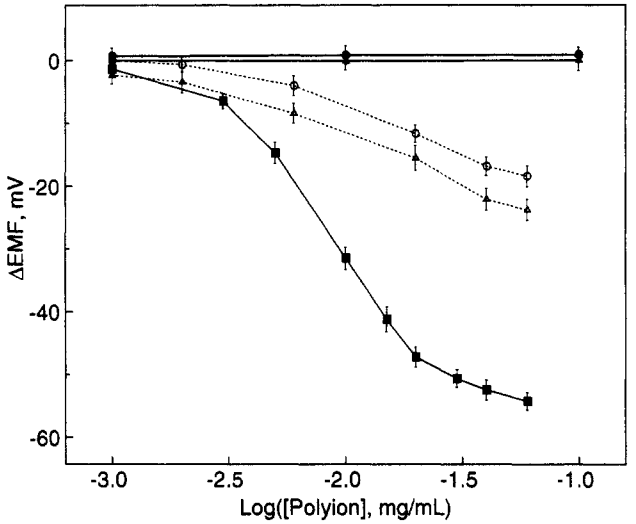

Figure 6. Potentiometric response of a PVC membrane doped with TDMAC toward polyanions in 15 mM (high-methylated pectin (○), low-methylated pectin (△)) and in 120 mM NaCl (high-methylated pectin (●), low-methylated pectin (▲), and LC-5 carrageenan (■)) background electrolyte solutions. The potential changes upon the addition of polyanions were normalized to the background potentials for 15 and 120 mM NaCl, respectively. The membrane composition was 66 wt % PVC, 32.5 wt % DOS, and 1.5 wt % TDMAC. The error bars represent the standard deviations of data from three different electrodes.

concentration is changed from 15 to 100 mM, a −29.4 mV potential change will be obtained. This potential change is more negative than the pectin's equilibrium response, which suggests that, thermodynamically, the membrane is not selective enough for pectin to exhibit any potentiometric response when a concentration of >100 mM NaCl solution is used as the background solution. An experiment was conducted to confirm this effect, and the results are shown in Figure 6. Indeed, a significant potentiometric response (steady state) to pectin is observed when a 15 mM NaCl solution is used as the background solution, although this polysaccharide has a relatively low charge density.[25] However, in a 120 mM NaCl solution, even the presence of 100 μg/mL of pectin (both high- or low-methylated pectins) yields no membrane EMF response and, consequently, the EMF response function toward carrageenan is unaffected by this possible interferent (Figure 6). It is anticipated that this concept will be generally useful for the development of analytical methods capable of detecting given polyions in the presence of others in real-world samples (e.g., carrageenan in ice cream, milk products, etc., in the presence of excess pectin).

Conclusions

The results presented herein demonstrate that the long-term potentiometric polyanion response of polymer membranes doped with quaternary ammonium exchangers is determined by the extraction thermodynamics of the polyanion from the aqueous sample phase into the organic membrane phase. This extractability controls the magnitude of the EMF response observed and is influenced by the structure of the specific ion-exchanger doped in the membrane phase as well as the charge density and molecular weight of the extracted polyanion. It has been shown that measurement of the EMF response of two different membranes doped with different ion-exchangers (TDMAC and TDAC) can be employed directly to estimate the free energy of ion-pair formation between polyanions and $TDMA^+$ or other quaternary ammonium species that form tight ion pairs with polyanions. It has further been shown that the EMF response of polymer ion-exchange membranes to certain polyanions can be controlled by judicious choice of the bathing electrolyte concentration, as the anion activity in the aqueous phase dictates the extent of the polyion extraction process. It is anticipated that the same type of potentiometric measurement technique can be used to examine ion-pair extraction thermodynamics of other polyanions and even polycations[3] into membranes doped with appropriate ion exchangers and that such fundamental information will be valuable in the design of new electrochemical and optical[19] sensors that can be used to conveniently detect various polyions in complex samples.

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