A Molecular Model of Proteoglycan-Associated Electrostatic Forces in Cartilage Mechanics

M. D. Buschmann
A. J. Grodzinsky

Introduction

Articular cartilage (AC) covers the ends of bones in synovial joints. Its main function is to bear compressive, tensile, and shear stresses during joint motion, and to provide the articulating surfaces with very low coefficients of friction. In doing so, the tissue is subjected to intermittent contact pressures reaching 3–18 MPa as many as a million times per year (Hodge et al., 1986). Cartilage cells (1–10 percent of tissue volume) synthesize a complex extracellular matrix (ECM); the weight-bearing and lubrication properties of cartilage are associated primarily with this matrix and its high water content (65–80 percent of tissue weight (Muir, 1979)). The main macromolecular constituents by mass are the large aggregating proteoglycan, aggrecan (30–35 percent of tissue dry weight), and the cross-linked network of collagen fibrils (50–60 percent of tissue dry wt), composed principally of collagen type II but also containing smaller amounts of types IX and XI (Buckwalter et al., 1988; Heinigard and Oldberg, 1989; Upholt and Olsen, 1991). Significant proportions of
smaller proteoglycans, noncollagenous proteins and glycoproteins are also present and are important in the assembly and maintenance of ECM (Heinegard and Oldberg, 1989). The aggrecan is comprised of a core protein (MW = 3 × 10⁶ Da) to which are attached glycosaminoglycans (GAGs) to form a highly charged macromolecule (MW = 2 × 10⁸ Da) due to ionized sulfate and carboxy groups on the GAGs. The aggrecans can interact with hyaluronate and link protein in the extracellular space to form large aggregates (MW = 2 × 10⁸ Da) which are enmeshed within the collagen network. In general, the collagen network resists tensile and shear stresses while the proteoglycans resist fluid flow and compressive stresses (Mow and Rosenwasser, 1988). This study focuses on a molecular model for the significant electrostatic contribution to the compressive stiffness of cartilage, associated with the charged PG.

In equilibrium, with no fluid flow, a compressive load is balanced by a restoring force which has both electrostatic and nonelectrostatic contributions. The nonelectrostatic contributions can include the configurational entropy of the network, and the effect of excluded-volume and nearest neighbor interactions. The latter two are related to Van der Waals forces, hydrogen bonding, hydration forces, and the mechanical stability of a molecule due to its covalent bond structure (Israelachvili, 1991). The electrostatic contribution arises from long range coulombic forces associated with the presence of fixed ionized charge groups on the GAGs. Macroscopically, the electrostatic contribution has been viewed as a Donnan osmotic swelling pressure (Maroudas, 1979) since the presence of fixed charge increases the total concentration of mobile ions within the tissue and thereby increases the intratissue osmotic pressure. On the molecular level, the electrostatic contribution to tissue stiffness arises from repulsive electrostatic forces between GAGs, which can be calculated from the fundamental laws of electrostatics and thermodynamics using the Poisson-Boltzmann (PB) unit-cell model (Marcus, 1955).

It is widely accepted that the macroscopic Donnan model and molecular level PB model are two different representations of the same electrostatic and osmotic phenomenon (Katchalsky et al., 1966; McLaughlin, 1989; Overbeek, 1956; Sanfeld, 1968). Moreover, the Donnan model has recently been derived as an approximate solution to a system of Poisson-Boltzmann equations using homogenization and scaling arguments (Basser and Grodzinsky, 1993). In addition to mobile ion osmotic effects, electrostatic forces are incorporated into the Donnan model through the electroneutrality condition. In the PB-cell model, osmotic (Brownian) and electrostatic interactions are included in the description of the thermodynamic equilibrium of mobile ions. Hence the swelling pressure predicted by both models is the consequence of highly coupled electrostatic and osmotic phenomena, and could be called equivalently an osmotic swelling pressure or an electrostatic swelling pressure.

One of the fundamental differences between the Donnan and PB-cell models is the characteristic length scale of the continuum (Fig. 1). In the Donnan approach, each "incremental volume" contains many macromolecules. There is no molecular level structure assumed; within a uniformly charged polyelectrolyte phase, the electrostatic potential Φ is assumed to be constant (and thus the electric field is zero). Even if the fixed charge density varied nonuniformly, but with a characteristic length scale much greater than a Debye length, the corresponding electric field within the matrix would still be orders of magnitude smaller than fields on the scale of a Debye length (i.e., the microscopic field between two adjacent GAG chains). In contrast, the length scale inherent to the Poisson-Boltzmann model contains many ions but can be much smaller than an individual polyelectrolyte molecule. Because the electrical Debye length at physiological ionic strength (~ 0.8 nm) is on the order of or less than the spacing between neighboring GAG chains in tissues such as cartilage, the electrical potential will vary steeply between GAG chains creating significant electric fields between GAGs. The inclusion of these molecular level electric fields in the quantitative prediction of pressures and moduli is inherent in the PB formulation and is enabled by microstructural modeling.

The importance of the GAG-associated fixed charge to the functional properties of articular cartilage has been long

**Nomenclature**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>σ(η)</td>
<td>GAG radius (normalized)</td>
</tr>
<tr>
<td>α</td>
<td>(cos(ϕ₁))/η²</td>
</tr>
<tr>
<td>b</td>
<td>interchange distance on GAG</td>
</tr>
<tr>
<td>β</td>
<td>moles of charge per gram of proteoglycan</td>
</tr>
<tr>
<td>C₀</td>
<td>bath ionic strength</td>
</tr>
<tr>
<td>C̅ₖᵢ</td>
<td>macrocontinuum ion concentration in polyelectrolyte phase</td>
</tr>
<tr>
<td>Cᵢₙ(r), Cᵢᵤ(r)</td>
<td>macrocontinuum ion concentration in unit cell</td>
</tr>
<tr>
<td>Cₑₕₑ</td>
<td>proteoglycan concentration</td>
</tr>
<tr>
<td>E(r)</td>
<td>microcontinuum electric field</td>
</tr>
<tr>
<td>ε(ε₀)</td>
<td>dielectric permittivity of medium (free space)</td>
</tr>
<tr>
<td>εₛ</td>
<td>permittivity in fluid phase of unit cell</td>
</tr>
<tr>
<td>εₑ</td>
<td>macroscopic compressive strain</td>
</tr>
<tr>
<td>F</td>
<td>Faraday constant</td>
</tr>
<tr>
<td>Φ(r)</td>
<td>microcontinuum (normalized) electrostatic potential</td>
</tr>
<tr>
<td>Φ₀</td>
<td>Φ(r) = Φ₀</td>
</tr>
<tr>
<td>Φ₀ₖₑ</td>
<td>dΦₙ/dFᵤ = Φ₀</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Φ₀ₕₑ</td>
<td>macrocontinuum Donnan electrostatic potential</td>
</tr>
<tr>
<td>Φ₉ₕₑ</td>
<td>bath electrostatic potential</td>
</tr>
<tr>
<td>Φ(ϕ)</td>
<td>osmotic coefficient in the polyelectrolyte phase (bath)</td>
</tr>
<tr>
<td>γᵢₙ, γᵢᵤ</td>
<td>activity coefficient of ions in bath</td>
</tr>
<tr>
<td>γᵢₙ, γᵢᵤ</td>
<td>activity coefficient of ions in polyelectrolyte phase</td>
</tr>
<tr>
<td>γₑₕₑ</td>
<td>mean electrolyte activity coefficient in polyelectrolyte phase (bath)</td>
</tr>
<tr>
<td>H₄ₑ</td>
<td>equilibrium confined compression modulus</td>
</tr>
<tr>
<td>H₄ₑₙₑₙₑ</td>
<td>electrostatic (non-electrostatic) component of equilibrium modulus</td>
</tr>
<tr>
<td>H₄ₑₘₑₑₙₑₙₑ</td>
<td>measured (model prediction of electrostatic component of equilibrium modulus at ionic strength i)</td>
</tr>
<tr>
<td>Iₙ(x)</td>
<td>modified Bessel function of order n</td>
</tr>
<tr>
<td>I₁</td>
<td>unit matrix</td>
</tr>
<tr>
<td>Kₙ(x)</td>
<td>modified Bessel function of order n</td>
</tr>
<tr>
<td>κ</td>
<td>inverse Debye length</td>
</tr>
<tr>
<td>λ</td>
<td>ratio of GAG strain in unit cell to macroscopic tissue strain</td>
</tr>
</tbody>
</table>
recognized (Maroudas, 1979). Recently, we also found a correlation between measured GAG concentration and the biomechanical properties of a developing ECM synthesized by chondrocytes cultured in agarose gel (Buschmann et al., 1992). To date, most models of cartilage swelling pressure have incorporated the Donnan theory directly (Maroudas and Bannon, 1981; Urban et al., 1979) or as part of a more general macroscopic continuum theory for cartilage behavior (Lai et al., 1991). Intratissue swelling pressures have been inferred from the Donnan theory and compared with measurements (Maroudas and Bannon, 1981; Maroudas et al., 1991; Urban et al., 1979), assuming previously reported virial coefficients for the osmotic swelling agent polyethylene glycol (Comper, 1991). The PB-cell model has been compared to the ideal Donnan model in the prediction of ion partitioning between tissue and bath and intratissue diffusivities of small ions (Maroudas et al., 1988; Parker et al., 1988). For these cases, the ideal Donnan model compared well to the PB-cell model (with exceptions for divalent ions) and was seen to be computationally simpler. To our knowledge, a molecular level model has not yet been used to explicitly estimate the electrostatic contribution to PG swelling pressure and cartilage compressive stiffness. Therefore, the objectives of this study were (1) to predict the swelling pressure of PG solutions and the electrostatic contribution to the equilibrium modulus of cartilage from the solution of the Poisson-Boltzmann (PB) equation within a unit cell containing a charged GAG molecule and its surrounding atmosphere of mobile ions, (2) to compare the predictions of this PB-cell model to data on PG swelling pressures (Williams and Comper, 1990) and the ionic strength-dependence of the equilibrium modulus of cartilage (Eisenberg and Grodzinsky, 1985), and (3) to compare and relate the predictions of the PB-cell model to those of the ideal Donnan model.

Theory

Due to the presence of charge on a polyelectrolyte there is a difference in the electrostatic potential between the polyelectrolyte (tissue) phase and the equilibrating bath (Fig. 1). The fundamental assumption of the Donnan model is that the potential within a homogeneous polyelectrolyte phase in equilibrium is spatially invariant. The Donnan model is therefore a macroscopic theory which does not include molecular structure, or molecular level electric fields. The Poisson-Boltzmann cell model considers an idealized molecular structure since it views the polyelectrolyte phase as composed of individual cylindrical cells, each containing a GAG chain (Fig. 1). The PB-cell model then requires the computation of the microscopically varying electrostatic potential and field between polyelectrolyte molecules in the prediction of ion concentrations and macroscopic thermodynamic properties like osmotic pressure and electrochemical potentials. The two models can be rigorously related by using the PB-cell model to compute activity and osmotic coefficients necessary to correct for the absence a microscopically varying potential in the Donnan model (Marcus, 1955).

Nomenclature (cont.)

- $M_{cs}$: molecular weight of chondroitin sulfate disaccharide
- $N$: Avogadro's number
- $n_i$: number of degrees of freedom for chi-square statistic
- $P_{pol} (P_{bath})$: osmotic pressure in polyelectrolyte phase (bath)
- $P_s$: osmotic swelling pressure: $P_{pol} - P_{bath}$
- $P_r (P_{unit})$: osmotic swelling pressure calculated from Donnan model (PB-cell model)
- $P_e$: microcontinuum Van't Hoff pressure in unit cell
- $q_s$: probability of chi-square being due to measurement variability
- $r (\hat{r})$: radius (normalized)
- $r_0 (\hat{r})$: bracketed interval of PB normalization
- $R (\hat{R})$: unit cell outer boundary radius (normalized)
- $R_0$: unit cell outer boundary radius at zero strain
- $R_{o_f}$: best fit unit cell outer boundary radius at zero strain
- $\kappa$: gas constant

\[
\begin{align*}
\rho_p &\equiv \rho_{m} = \text{microcontinuum fluid (macrocontinuum fixed) charge density} \\
\rho_{m} &\equiv \rho_{m} = \text{macrocontinuum (best fit) fixed charge density at zero strain} \\
\rho_{pg} &\equiv \rho_{pg} = \text{macrocontinuum fixed charge density calculated from PB-cell model} \\
\sigma (\hat{\sigma}) &\equiv \sigma_{\text{unit cell}} = \text{surface charge density (normalized) on GAG in unit cell} \\
\sigma_{\text{norm}} &\equiv \sigma_{\text{norm}} = \text{normalization factor for surface charge} \\
\sigma_i (\hat{\sigma}) &\equiv \sigma_i (\hat{\sigma}) = \text{standard deviation of measured electrostatic component of equilibrium confined compression modulus at ionic strength } i \\
T &\equiv T = \text{absolute temperature} \\
T_e &\equiv T_e = \text{Maxwell stress tensor} \\
\hat{\sigma} &\equiv \text{error parameter in numerical solution: } 10^{-6} \\
u &\equiv \text{partial specific volume} \\
V &\equiv \text{solid volume fraction} \\
\gamma &\equiv \text{thermal voltage: } 0T/F \\
\hat{\psi} (\hat{r}) &\equiv \text{incremental microcontinuum normalized electrostatic potential} \\
\hat{\psi} (\hat{r}) &\equiv \text{incremental microcontinuum normalized electrostatic potential} \\
X^2 &\equiv \text{chi-square statistic}
\end{align*}
\]
The Donnan Model for Osmotic Swelling Pressure. For a polyelectrolyte phase in equilibrium with a bath containing 1:1 electrolyte such as NaCl, the distribution of mobile ions between the two macroscopic phases is described by the Donnan equilibrium condition (Overbeek, 1956).

\[
(\gamma_+^2 \bar{C}_{Na^+} + \bar{C}_{Cl^-}) = (\gamma_-^2) \bar{C}_0
\]

(1)

where \( \bar{C}_{Na^+} \) (mol/L-fluid) and \( \bar{C}_{Cl^-} \) are the mobile ion concentrations in the polyelectrolyte phase, \( \bar{C}_0 = C_{Na^+} = C_{Cl^-} \) is the bath salt concentration, and \( \gamma_+ \) and \( \gamma_- \) are the mean activity coefficients in the polyelectrolyte and bath phases, respectively. If the electrical potential in the bath is taken as the reference potential, the potential in the polyelectrolyte phase is the equilibrium Donnan potential, \( \Phi_D \), which can be written in terms of \( C_{Na^+} \) or \( \bar{C}_{Cl^-} \), e.g. (Overbeek, 1956):

\[
\Phi_D = \frac{RT}{F} \ln \frac{\gamma_+ \bar{C}_0}{\gamma_- \bar{C}_{Na^+}}
\]

(2)

where \( F = 96,487 \) coul/mol is the Faraday constant, \( R' = 8.314 \) J/(K-mol) is the gas constant, and \( T = 293 \) K is the absolute temperature. Activity (and osmotic) coefficients are not fundamental, structural parameters; rather, they are strictly empirical quantities which are used to account for deviations from ideal model behavior often observed with highly charged polyelectrolyte solutions (Overbeek, 1956) (see Appendix II). These coefficients would have to be measured at each PG concentration, cartilage compressive strain, and ionic strength of interest, and then inserted into Eq. (1). Therefore, we first compare the PB-model to the ideal Donnan model, thereby avoiding the use of empirical activity and osmotic coefficients in a parallel manner in both models. Activity and osmotic coefficients will, however, be calculated from the solution of the PB-cell model which may then be inserted into the Donnan model to account for non-ideal (i.e., microstructural electrostatic) interactions not included in the Donnan model. In ideal Donnan equilibrium then, \( (\gamma_+^2 (\gamma_-^2) = \bar{C}_{Na^+} = \bar{C}_{Cl^-} \) to Eq. (1) to give the relation:

\[
\bar{C}_{Na^+} + \bar{C}_{Cl^-} = \bar{C}_0
\]

(3)

Bulk electroneutrality within the macroscopically smoothed polyelectrolyte phase having fixed charge density \( \rho_m \) (coul/L-fluid) requires

\[
\frac{\rho_m}{F} + \bar{C}_{Na^+} + \bar{C}_{Cl^-} = 0
\]

(4)

Combining Eq. (4) with Eq. (3) yields the ion concentrations in the polyelectrolyte phase for ideal Donnan equilibrium,

\[
\bar{C}_{Na^+} = \left( \frac{\rho_m}{F} \right) \bar{C}_0
\]

(5)

Because there is a difference in concentration of mobile ions in the bath and polyelectrolyte phases, there will be an osmotic pressure difference between the phases. The osmotic pressure in the polyelectrolyte and bath phases, respectively, is (Katchalsky, 1971; Overbeek, 1956)

\[
P_{poly} = \bar{C}_o \Phi_D (\bar{C}_{Na^+} + \bar{C}_{Cl^-})
\]

(6)

\[
P_{bath} = 2 \Phi_D \bar{C}_0
\]

(7)

where the osmotic coefficients (\( \bar{C}_o, \Phi_D \)) account for non-ideal. The osmotic swelling pressure, \( P_s \), is defined as the difference between the osmotic pressure in the polyelectrolyte phase and that in the bath,

\[
P_s = P_{poly} - P_{bath}
\]

(8)

The osmotic swelling pressure calculated from the ideal Donnan model, \( P_s^d \), is obtained by combining Eqs. (5)–(8) with \( \bar{C}_o = \bar{C}_0 = 1 \):

\[
P_s^d = 2 \Phi_D \bar{C}_0 \left( \left( \frac{\rho_m^2}{4F^2 \bar{C}_0^2} + 1 \right)^{1/2} - 1 \right)
\]

(9)

Thus, in the Donnan model, mobile ion osmotic effects and electrostatic interactions between fixed charge groups are included via \( \rho_m \) in Eq. (9).

Poisson-Boltzmann Cell Model for Swelling Pressure. The Poisson-Boltzmann unit cell model, composed of a cylindrical-shaped polyelectrolyte molecule and its surrounding aqueous electrolyte, is a widely used molecular-level model that predicts electrostatic interactions, osmotic pressures, ion distributions, and ion activity coefficients for polyelectrolyte solutions (Einovoll and Hemmer, 1988; Fuoss et al., 1951; Katchalsky, 1971; Marcus, 1955; Ramananathan, 1965; Wenerstrom et al., 1982). Figure 1 shows the model unit cell composed of a charged cylinder of radius \( a \) representing a GAG molecule surrounded by an aqueous solution of electrolyte out to radius \( R \), the radius corresponding to the average proximity of neighboring GAGs. In such models, the polyelectrolyte chains are pictured as locally rigid but globally flexible (de Gennes, 1978). Moreover, the cell model can represent an isotropic polyelectrolyte on the macroscopic scale since there is no required periodicity or preferred direction of orientation of the molecules (de Gennes, 1978).

In applying the cell model to proteoglycans in solution and in cartilage matrix, it is important to note that the characteristics decay length of an electrostatic field in an electrolyte solution, the Debye length, is \( \sim 0.8 \) nm in physiological salt solution (Eq. (20)). Since inter-GAG chain spacings on PG monomers on the order of several Debye lengths have been visualized using electron microscopy (Morgelin et al., 1989), the presence of significant electrostatic interactions between GAGs may be expected. In addition, it has been reported that the osmotic swelling pressures of solutions of chondroitin sulfate GAG, PG, and PG-aggregates are nearly identical when expressed as a function of mass concentration (Comper and W., 1987; Urban et al., 1979). This has suggested to these investigators that most of the osmotic pressure of PG in solution and in cartilage results from local interactions between segments of chondroitin sulfate GAG molecules. Therefore, there is strong evidence to suggest that the GAG chain is the appropriate biochemical constituent upon which to base a molecular model of PG-associated electrostatic forces. In this context, we identify the unit cell as a two-dimensional representation of the local electrostatic environment of a GAG chain. On the microscale, the unit cell accounts for electrostatic interactions between locally cylindrical segments of adjacent GAG chains that are nearest one another, where the electrostatic repulsive force is maximal. In Fig. 1, these segments may be pictured locally as having a rigid rod-like conformation analogous to the models of Katchalsky (1971) and de Gennes (1978). On the macroscopic scale, the GAG chains may be randomly oriented; the model matrix described by the unit cell can therefore be isotropic.

The ionized negative charge groups on the GAG molecule are represented as a uniform surface charge density \( \sigma \) at \( r = a \) which can be expressed in terms of the average inter-charge distance along the molecule, \( b \),

\[
\sigma = \frac{-e}{2\pi ab}
\]

(10)

182 / Vol. 117, MAY 1995
where \( e = 1.6 \times 10^{-19} \) coulomb is the electronic charge. The electric field laws in the electroquasistatic formulation of Maxwell's equations (Melcher, 1981) are Gauss' law relating the total charge density \( \rho \) in a medium, to the electric field:

\[
\nabla \cdot \mathbf{E} = \rho
\]

(11)

and Faraday's law which states that \( \mathbf{E} \) is irrotational in the absence of time-varying magnetic fields:

\[
\nabla \times \mathbf{E} = 0
\]

(12)

The electric field can then be represented by a scalar potential, \( \Phi \), consistent with Eq. (12):

\[
\mathbf{E} = -\nabla \Phi
\]

(13)

In the fluid phase, Eqs. (11) and (13) combine to give Poisson's equation

\[
\nabla^2 \Phi = \frac{-\rho_{f}}{\varepsilon_f}
\]

(14)

with the fluid permittivity \( \varepsilon_f \) taken to be that of water at 20°C; \( \varepsilon_f = 78.3 \times 6.0 \) (c = 8.85 \times 10^{-12} \) farad/m). In thermodynamic equilibrium, the mobile ion concentrations are related to the potential by the Boltzmann distribution, representing the balance between electrical migration and ionic diffusion,

\[
C_+ (r) = C_0 \exp \left( \frac{F \Phi (r)}{RT} \right)
\]

(15)

In Eq. (15), ions are treated as point charges, and the potential of the average force on the ions is assumed to be the electric potential of the mean field in Poisson's equation (Fixman, 1979). The fluid charge density \( \rho_f \) in Eq. (14) is then

\[
\rho_f = F(C_+ (r) - C_- (r)) = -2FC_0 \sinh \left( \frac{F \Phi}{RT} \right)
\]

(16)

where \( C_0 \) is the bath NaCl concentration (\( \Phi = 0 \)). The Poisson-Boltzmann equation, which accounts for electrostatic interactions between all the ions, is then obtained by combining Eqs. (14) and (16) as

\[
\nabla^2 \Phi = \frac{2FC_0}{\varepsilon_f} \sinh \left( \frac{F \Phi}{RT} \right)
\]

(17)

It has been shown (Carnie and Torrie, 1984; Fixman, 1979) that the PB Eq. (17) is the mean field, point ion approximation of the exact statistical mechanical description of monovalent ions in a continuum of constant dielectric permittivity, near a charged surface. More detailed descriptions than the PB-cell formulation for ion-ion, ion-polyion, polystyrene-polyion interactions and solvation effects have been used (Carnie and Torrie, 1984). In the PB approximation, the solvent is treated as an incompressible fluid dielectric having a constant permittivity, \( \varepsilon_f \), and the potential of the mean force on an ion is equated with the electrostatic potential. The former assumption excludes effects such as dielectric saturation, electrostriction, and hydrophobic forces while the latter excludes polarizability of mobile ions, short range interionic repulsion, and any other two-particle correlations beyond the mean field, point ion approximation. Even with these assumptions, many theoretical and experimental studies have confirmed the accuracy of the PB equation in a variety of situations (Jonsson et al., 1980; McLaughlin, 1989; Svensson et al., 1990; Wennerstrom et al., 1982). Measured surface potentials and forces between charged surfaces have agreed well with PB theory when the concentration of divalent ions is low enough (~ 1mM) (McLaughlin, 1989). The mean field, point ion approximation inherent to the PB theory has also been shown to be valid provided the ion size does not exceed the Debye length (valid even at very high ionic strengths (1M)) (McLaughlin, 1989). Condensation theory, another approach to the properties of polyelectrolytes, has been formulated using empirical rules by Manning (Manning, 1969) and Oosawa (Oosawa, 1971). Condensation theory has been shown to be a special case of the Poisson-Boltzmann formulation (Fixman, 1979; Zimm and Le Bret, 1983); thus the results of the PB model can be more generally useful. For these reasons, the PB-cell model appears to be a reasonable starting point for our molecular-level description of electrostatic forces in articular cartilage.

The GAG cylinder is modeled as having a surface and not a volume charge density; with azimuthal symmetry, the electric field is zero within the GAG. Therefore, the electrical boundary condition from Eq. (11) at the GAG surface \( (r = a) \) relating the GAG surface charge to the potential in the fluid is:

\[
\sigma = -\varepsilon_f \frac{d\Phi}{dr} \bigg|_{r=a}
\]

(18)

The boundary condition at \( (r = R) \) is determined from continuity of the electric field between adjacent cylindrical cells and symmetry,

\[
\frac{d\Phi}{dr} \bigg|_{r=R} = 0
\]

(19)

With Eqs. (17) and (18), Eq. (19) ensures that the GAG fixed charge at \( r = a \), is balanced by the ionic space charge in the fluid region \( (a < r < R) \), thereby ensuring electroneutrality of the unit cell as a whole. Equation (19) also incorporates the presence of other charged GAGs and hence inter-GAG repulsion; if other GAGs were not present (Eq. (19)) would be applied at \( r = \infty \), Equation (17) may be solved subject to the boundary conditions Eqs. (18) and (19) once \( a, h, R, \) and \( C_0 \) have been specified. The PB cell model therefore requires 3 structural molecular parameters \( (a, b, R) \) whereas the Donnan model requires a single macroscopic parameter \( (\rho_m) \).

The characteristic length of decay of the potential from the charged surface is called the Debye length, \( 1/\kappa \), and can be found by normalizing \( \Phi \) in Eq. (17) to the thermal voltage \( RT/F \):

\[

\frac{1}{\kappa} = \left[ \frac{\varepsilon_f RT}{2FC_0^2} \right]^{1/2}
\]

(20)

**Force Densities, Stresses and Swelling Pressure in the PB-Cell Model.** Within the unit cell, the local force density on an infinitesimal volume of the fluid can be considered as the sum of an electrical force and a fluid pressure. For example, by multiplying the PB Eq. (17) by \( \nabla \Phi \) and integrating the right-hand side, an expression of mechanical equilibrium is obtained

\[
0 = \rho_f \mathbf{E} - \nabla \Phi \left( C_+ (r) + C_- (r) \right)
\]

(21)

Equation (21) may be written in tensorial form,

\[
\nabla \cdot \left( \mathbf{T} - P \mathbf{I} \right) = 0,
\]

(22)

where \( I \) is the unit matrix and \( \mathbf{T} \) is the Maxwell stress tensor corresponding to the Lorentz force density \( \rho_f \mathbf{E} \) (for constant \( \varepsilon_f \)) (Melcher, 1961).

\[
\mathbf{T} = \varepsilon_f \mathbf{E} \mathbf{E} - \frac{\varepsilon_f}{2} \mathbf{E} \mathbf{E}^2 \]

(23)

which will only contain a normal (radial) component within the unit cell of Fig. 1. In equilibrium, the local stress on a fluid element within the cell is described in Eq. (22) as due to spatially varying electrostatic and pressure terms whose sum...
is divergence-free within the cell. At the outer cell boundary \((r = R)\), \(E = 0\) from Eq. (19) and the Maxwell stress (Eq. (23)) is also zero. Therefore, the total stress at \(r = R\) is represented by the osmotic pressure at the cell boundary, referred to as the osmotic pressure of the polyelectrolyte phase:

\[
P_{\text{poly}} = \frac{\partial T (C_+ (R) + C_- (R))}{\partial T} = 2 \partial T C_0 \cosh \left( \frac{F \Phi (R)}{\partial T} \right)
\]

(24)

Equation (24) has been derived previously by differentiating the free energy in the unit cell (Marcus, 1955), or the partition function (Wenerstrom et al., 1982), with respect to cell volume. The measurable osmotic swelling pressure in the PB-cell model, \(P_{\text{poly}}\), is the difference between the osmotic pressure in the polyelectrolyte, \(P_{\text{poly}}\), and the osmotic pressure of the bath, \(P_{\text{bath}} = 2 \partial T C_0\), giving

\[
P_{\text{poly}} = 2 \partial T C_0 \left( \cosh \left( \frac{F \Phi (R)}{\partial T} \right) - 1 \right)
\]

(25)

Equation (25) shows that the swelling pressure or "electrical double layer repulsion" (Overbeek, 1956), is of electrostatic origin through its dependence on \(\Phi (R)\).

**Solution of the Poisson-Boltzmann Equation.** The PB Eq. (17) is now solved within the unit cell subject to boundary conditions Eqs. (18) and (19). Various analytical approximations to the solution have been devised, including that of Katchalsky (1971); in contrast, we have developed a numerical solution to the exact problem and, further, have compared the predictions to those of the Donnan model. With the normalization

\[
\frac{\Phi}{\partial r} = \Phi; \quad \sigma = \frac{\sigma_0}{r} = \left( \frac{F \partial T}{\Phi} \right) \kappa
\]

(17), (18), and (19) become

\[
\frac{d^2 \Phi}{dr^2} + \frac{1}{r} \frac{d \Phi}{dr} = \sinh (\Phi)
\]

(27)

\[
\frac{d \Phi}{dr} \bigg| _{a} = - \sigma; \quad \frac{d \Phi}{dr} \bigg| _{R} = 0
\]

(28)

There is no known general analytic solution to (27) (McCaskill and Fackerell, 1988) and numerical solutions (shooting point Runge-Kutta) can be nonconvergent in the nonlinear region, even with very small steps (Gur et al., 1978). An efficient and stable numerical solution was formulated by solving (27) in intervals where the change in \(\Phi\) is \(<< 1\). Considering an interval \([\bar{r}_0, \bar{r}_1]\), (27) is linearized to

\[
\tilde{\Phi} = \tilde{\Phi} - \tilde{\Phi}_0
\]

(29)

where \(\tilde{\Phi}_0 = \tilde{\Phi}(\bar{r}_0)\). \(\tilde{\Phi}\) is then found to be,

\[
\tilde{\Phi} = A \tilde{J}_0 (a \bar{r}) + BK_0 (a \bar{r}) - \tanh (\tilde{\Phi}_0)
\]

(30)

where \(J_0\) and \(K_0\) are modified Bessel functions of order \(n\), and \(a^2 = \cosh (\tilde{\Phi}_0)\). \(A\) and \(B\) can be found with knowledge of \(\tilde{\Phi}_0\) and \(\tilde{\Phi}_0\), yielding

\[
A = \frac{\tilde{\Phi}_0 + K_0 (a \bar{r}) \tanh (\tilde{\Phi}_0)}{K_0 (a \bar{r})} \frac{2}{\tilde{I}_0 (a \bar{r}) - K_0 (a \bar{r}) I_0 (a \bar{r})}
\]

(31)

\[
B = \frac{\tanh (\tilde{\Phi}_0) - A \tilde{\Phi}_0 (a \bar{r})}{K_0 (a \bar{r})}
\]

(32)

A step is taken to the new point \(\bar{r}_1\) by calculating \(\tilde{\Phi}(\bar{r}_1)\) and \(\tilde{\Phi}'(\bar{r}_1)\) from Eq. (30), which then serves as the starting point \(\bar{r}_0\) for the next step, with a restriction on the nonlinearity (second order term in the linearization using Eq. (29)),

\[
\tilde{\Phi}^2 (\bar{r}_1) < \tilde{\Phi}^2 (\bar{r}_0) - \frac{2}{\theta}
\]

(33)

where \(\theta = 10^{-6}\). If the trial step violates (33), the step size is reduced until (33) is satisfied. The complete integration begins at the outer boundary \((r = R)\) where \(\tilde{\Phi}_0 = 0\) and a guess is taken for \(\tilde{\Phi}_0\) is \(\tilde{\Phi}(R)\). Eq. (28a) is satisfied by repeating the integration with different values of this initial \(\tilde{\Phi}(R)\) determined by polynomial interpolation (order = iteration number completed) of the pairs \((\tilde{\Phi}(R), \tilde{\Phi}'(R))\) until \(d \tilde{\Phi} / dr |_{R} = 0\) to within a specified accuracy \((\sim 10^{-6})\). By using a five point discrete differentiation formula to calculate the error in the solution to (27),

\[
\frac{d^2 \tilde{\Phi}}{dr^2} + \frac{1}{r} \frac{d \tilde{\Phi}}{dr} - \frac{1}{\sinh (\tilde{\Phi})}
\]

(34)

the error was found to be of the same order as \(\tilde{\Phi} (10^{-6})\).

**Use of PB-cell Model to Calculate Activity and Osmotic Coefficients.** In the ideal Donnan model, the assumption of a spatially invariant electrosstatic potential has often necessitated the introduction of osmotic and activity coefficients in order to account for experimentally observed deviations from the ideal case (Overbeek, 1956). Semi-empirical formulas for the activity coefficients and osmotic coefficients have been derived to account for non-ideality (Manning, 1969; Wells, 1973b). They have been used with the Donnan model to determine intratissue ion concentrations and partition coefficients (Maroudas, 1979; Presten et al., 1972) and osmotic swelling pressures (Maroudas, 1979; Maroudas and Bannor, 1981; Urban et al., 1979) in articular cartilage or PG solutions. An alternative approach is to use the PB cell model to calculate the intratissue osmotic and activity coefficients for the Donnan model (Einsevoll and Hemmer, 1988; Marcus, 1955). The utilization of these calculated activity and osmotic coefficients in the Donnan Model accounts for the microstructure present in the PB-cell model. They may therefore be used in conjunction with the Donnan model to achieve the result of the PB-model calculation without having to solve the PB equation again (see Appendix II).

The activity coefficients of the ions in the polyelectrolyte phase have been derived previously from thermodynamic principles by differentiating the free energy of the unit cell with respect to ion concentration (Einsevoll and Hemmer, 1988; Marcus, 1955),

\[
\bar{y}_+ = \frac{C_+ (R)}{C_{\text{Na}^+}}; \quad \bar{y}_- = \frac{C_- (R)}{C_{\text{Cl}^-}}
\]

(35)

where \(C_{\text{Na}^+}\) and \(C_{\text{Cl}^-}\) are the average ion concentrations in the polyelectrolyte phase. In Eq. (35), the ion concentrations at the outer cell boundary, \(C_+ (R), C_- (R)\), can be found from the Boltzmann distribution Eq. (15) using the solution for \(\Phi (R)\) of the PB Eq. (17), and the average ion concentrations are,
The intratissue activity coefficients computed from Eq. (35) do not account for two particle ion-ion correlations (Carnie and Torrie, 1984). By using the PB-cell model, they account for the interaction between the charged macromolecule and the ions, that part of the ion-ion interaction which is described by the mean electric field, and the macromolecule-macromolecule interaction described by the cell boundary. In the bath, the absence of a charged surface yields activity coefficients equal to unity, in this approximation. Equivalently, the activity coefficients computed by Eq. (35) can be interpreted as the intratissue to bath activity coefficients.3 Equation (24) gives a physical interpretation of the activity coefficients in Eq. (35): the concentrations at the outer cell boundary in Eq. (24) may be replaced by the product of the average ion concentration in the unit cell and its activity coefficient (C*(R) = γCNa* + γCCl−). Using Eqs. (24), (35), and (36), the osmotic coefficient in the polyelectrolyte phase given by the PB-cell model can be related to the activity coefficients (Katchalsky, 1971)

\[ \bar{\psi} = \frac{C_*(R) + C_*(R)}{C_{Na*} + C_{Cl-}} = \frac{\gamma Na* + \gamma Cl-}{C_{Na*} + C_{Cl-}} \]  

(37)

The osmotic coefficient in the bath (Eq. (7)) is unity in order to be consistent with the PB treatment of the unit cell (Guldbrand et al., 1986; Marcus, 1955). (The nonideality in the bath arises from two particle ion-ion correlations which have been neglected in the PB description of the cell and hence must be neglected in the bath as well in order to describe the equilibrium between the two phases (Carnie and Torrie, 1984).)

Results: Comparison of Theory and Experiments

The Donnan model and the PB-cell model are now compared in their ability to predict: (1) the swelling pressure of proteoglycan solutions of different concentrations in 0.15 M phosphate-buffered saline (PBS) (Williams and Comper, 1990) and (2) measurements of the ionic strength-dependence of the confined compression equilibrium modulus of adult bovine articular cartilage equilibrated in NaCl baths varying from 0.005 M to 1.00 M (Eisenberg and Grodzinsky, 1985). These data set focus on two different consequences of electrostatic interactions in polyelectrolytes: the electrostatic contribution to the swelling pressure of a solution (or tissue), and that part of the modulus which results from increases in this swelling pressure with increasing compressive strain. These data also provide two fundamentally different tests for the Donnan and the PB-cell models. In the prediction of swelling pressures, the PB-cell radius, R, will decrease for increasing PG concentration while the bath ionic strength, C0, and therefore the Debye length are constant; in the Donnan model the fixed charge, \( p_m \), increases with increasing PG concentration. In the prediction of the modulus, the PB-cell model involves a constant zero-strain R with decreasing Debye length for increasing ionic strength; in the Donnan model the zero-strain \( p_m \) is constant and \( C_0 \) is varied directly. In the prediction of PG swelling pressures at different PG concentrations, all Donnan and PB model parameters are known from experiments, so there are no adjustable parameters. In the prediction of the equilibrium modulus of cartilage at varying ionic strengths, one adjustable parameter in each model is varied to obtain the best fit to the data. The resulting best fit parameter is compared to the range of literature values from other experiments.

Swelling Pressure of Proteoglycan Solutions. The measured swelling pressures of PG solutions are compared to the predictions of the ideal Donnan model (Eq. (9)) and the PB-cell model (Eq. (25)) in Fig. 2. The data are from curve 1 of Fig 4 of Williams and Comper (1990); the 10 percent measurement variability was from Comper (personal communication). Williams and Comper used sedimentation-diffusion experiments to measure the osmotic swelling pressure of aggrecan (aggregating chondroitin sulfate proteoglycans) from Swarm rat chondrosarcoma equilibrated in 0.15 M ionic strength PBS.4 These PG are of order 2 x 105 Da molecular weight, and are known to contain ~ 86% chondroitin sulfate (CS) as the primary GAG constituent with little other charged moieties (Kimura et al., 1984). The similarity of these PG to native cartilage PG in structure, function, and properties has led to their wide use in model systems (Kimura et al., 1984).

The parameters used initially in each model are the most frequently reported values in the literature for chondroitin sulfate GAG. Model predictions for parameter values covering a range centered about these most frequently reported values were then calculated to examine the sensitivity of the models to such parameter variations. While one could treat all parameters in both models as adjustable and curve-fit the theories to the data, we believed that it would be a more severe test of the individual models and of the comparative physical behavior of the models to use reported (measured) parameter values; thus, we did not adjust parameter values to fit the data in either the Donnan or PB-model for swelling pressure. Since the pH of the PG/PBS solutions was constant, the total number of ionized charge groups was constant for a given mass of PG. Then, in the Donnan model, \( p_m \) is proportional to the concentration of PG (\( C_0 \) in Fig. 2):

\[ p_m = \beta \times C_0 \]  

(38)

\footnote{Most other direct measurements of PG swelling pressures (Comper and Preston, 1974; Shaw, 1976; Wells, 1972) involved PG concentrations below the range found in articular cartilage of 20–80mg/ml (Maroudas and Bannan, 1981; Urban et al., 1979).}
The proportionality constant, \( \beta \), is obtained from the structure of the chondroitin sulfate GAG assuming \( \sim 86 \) percent of the PG to be GAG. The molecular weight of a dissociated CS disaccharide is \( \sim 458 \) g/mol and on the average, it contains two ionized charge groups at pH 7.4 (Comper and Laurent, 1978; Muir, 1979) so that \( \beta = 0.86 \times 2 \) mol-charge/458gPG = 1 mol-charge/266g PG. The values of \( \rho_m \) at different PG concentrations from Eq. (38) were used in Eq. (9) to predict the ideal Donnan swelling pressure in Fig. 2. (Here, \( \rho_m \) is the fixed charge density based on total solution volume. By introducing some molecular level structure, \( \rho_m \) in the Donnan model could be based on fluid volume, which is more appropriate. This correction would be a function of concentration and would increase \( \rho_m \) by 1–10 percent, further overpredicting the measured pressures in Fig. 2.)

In the PB-cell model, the GAG radius was set to \( a = 0.55 \) nm (Ogsten et al., 1973) and the interchange distance \( b \) to 0.64 nm (Comper and Laurent, 1978; Preston et al., 1972). This interchange distance, 0.64 nm, corresponds to a value obtained experimentally for chondroitin sulfate (Preston et al., 1972), which compares with 0.51 nm for a structural model (Comper and Laurent, 1978; Preston et al., 1972) and 0.8 nm from an earlier experiment (Nagasawa and Kagawa, 1957). The chondroitin sulfate radius, 0.55 nm, is the value reported most often from sedimentation and diffusion studies (Ogsten et al., 1973), and lies in the middle of the 0.3 to 0.9 nm range reported for a variety of different GAGs and synthetic polymers (e.g., hyaluronate, dextrins, polyacrylamide, etc.) (Ogsten et al., 1973). The cell radius, \( R \), is determined from the PG concentration, by assigning a cylindrical volume of length \( 2b \) and radius \( R \) to each CS disaccharide,

\[
R = \left( \frac{\pi 2bN C_{\text{PB}}_{M_c}}{M_c} \right)^{1/2} \tag{39}
\]

where \( N = 6.02 \times 10^{23} \) is Avogadro's number, \( M_c = 458 \) g/mol is the molecular weight of a dissociated CS disaccharide, and \( C_{\text{PB}} \) in Eq. (39) is the PG concentration in g/m³. Given \( a \) and \( b \) as the quoted literature values above, and \( R \) as a function of PG concentration from Eq. (39), the PB equation (Eq. (17)) subject to the boundary conditions (Eqs. (18) and (19)) was numerically solved, and the swelling pressures found from Eq. (25).

The concentrations of counter-ions and co-ions are shown in Fig. 3 calculated from the PB model (Eq. (15)) and from the Donnan model (Eq. (5)). These calculations correspond to PG concentrations of 30 and 70 mg/ml. In the ideal Donnan model, the swelling pressure is computed from these spatially invariant concentrations (Eq. (9)), while in the PB-cell model, the swelling pressure can be computed from the values at the outer cell boundary (Eq. (24)). Since the counter-ion concentration predominates, the lower values of \( C_{Na^+}(R) \) from the PB-cell model compared to the Donnan \( C_{Na^+} \) in Fig. 3 are consistent with the lower value of swelling pressure predicted by the PB-cell model in Fig. 2.

The sensitivity of the calculated pressures to the values of the model parameters is shown in Fig. 4. The curve shown for \( \beta = 1 \) mol-charge/433g (Fig. 4(a)), is the best fit of the ideal Donnan model; this value of \( \beta \) is \( \sim 1/2 \) that obtained from the known CS GAG charge, assuming that 86 percent of the PG is CS GAG (Kimura et al., 1984). The curves of Fig. 4(a) also show that the ideal Donnan model cannot simultaneously predict lower pressures and the very steep increase in pressure with PG concentration. In contrast, the PB-cell model does simultaneously predict lower pressures and the nonlinear pressure-concentration behavior for reasonable values of model parameters (Fig. 4). This may be an important functional property of PG since the ability of a tissue to resist load in equilibrium is related more to the increase in pressure with compressive strain (or PG concentration) than to absolute pressure. The steeper the increase in pressure with PG concentration, the more the tissue is able to resist compression. The shape of the pressure vs. concentration curves in Fig. 4(b) is a consequence of the repulsive interaction between two highly charged surfaces spaced several Debye lengths apart in an electrolyte solution. The macroscopic Donnan model does not similarly predict this molecular-level behavior.

**Cartilage Equilibrium Modulus.** The equilibrium confined compression modulus, \( H_A \), of adult bovine articular cartilage has been measured in uniaxial confined compression for specimens equilibrated in NaCl baths ranging from 0.005 to 1.0 M (Eisenberg and Grodzinsky, 1985). The data (Fig. 5) show that at neutral pH, \( H_A \) is relatively insensitive to NaCl concentration at the highest concentrations used. This suggested (Eisenberg and Grodzinsky, 1985) that the electrostatic contribution to \( H_A \) was essentially shielded by
1.0 M NaCl, and that such electrostatic repulsive interactions between GAG charge groups account for at least half the modulus at physiological ionic strength. Thus, the modulus at 1.0 M NaCl approached an asymptote which is numerically equivalent to the nonelectrostatic contribution to the modulus, $H^*_0$. As the ionic strength is lowered, electrostatic repulsion forces between neighboring CS molecules come into play and add an electrostatic component, $H^*_e$, as indicated in Fig. 5. The modulus itself is then the sum of these two components,

$$H = H^*_0 + H^*_e.$$  \(40\)

$H^*_e$ can be estimated using the Donnan and PB-cell models. The measurements of Fig. 5 were performed at strains between 10 and 25 percent, and the stress-compression curves were seen to be essentially linear in this range (Eisenberg and Grodzinsky, 1985). The electrostatic component of the modulus, $H^*_e$, was found from both models by computing the swelling pressures at 10 and 20 percent compression and then using the relation,

$$H^*_e = \frac{P(e = 0.2) - P(e = 0.1)}{\Delta e = 0.1},$$  \(41\)

where $e$ is the compressive strain and $P$ is obtained from Eqs. (9) and (25), for the Donnan and PB-cell models, respectively. The theoretical calculations of $H^*_e$ are compared to the data in Fig. 5. An assumption inherent in Eq. (41) is that any change in tension in the collagen network when the tissue is compressed from 10 to 20 percent compression is not a function of the osmotic swelling pressure (a change in tension is assumed to be ionic-strength independent and its effect would be considered as part of $H^*_0$). In the Donnan model, compressive strain increases the pressure by increasing the fixed charge density, $\rho_m$,

$$\rho_m = \frac{\rho_{m0}}{(1 - \epsilon)},$$  \(42\)

where $\rho_{m0}$ is the fixed charge density at zero strain. In the PB-cell model, compressive strain reduces the cell radius, $R$, through the relation,

$$R = R_0\sqrt{1 - \epsilon},$$  \(43\)

where $R_0$ is the cell radius at zero strain.

The chi-square statistic, $\chi^2$, for each model is defined as,
the Donnan model. Figure 6 shows the sensitivity of the predicted moduli to the fitted parameters in each model. The PB-cell model is very sensitive to the best fit $R_0$ (Fig. 6(b)) due to the nonlinearity of the PB equation, and the fact that the solid volume ratio, $V_s = a \sigma^2 / R^2$.

Activity and Osmotic Coefficients. The ideal Donnan model was acceptable in determining bulk small ion partitioning between the polyelectrolyte phase and the bath. However, the ideal Donnan model was seen to be significantly less accurate than the PB-cell model in the calculation of electrostatic swelling pressures and moduli. Therefore, activity and osmotic coefficients were computed from the PB-cell model (Fig. 10), after its numerical solution, to use in the nonideal Donnan model. When swelling pressures and average ion concentrations are calculated using the nonideal Donnan model with these coefficients calculated from the PB-cell model, the predicted pressures and average concentrations are necessarily identical to those of the PB-model (see Appendix II). Therefore the complexity involved in numerically solving the PB equation may be avoided by utilizing the nonideal Donnan model with the activity coefficients of Fig. 10.

Discussion

The range of PG concentration found in adult bovine articular cartilage is 20–80 mg/ml (Comper, 1991; Maroudas and Bannan, 1981; Urban et al., 1979). Given the good agreement between the PB-cell model prediction, with no adjustable parameters, and the measured pressures (Fig. 2) it would appear that within this concentration range, the swelling pressure of the PG is predominantly of electrostatic origin and is well described by the interactions included in the PB-cell model. At 30mg/ml the cell boundary radius from Eq. (39) is $R = 2.51$ nm, resulting in a surface to surface distance between adjacent GAGs of $\sim 5$ Debye lengths (1/$\kappa = 0.8$ nm from Eq. (20)). This is the critical distance of separation since significant electrostatic repulsion forces will only occur when intermolecular distances are $\sim 5$ Debye lengths or less. The steep rise in pressure predicted by the PB-cell model between 30 and 70mg/ml (Fig. 2), from 11 kPa to 173 kPa, is a consequence of decreasing the distance between these highly charged surfaces from $\sim 5$ Debye lengths at 30 mg/ml to $\sim 2.8$ Debye lengths at 70 mg/ml (as predicted through Eq. (39)). The predictions of the PB-cell model slightly underestimate the swelling pressures at 70mg/ml, and at 30mg/ml and below (Fig. 2). At PG concentrations below 30 mg/ml the repulsive electrostatic interaction becomes relatively weak. The entropic and excluded volume contributions to the swelling pressure, which are not calculated here, are then likely to become significant and exceed the electrostatic component. At 70mg/ml, the discrepancy between the PB-cell model and the measured pressure may be due to the excluded volume contribution, significant ion-ion correlations, or hydration forces (Rau and Parsegian, 1990) not accounted for in the PB-cell model. In light of the PB-cell model, the disagreement between the predictions of the Donnan model and the measured pressures (Figs. 2 and 4(a)) is likely due to the absence of molecular structure and molecular level electrostatic fields in the Donnan model (i.e., a spatially invariant electrostatic potential within the solution phase (Fig. 1)). In particular, the very steep nonlinear increase in electrostatic swelling pressure with PG concentration (beginning at 20mg/ml), is independent of the model parameters used for Fig. 4, and can only be described by the PB-model which incorporates molecular level spacings and electric fields, Fig. 4. Donnan theory has also been seen to predict higher pressures than those that are measured for other GAGs (hyaluronate and heparin) (Pittsche and Reed, 1992), this phenomenon has been termed the Hammarsten effect (Overbeek, 1956).

The PB-cell model also predicted the cartilage modulus data more accurately than the Donnan model (Figs. 5, 6). The fixed charge density for adult bovine articular cartilage is in the range (0.1 to 0.3 mol/liter tissue water) (Frank et al., 1990; Lesperance et al., 1992; Maroudas et al., 1991). The best fit value of the zero-strain fixed charge density in the Donnan fit $\rho_{P0}/F$ was $= 0.206$ M. The value computed from the PB-cell fit of Fig. 5 is $\rho_{P0}/F = 0.246$ M, using

$$\rho_{P0} = \frac{2a \sigma}{(R_0^2 - \sigma^2)}.$$  

Both of these values of fixed charge density are therefore within the range determined by titration (Frank et al., 1990), radiolabel equilibration (Maroudas, 1979) and more recently by NMR spectroscopy (Lesperance et al., 1992). Using Eq. (39), the PG concentration corresponding to the best fit zero-strain cell radius ($R_0 = 1.91$ nm) is 52mg/ml, which is within the 20–80mg/ml range found in bovine articular cartilage (Comper, 1991; Maroudas and Bannan, 1981; Urban et al., 1979). Although the PG content of the cartilage disks used in these experiments was not measured (Eisenberg and Grodzinsky, 1985), later measurements (Sah, 1991) in a similar tissue using the dimethylmethylen blue dye assay resulted in values near 30 mg/ml tissue water). The higher predicted PG concentration of 52 mg/ml is suggestive of the observation that PGs are excluded from the tissue's collagen volume, increasing its effective concentration in the extrafibrillar space (Grynpas et al., 1980; Maroudas et al., 1991). Also, the solid volume fraction $V_s = \sigma / R^2 = 0.083$ compares well with solid volume fractions computed previously ($V_s = 0.09$) (Eisenberg and Grodzinsky, 1986) by taking into account the different amounts of water in the proteoglycan compartment ($\sim 65$ percent total water) and the collagen compartment ($\sim 35$ percent total water) in articular cartilage (Grynpas et al., 1980; Maroudas et al., 1991).

Electrostatic Potentials. When $1/\kappa >> R$ the two models could be expected to behave in similar fashions since the flat Donnan potential will be a closer approximation to the actual microscopic potential. However, when $1/\kappa < R$ the potential of the PB-cell model will vary steeply in the radial direction as seen in the potentials of Fig. 7 for 30mg/ml and 70mg/ml PG in 0.15 M NaCl. At 0.15 M ionic strength, $1/\kappa = 0.8$ nm, and the outer cell boundary at 70 mg/ml in $R = 1.64$ nm $= 2$ ($1/\kappa$). Hence, a flat Donnan potential would not be expected to reasonably match the microstructural electric potential. Swelling pressures (Fig. 2) and electrostatic potentials (Fig. 7).

![Fig. 7 Electric potential within the PB unit cell and the homogenous Donnan phase, corresponding to the swelling pressures and ion concentrations of Figs. 2 and 3 where $V_s = a \sigma / F = 25.3 mV$](image-url)
can be further related by considering together Eqs. (9) and (25) and recognizing that the Donnan potential is defined implicitly by:

$$
\left( \frac{\rho^2}{4F^2C_0} + 1 \right)^{1/2} = \cosh \left( \frac{F\Phi_D}{kT} \right)
$$

Then it can be seen that the lower pressures in the PB-cell model are due to the boundary potential $\Phi(R)$ always being lower than the Donnan potential $\Phi_D$ (Overbeek, 1956) (Fig. 7). Furthermore, the PB-cell model accounts for the very strong nonlinearity of the pressure with concentration (Fig. 2) because the boundary potential, $\phi(R)$, undergoes relatively larger increases than the Donnan potential when PG concentration is increased (R is reduced in the PB-cell model; $\rho_0$ is increased in the Donnan model). In Fig. 5, the Debye length becomes shorter with increasing ionic strength, making the flat Donnan potential approximation less valid. Above $C_0 = 0.025M$ (the approximate point of divergence of the two models in Fig. 5), $\lambda < R$. The low ionic strength region ($C_0 < 0.025M$) is not well described by either model. One possible explanation is that the assumption regarding collagen tension between 10 percent strain and 20 percent compression in Eq. (41) is less accurate at low ionic strength when the swelling pressures in the PG phase will be very high, tending to stress the collagen network more than at higher ionic strengths.

**PG Strain Versus Tissue Strain.** In Eq. (43), bulk strain in uniaxial confined compression is assumed equal to strain on the unit cell. Recent studies (Maroudas et al., 1991) have suggested that during compression, fluid may not be lost from collagen and PG compartments by equivalent amounts. If collagen intracellular water ($\sim 35$ percent total water (Grynopoulos et al., 1980; Maroudas et al., 1991)) is not expressed, then the strain on the PG compartment would be greater than the average tissue strain (because the collagen strain is zero) by a factor $\lambda = 1/0.65 = 1.54$. The contribution of electrostatic repulsion to the modulus would be enhanced because of the larger strain in the PG compartment. A fit was performed (Fig. 8) which was identical to that shown in Fig. 5 but with a factor $\lambda$ inserted into Eq. (43), $R = R_0/(1 - \lambda)$. We chose $\lambda = 1.3$ to represent an intermediate situation where water was preferentially expressed from PG but still partially from collagen, and $\lambda = 1.6$ to represent the case where no water is lost from the collagen. The model fit the data better when $\lambda$ was increased above 1, even in the low ionic strength range. This resulted from increasing the best fit zero-strain boundary $R_0$, which gives lower absolute pressures (Fig. 8), but maintains the large increase in pressure with strain (which constitutes the modulus).

**Swelling Pressures Versus Modulus.** The calculated swelling pressure at 10 percent strain for the best fit computations of the two models shown in Figs. 5 and 6 is plotted in Fig. 9. Unlike the case of PG solutions, this swelling pressure cannot be measured directly since there are restraining tensile stresses in the tissue (usually associated with the collagen network) which have not been quantified. The swelling pressure predicted by the Donnan model is always higher than that of the PB-cell model. In particular, at physiological ionic strength (0.15M), the Donnan pressure (201 kPa) is twice the PB-cell pressure (99 kPa). Even though the PB-cell model predicts lower absolute swelling pressures it still allows for a very significant electrostatic contribution to the modulus. For example, the swelling pressure increases from 99 kPa at 10 percent compression to 139 kPa at 20 percent strain yielding

---

Journal of Biomechanical Engineering

MAY 1995, Vol. 117 / 189
an electrostatic contribution to the modulus of 399 kPa from Eq. (41) (higher than the measured value of 286 kPa but within the standard deviation range). In contrast, the Donnan model requires swelling pressures of 201 kPa at 10 percent strain and 247 kPa at 20 percent strain to produce an electrostatic contribution to the modulus of 464 kPa, outside the measured range in Fig. 5. The divergence of the Donnan model from the PB-cell model is less drastic in the computation of the modulus than the pressure since the modulus is generated from increases in the swelling pressure with strain which can be less sensitive than the absolute pressure to the details of the microscopic curvature of the potential. The PB-cell model yields substantial electrostatic contributions to the modulus with only ~1/2 the absolute swelling pressures (a reflection of Fig. 2). Functionally, it is important that the modulus of cartilage be high (not the swelling pressure) in order to resist compression. The ability of a molecular-level model to simultaneously predict both the observed high values of the modulus and lower swelling pressures is consistent with these important attributes of GAG-associated electrostatic forces in forming the functional properties of cartilage.

Molecular-level models of intermolecular forces can be used not only to account for bulk material properties, but also for giving insight into the environment of an individual cell in connective tissues. In place of a diffuse pressure acting within the tissue, discrete forces can be considered which act on a limited distance scale. The electrostatic forces modeled in this paper, for example, are only active at a distance within a few nanometers from the charged molecule. Such considerations may be important in conceptualizing the types of mechanical signals which could affect cell behavior. Extensions of the model used here have suggested that the presence of divalent ions in a spatially ordered system can produce a collapse of the charged molecules to a condensed state due to long range correlations between the mobile divalent ions (Guilbrard et al., 1986). There appears to be significant advantages to molecular level modeling of physical forces in the understanding of a variety of biological phenomena.

Conclusions

A PB unit cell approach was used to develop a microstructural model of electrostatic and osmotic interactions between adjacent glycosaminoglycans in an aqueous electrolyte and to predict (a) the osmotic swelling pressures of proteoglycan solutions at physiological concentrations, and (b) the electrostatic component of the equilibrium modulus of articular cartilage. With no adjustable parameters in the calculation, the PB-cell model accounted for the very steep increase in pressure with the concentration of PG. In contrast, the ideal Donnan model overestimated the pressure by up to three-fold and was unable to account for the very nonlinear pressure versus concentration behavior. The PB-cell model also quantitatively fit the ionic strength dependence of the confined compression equilibrium modulus of articular cartilage (Eisenberg and Grodzinsky, 1985) for a reasonable choice of one adjustable parameter. There are certain phenomena that can be very well described by the Donnan model. It is known, for example, that the Donnan model can accurately predict ion partition coefficients in tissue (Marchiadas et al., 1988, Flory et al., 1988), and it is much simpler to use than the PB model. However, the Donnan model appears less accurate than the PB-cell model in predicting electrostatic swelling pressures of polyelectrolyte solutions and the electrostatic component of the modulus of highly charged connective tissues. Here, the question is whether a model that incorporates an important microstructural feature of the matrix can predict and give insights on how electrostatic forces are organized at the molecular level to provide the matrix with specific biomechanical properties. Since the Donnan model does not include microstructural features by design, it can not be expected to predict the same functional behavior as the PB model when the distance between GAG chains is on the order of a Debye length. Activity and osmotic coefficients computed from the PB-cell model in this paper may be used in the nonideal Donnan model to improve the accuracy of the Donnan model without necessitating again the numerical solution of the PB equation. These computations with the molecular PB model suggest an important functional role for electrostatic repulsion in aiding articular cartilage to resist load by supplying a large electrostatic contribution to the equilibrium modulus with relatively low absolute swelling pressures.

Acknowledgments

This research was supported by NIH Grant AR33236 and AFOSR Grant 91-0153. MDB also received fellowship support from the Whitaker Health Sciences Fund and the Natural Sciences and Engineering Research Council of Canada. The authors thank Drs. Eliot Frank; Peter Baxeser, Young-jo Kim, and Tom Quinn for many helpful discussions and critical comments.

References


APPENDIX I

Calculation of Cell Boundary: R

Equation (39) has been found to agree with an experimentally observed correlation length in small angle neutron scattering of polyelectrolytes (Rinaudo, 1978). It was proposed that this observation provides support for the isotropic cell model of linear polyelectrolytes (Rinaudo, 1978). An alternative method of calculating R (Park et al., 1988) is by use of the partial specific volume, \( \upsilon_s = 0.49 \text{ml/g} \) (Williams and Comper, 1990), such that

\[
\frac{a^2}{R^2} = \upsilon_s C_p g
\]

Eq. (47) can lead to much larger cell boundaries (and lower swelling pressures) than Eq. (39). One consideration involved in the choice of either Eq. (39) or Eq. (47) to calculate R from \( C_p \) is the technique used to measure \( a \), the GAG radius. Typically, it is measured by sedimentation or diffusion (Oppen et al., 1973) and is therefore an equivalent hydrodynamic radius (Engel and Furthmayr, 1987; Tanford, 1961).
The experimentally derived \( A \) would then include any solvent associated with particle solution, which is likely to be near a monolayer of water (Fletcher et al., 1988; McLaughlin, 1989) (of thickness \( \sim 0.25 \text{nm} \)), accounting for approximately 70 percent of the solvated particle volume. The partial specific volume characterizes the volume of the unsolvated particle, whose radius is unknown (but likely closer to 0.3nm than 0.55nm). Therefore, with a hydrodynamically measured \( A \) an Eq. (47) may be less consistent than Eq. (39). It is interesting to note that a radius of 0.3nm was seen to more closely predict ion partition data than a radius of 0.55nm when Eq. (47) was used (Parker et al., 1988). It was also previously observed (Parker et al., 1988) that the partition coefficients calculated from the PB-cell model are very sensitive to the exact choice of \( A \) when Eq. (47) was used. The osmotic pressure is even more sensitive to choice of parameter values than the partition coefficients because it is related to the potential at \( R \) rather than an average throughout the volume of the cell. Here, however, this sensitivity is partially removed by using Eq. (39) instead of Eq. (47), where an increase in \( A \) will not increase the cell boundary. This is apparent in Fig. 8(c). Equation (47) can be seen to be equivalent to Eq. (39) using \( v_r = 1.60 \text{mol/g} \) if \( A = 0.55 \text{nm} \), and \( b = 0.64 \text{nm} \).

**APPENDIX II**

**Activity and Osmotic Coefficients**

The PB-cell model does not require the use of activity and osmotic coefficients (which are empirical quantities (Overbeek, 1956)) in order to account for the swelling pressure of protolyte solutions (Fig. 2) or the electrostatic component of the equilibrium modulus (Fig. 5). However, the PB-cell model can be used to calculate activity and osmotic coefficients for use in a nonideal Donnan model (Marcus, 1955). Hence a theoretical foundation based on structural parameters exists for the "nonideal" behavior often seen with highly charged polyelectrolytes (Overbeek, 1956). Once obtained in this manner, the nonideal Donnan model is mathematically simple to use. The resulting electrostatic swelling pressures will then be necessarily identical to those computed from the PB-cell model directly; however, repeated numerical integration of the PB equation is not required. Figure 10(a) shows the activity coefficients and the osmotic coefficient, calculated from the PB-cell model using Eq. (35) to (37), for the range of PG concentration tested. Since the co-ion is excluded, its activity coefficient (\( \gamma^- \)) is increased from ideality (\( \gamma^-
\)) 1), the counter-ion is attracted and its activity coefficient (\( \gamma^+ \)) is reduced. The mean activity coefficient for the salt, \( \gamma_{s} \), remains nearly constant within the range 0.94 < \( \gamma^- \) < 0.97. Given that the PB-cell model is equilibrated with an ideal bath, the mean activity coefficient computed here corresponds to the ratio (\( \gamma_{s}/\gamma^+ \)) in the Donnan model (Eq. (1)). The computed value is reasonable considering values obtained from measurements of partition coefficients of 0.89 < \( \gamma^- \) < 0.95 (Maroudas, 1979). Since the expression for ideal Donnan equilibrium (Eq. (3)) only approximates the mean activity coefficient, \( \gamma_{s} \), as unity, this assumption will lead to relatively small errors in the order of \( (1/\gamma_{s}) < 1 < 10 \text{ percent} \) in underestimating the intratissue concentrations, when compared to the PB-cell model.

The assumption of ideality in the expression of swelling pressure (Eq. (9)), however, requires that individual activity coefficients, \( \gamma^+ \) and \( \gamma^- \), be unity according to (Eq. (37)), which does lead to large errors in the computed swelling pressure. Because the counter-ion concentration is enhanced, the reduction in its activity coefficient is of greater consequence than the increase in activity coefficient of the co-ion, resulting in much lower pressures, consistent with considerations based on electrostatic potential profiles above. These lower pressures can also be inferred from the osmotic coefficient, \( \Phi \), which is the ratio of outer cell boundary ion concentration to average cell ion concentration (Eq. (37)). It is a weighted average of counter-ion and co-ion activity coefficients, weighted by the average counter-ion and co-ion concentrations, and decreases substantially from ideality (\( \Phi = 1 \)) due to the predominance of counter-ions and their reduced activity coefficients. Figure 2 shows substantial deviations of the ideal Donnan prediction at low concentrations where (\( \Phi = 1 \)). This is due to the relatively small swelling pressures at these concentrations so that small deviations of \( \Phi \) from ideality create large changes in swelling pressures according to Eqs. (6), (7), and (8) with \( \Phi = 1 \). Figure 7 can also be related to the failure of the assumption of ideality in the calculation of swelling pressures and the relative success of the assumption of ideality in determining ion partitioning. The latter is true because average ion concentrations are a weighted average of the potential in the cell (i.e., close to the Donnan potential; consider Eqs. (36) and (15) and Fig. 7), while the former is a function of the minimum potential in the cell, the potential at the cell boundary. The ideal Donnan model can therefore successfully approximate spatially averaged ion distributions but not osmotic swelling pressures.

The activity and osmotic coefficients computed here, \( \gamma^+ \) and \( \gamma^- \) and \( \Phi \), could be used to account for non-ideality in Donnan equilibrium given the apparent success of the PB-cell model in accounting for the measured pressures. The activity coefficients are relatively constant at (\( \gamma^+ = 0.72 \)), \( \gamma^- = 1.22 \) for \( C_{pg} > 30 \text{mg/ml} \) and the ionic strength kept constant at 0.15M. In general, however, these activity coefficients are functions of all 4 parameters in the PB-cell model. To be accurate, one would have to also compute \( \rho_m \) slightly differently than is done in Eq. (38) using the value of \( \beta \) for CS, \( \beta = 1 \text{mol/229g} \), and basing the charge density on fluid volume, not tissue volume, so that,

\[
\rho_m = \beta \frac{R^2}{R^2 - a^2} \times C_{pg}
\]

where \( R \) is determined by Eq. (39). When the activity and osmotic coefficients of Fig. 10 are incorporated into the nonideal Donnan model, the predicted swelling pressure necessarily coincides with that predicted by the PB-cell model.

Figure 10(b) shows the activity coefficients and osmotic coefficient computed from Eqs. (35) to (37) for the PB-cell best fit to the modulus data. Since 0.93 < \( \gamma^- < 0.98 \) it is clear that the assumption of ideality in Donnan equilibrium will not lead to large errors for the average intratissue ion concentrations or equivalently for ion partition coefficients when compared to the PB-cell model. Once again, however, the assumption of ideality in the expression for swelling pressure will lead to more serious errors. Even at high ionic strength, \( C_{pg} = 1 \text{M} \), the fact that \( \gamma^- = 0.971 \) may lead one to think that the assumption of ideality would be sufficient. However, it leads to even larger fractional errors than the low salt case, which should be expected from \( 1/\gamma^- \times R \).