Ion mediated cross-shield driven mucous swelling kinetics

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Abstract

We present an experimentally guided, bi-phasic, multi-species ionic gel model to compare and make quantitative predictions on the viscoelastic properties of healthy mucus versus Cystic Fibrosis infected mucus. The mixture theory consists of the mucus (polymer phase) and water (solvent phase) as well as several different ions: H\textsuperscript{+}, Na\textsuperscript{+}, Cl\textsuperscript{−} and Ca\textsuperscript{2+}. The model is calibrated with the experimental data of mucus diffusivities. Numerical studies reveal that the Donnan forces are the dominating mechanism driving the mucus swelling/deswelling transition. However, these transitions could be continuous or discontinuous, depending on the complex interplay between ionic bath concentrations, pH, polymer mass in the solvent and the average charge per monomer.

Keywords: Donnan potential, hydrogel, Cystic Fibrosis, diffusivity

1 Introduction

Mucous are glycoproteins of gigantic dimensions, with thousands of amino acid residues per monomer and molecular weight in millions [Kuver et. al, 2000]. Their primary structure consists of a linear peptide backbone, to which are attached short polysaccharide side branches [Perez-Vilar, 2007]. Mucus plays a critical role as a protective, exchange and transport medium in the digestive, respiratory and reproductive systems of humans and other vertebrates [Verdugo, 1984]. Naturally, its swelling mechanism are of special interest, e.g. in describing the locomotion of single cell invertebrates [Wolgemuth et al, 2004] and in understanding the pathophysiology of respiratory diseases like cystic fibrosis (CF) [Barasch et. al, 1991].

Major experimental findings explain mucus swelling as a balance between the osmotic forces, created due to the difference in the solvent concentration across the mucus mesh, and Donnan driving forces [Tam and Verdugo, 1981]. It is argued that the ‘ensemble of entangled polymer mesh’ prevents the residual charges on the mucin chains from migrating out of its matrix and hence the polymer entangles virtually functions as its own semipermeable membrane [Verdugo, 1998]. However, the osmotic pressure difference could not explain the recent experimental findings of the massive and the explosive post-exocytotic swelling observations [Verdugo et. al, 1987]. Furthermore, while it is certain to have some Donnan-like effect if there are rapid spatial changes in the polymer concentration, overall, one expects
electro-neutrality to be enforced, with the result that the electrical potential is not a significant driving force for mucus hydration [Wolgemuth et al., 2004]. Hence we suggest that, instead, the mucus swelling might be due to the steric repulsive interaction within its poly-ionic chains, caused due to a change in its cross-shielding structure during the hydration process.

A mucus gel has negatively charged poly-ionic chains and in a neutral medium all charges must be balanced cations. There are several possible cations, including sodium, calcium, potassium and hydrogen. The important difference between these is that calcium is divalent and so it must shield two negative charges rather than one. Hence, the divalent Ca$^{2+}$ ions act as a cross shielding agent between two polymer strands, allowing much tighter condensation than when the negative charges of these network are shielded by monovalent ions, e.g. Na$^+$. An exchange of the divalent ions with the monovalent ions, unshields the polyionic charges of the mucins, driving the mutual repulsion of the polymer chains (see Fig 1a) and triggering a quick expansion of the mucin network [Crowther and Marriott, 1983].

If this is the swelling mechanism, then it follows that a change in the ionic affinity of the mucins or changes in the ionic concentrations in the medium, drastically alters the condensation/hydration process. It has been confirmed experimentally that an incomplete mucus hydration is a result of either a faulty electrolytic composition of water on the mucosal surface [Baconnais et. al, 2005] or an increased mucin sulfation leading to a higher affinity for cations [Cheng et. al, 1989].

Early theories have addressed the mechanical aspects of mucus gel swelling by treating it as a neutral, linear, elastic solid immersed in a viscous fluid [Tanaka and Fillmore, 1979; Tanaka et al, 1980]. The characteristic time of swelling was shown proportional to the square of the linear dimension of the gel and inversely proportional to the diffusion coefficient of the gel network, also termed as the diffusivity. More recently, Wolgemuth and colleagues [Wolgemuth et al., 2004] describe the hydration dynamics of poly-ionic gels by appealing to a Flory-Higgins free energy model [Flory, 1953]. However, the flory interaction parameter in their model is designed to just approximate the swelling of neutral gels. Further, the diffusivity is calculated by linearizing their constitutive equations about an initial isotropic volume fraction, and not the final (equilibrium) conditions.

2 Model

Drawing on the work cited above, we suggest a simplified model by uniting the mucus gel-ionics with a two phase fluid dynamics. The polymer and the solvent phase (described by their respective volume fractions $\theta$ and $1-\theta$) satisfy their respective conservation and force balance equations in the low reynolds number limit, such that the total volume of the system is conserved. Readers are requested to refer [Sircar et. al, 2011] for the details.

The solvent may contain a variety of ionic species. It is easy to include all of these ions (e.g. Ca$^{2+}$, H$^+$, K$^+$, Cl$^-$ [Verdugo et. al, 1998]) and study their combined effect. However, in this setup, we assume that there are just two cations, the divalent calcium, Ca$^{2+}$, and the monovalent sodium, Na$^+$, and two anions, the mucus gel monomer, M, (a segment of the mucus containing a single negative charge) and chloride, Cl$^-$. Because of the requirement for charge neutrality, it must be that

$$[M] + [Cl^-] = 2[Ca^{2+}] + [Na^+] .$$ (1)
The shielding charges can be exchanged according to

\[ \text{M}_2\text{Ca} + 2\text{NaCl} \xrightleftharpoons[k_+]{k_-} \text{CaCl}_2 + 2\text{NaM.} \quad (2) \]

with \( k_\pm \) the forward/backward reaction rate constants. Fig. 1b shows a schematics of this displacement reaction. If we let \( x = [\text{M}_2\text{Ca}] \), \( c = [\text{CaCl}_2] \), \( u = [\text{NaM}] \) and \( n = [\text{NaCl}] \) be concentrations of the species in moles per total volume, then the forward and the backward reaction rates are

\[ R_+ = k_+ xn^2, \quad R_- = k_- cu^2. \quad (3) \]

In a chemical equilibrium, \( R_+ = R_- \), or

\[ \left( \frac{\gamma \theta}{1 - \theta} m^2 + u - m \right) = \left( \frac{\gamma \theta}{1 - \theta} (1 - \alpha)^2 - \alpha \right) = 0, \quad (4) \]

where \( u = (1 - \alpha)m \), \( m = 2x + u \) being the total mucus concentration and \( \alpha = \frac{2x}{m} \) is the fraction of the mucus shielded by the divalent calcium ions. The gel chemistry parameter, \( \gamma = 2\frac{K_{eq}c_b}{\nu_m N_A \nu_m} \) where \( K_{eq} = \frac{k_+}{k_-} \), \( N_A \) is the Avogadro’s number, \( \nu_m \) is the mucin monomer volume and \( n_b = \frac{n}{1-\theta}, c_b = \frac{c}{1-\theta} \) are the concentrations of NaCl and CaCl\(_2\) in the ionic bath, respectively. We assume that the size of the ionic bath is large compared with the size of the gel, and so that \( n_b \) and \( c_b \) are fixed parameters.

Further, in a radially symmetric mucus blob (with radius \( r \)) immersed in a pure solvent, the interface between the mucus gel and the pure solvent moves such that it satisfies the interface condition

\[ (\sigma_p - \sigma_s) \cdot \hat{r} = \frac{k_B T_0}{\nu_m} \frac{\partial f}{\partial \theta} \quad (5) \]
where \( \sigma_{p,s} = \frac{2\nu_m}{3}(\nabla v_{p,s} + \nabla v_{p,s}^T) + \lambda_{p,s}(\nabla \cdot v_{p,s})I \) are the polymer and the solvent viscous stress tensors with \( \eta_{p,s}, \lambda_{p,s} \) and \( v_{p,s} \) being their respective viscosities and the radial component of the velocities, \( T_0 \) is a reference temperature determined by the experimental conditions and \( k_B \) is the Boltzmann constant. \( f \) is the total non-dimensional free energy density, given by

\[
f(\theta, \alpha) = \frac{T}{T_0} \left( \frac{\theta}{N} \ln(\theta) + (1 - \theta) \ln(1 - \theta) \right) + \frac{1}{2} \left( z(\epsilon_2 - \epsilon_1) + \epsilon_3 \alpha \right) \theta + \frac{1}{2} \left( z(\epsilon_2 + \epsilon_1) - \epsilon_1 \alpha \right) \theta(1 - \theta). \tag{6}
\]

where the non-dimensional interaction parameters, \( \epsilon_{1,2,3} \), measure the relative interaction strength among the various species (cross-shielded monomers, uncross-shielded monomers and the solvent molecules), \( z \) is the coordination number and \( N \) is the number of monomers in one mucin chain (arbitrarily fixed to a large value). The mucus-solvent equilibrium is obtained if the forces at the interface disappear, (Eqn. 5), i.e.,

\[
\frac{\partial f}{\partial \theta} = 0. \tag{7}
\]

The time constant of the swelling dynamics, \( \tau_s \), is determined through the linearized analysis of the equilibrium solutions (i.e. the solution of Eqns. 4, 7). Assuming that the variations about the equilibrium \([\theta^*, v_{p,s} = 0, \alpha^*]\) are small, we find the solutions using appropriate eigenfunctions [Sircar et. al, 2011],

\[
\theta = \theta^* + e^\xi f_1(r), \quad v_{p,s} = \frac{g_{p,s}(r)}{r}, \quad \alpha = \alpha^* + e^\xi f_2(r). \tag{8}
\]

where \( f_{1,2}(r), g_{p,s}(r) \) are radially symmetric functions of \( r \). In the limit of fast chemistry (or \( k_s n^2 \rightarrow \infty \)), the eigenvalue \( (\lambda = \lambda^\infty(\beta)) \) closer to zero is given by

\[
\lambda^\infty(\beta) = -y(\beta)\theta^* \left| f_{\theta \theta} + f_{\theta \alpha} \frac{\gamma (1 - \alpha^*)^2}{(1 - \theta^*) (2\gamma \theta^* (1 - \alpha^*) + 1 - \theta^*)} \right|, \tag{9}
\]

where \( f_{\theta \theta} = \frac{\partial^2 f}{\partial \theta^2}, \quad f_{\theta \alpha} = \frac{\partial^2 f}{\partial \theta \partial \alpha}, \quad y(\beta) = \frac{\beta^2 k_B T_0 (1 - \theta^*)}{\nu_m \eta_s \beta^2 + \xi r_f^2}, \quad \eta_c = \eta_s \theta^* + \eta_p (1 - \theta^*), \quad \xi \) is the drag coefficient [Keener et. al, 2011] and \( r_f \) is the equilibrium gel radius. The swelling time constant is determined by the smallest admissible value of \( \beta = \beta_s = 1.7374 \) (satisfying the interface condition, eqn. 5), i.e.

\[
\tau_s = 1/|\lambda^\infty(\beta_s)| = \frac{1}{D} r_f^2 + \kappa. \tag{10}
\]

Thus, the swelling diffusivity, \( D \), is given by

\[
D = \frac{\xi \nu_m}{k_B T_0 \beta_s^2 (1 - \theta^*) \left| f_{\theta \theta} + f_{\theta \alpha} \frac{\gamma (1 - \alpha^*)^2}{(2\gamma \theta^* (1 - \alpha^*) + 1 - \theta^*)} \right|}. \tag{11}
\]

3 Results

Fig. 2h shows the diffusivity (\( D \) ‘vs’ \( \gamma \)) curve passing through the diffusivity data in various swelling literature. The parameters (in eqn. 11) are chosen as \( T/T_0 = 1.0, \epsilon_1 = -1.0, \epsilon_2 = -
0.65, $\epsilon_3 = -10.0$, $N = 1000$, $z = 4$, $\frac{\kappa_0 \xi_2^z}{k_B T_{0\gamma}} = 0.001$ where $l, \tau$ are the characteristic length and time scales (chosen as $l = r_f$ and $\tau = 1$ sec.). The reference temperature, $T_0$ is chosen at 310K (or 37° C) since experiments are reported at this temperature [Verdugo et al. 1987; Kuver et al. 2006].

Verdugo reports that the swelling diffusivity in the mucous from a normal patient changes (or Wild-Type mucous) from $3.48 \times 10^{-5}$ to $2.99 \times 10^{-6}$ cm$^2$/s when the extracellular $[Ca^{2+}]$ changes from 1mM to 4 mM [Verdugo et al. 1987]. These are the points 1 and 4 in Fig. 2b (denoted by the symbol ○). Notice that the gel chemistry parameter, $\gamma \propto [Ca^{2+}]$, is such that $\gamma_4/\gamma_1 = 4$, on this curve. In different experimental conditions, he reports that the diffusivity in the Wild-Type mucous changes from $3.17 \times 10^{-7}$ to $2.56 \times 10^{-7}$ cm$^2$/s when $[Ca^{2+}]$ changes from 1mM to 2.5 mM (points 5,6 on the curve and denoted by the symbol ○). For CF infected mucous, a similar change in $[Ca^{2+}]$, changes the diffusivity from $7.41 \times 10^{-8}$ to $9.11 \times 10^{-9}$ cm$^2$/s (points 7,8 and highlighted by the symbol □). Again, both sets of values is spaced on the curve such that $\gamma_6/\gamma_5 = \gamma_8/\gamma_7 = 2.5$. It is argued that under identical conditions of extra-cellular ionic composition, the decrease in diffusivity between the normal and the CF infected mucous is attributed due to an increased sulfation or a higher affinity for cations within the mucin chains [Cheng et al. 1989] (i.e. when the ionic conditions in the surrounding medium are held constant, then a change in $\gamma$, $\delta \gamma \propto \delta K_{eq}$). This claim is highlighted in Fig 2b, as well, since under identical ionic bath conditions, $\gamma_7/\gamma_5$ (when $[Ca^{2+}] = 1$ mM) = $\gamma_8/\gamma_6$ (when $[Ca^{2+}] = 2.5$ mM). In yet another different experimental conditions, Kuver reports a drop in Wild-Type mucous diffusivity value of $3.12 \times 10^{-5}$ cm$^2$/s to a CF-infected mucous diffusivity value of $1.92 \times 10^{-5}$ cm$^2$/s (points 2,3 on the curve and shown by the symbols ○ and □ respectively) [Kuver et al. 2006].

Almost all articles in gel swelling predict that a spherical mucus ball swells in accordance with the linear theory of swelling of neutral hydrogels, proposed by Tanaka [Tanaka and Fillmore, 1973], which states that the swelling time constant is proportional to the square of the final radius, i.e. $\tau_s = r_f^2/D$. In particular, this theory suggests that a plot of $\tau_s$ ‘vs’ $r_f^2$ passes through the origin ($\tau_s = 0$ when $r_f = 0$). However, the experimental observations suggests otherwise (See, for example, Fig. 5b in [Kuver et al. 2006] and Fig. 4 in [Verdugo, 1998]). It is further argued by Verdugo and colleagues that the diffusivity of the mucus gel network cannot be precisely determined from the slope of $r_f^2$ as a function of $\tau$ using Tanaka’s theoretical formulation since it is not suitable for phase equilibria in gels containing an entangled poly-ionic polymer network, and also because of a rather large degree of swelling observed in these gels (page 218, Verdugo, 1984). The model presented in this paper suggests that a plot of $\tau_s$ ‘vs’ $r_f^2$ need not pass through the origin (i.e. in general $\kappa \neq 0$ in eqn. 10).

Fig. 2b highlights the $\theta^*$ ‘vs’ $\gamma$ volume transition curve corresponding to the curve matching the diffusivity data. While the various mucus swelling literature are inconclusive on whether the volume gel phase transitions are discontinuous (first order transitions) or continuous (second order transitions) [Perez-Vilale, 2007]; based on our match with the limited available experimental data we deduce that this curve is continuous and the transition type is of second order. The two points on the curve in Fig. 2b, at $\gamma = 1.0$ and $\gamma = 25.0$, show a typical swelled and a deswelled volume phase respectively. Note that the volume ratio at these two points $\theta^*_{\gamma=25}/\theta^*_{\gamma=1} \approx 61.3$, is well within the range of experimentally reported values (600 in Verdugo, 1984, 650 in Kuver et al. 2006).
Figure 2: (a) Plot of diffusivity, D, (refer eqn. 11) ‘vs’ the gel-chemistry parameter, γ. The experimental data being matched are given in Fig. 3 in Verdugo et. al. [1987], in Fig. 5b in Kuver et. al. [2006] and in Table 1 in Verdugo, 1998. ‘WT’ refers to Wild-Type or normal mucus and ‘CF’ refers to Cystic-Fibrosis infected mucus. (b) The corresponding equilibrium volume transition curve in the θ∗ ‘vs’ γ plane, passing through the diffusivity data. Parameters (in eqn. 5) are chosen at $T/T_0 = 1.0$, $\epsilon_1 = -1.0$, $\epsilon_2 = 0.65$, $\epsilon_3 = -10.0$, $N = 1000$, $z = 4$. Solid curves denote stable equilibria while dashed curves denote unstable equilibria. Two points chosen on the curve, represent a typical swelled ($\gamma = 1.0$) and a deswelled state ($\gamma = 25.0$). Notice that $\theta^*_1/\theta^*_2 \approx 613$, a ratio within the range of experimentally reported values in Verdugo [1984]; Kuver et. al [2006].
4 Conclusion

In this paper, we have developed a bi-phase, multi-species model to quantify the swelling / deswelling mechanism for polyelectrolyte gels. Using this model we have quantified the effects of the changes in bath concentration of monovalent solute (i.e. [NaCl]), the average charge per monomer on the equilibrium swelled/de-swelled configuration. We learned that, generally speaking, increasing the bath concentration of the ion species as well as the cross-link fraction of the gels per volume of total solution leads to deswelling while increasing the average charge per monomer leads to swelling, in agreement with experimental observations. However, because of complex interactions between competing forces (e.g. Donnan forces which aids swelling and the elastic forces from covalent cross-links which helps de-swelling), the swelling/de-swelling mechanism is non-linear (or hysteretic) exhibiting a first order (or discontinuous) transition or a second order (or continuous) transition in many situations. A change in the solute concentration leads to changes in the equilibrium swelling of the gel which is either irreversible (in the case for first order transition) or reversible (for second order transitions).

Acknowledgment and Disclaimer

The authors thank Dr. R. Kuver for useful discussions and the image in Fig. 1a. This research is partially supported by National Science Foundation through grants DMS0540779 and DMS0718036 and by NIGMS grant R01GM090203.

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