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Modeling of Active Transmembrane Transport in a Mixture Theory Framework

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Abstract

This study formulates governing equations for active transport across semi-permeable membranes within the framework of the theory of mixtures. In mixture theory, which models the interactions of any number of fluid and solid constituents, a supply term appears in the conservation of linear momentum to describe momentum exchanges among the constituents. In past applications, this momentum supply was used to model frictional interactions only, thereby describing passive transport processes. In this study, it is shown that active transport processes, which impart momentum to solutes or solvent, may also be incorporated in this term. By projecting the equation of conservation of linear momentum along the normal to the membrane, a jump condition is formulated for the mechano-electrochemical potential of fluid constituents which is generally applicable to nonequilibrium processes involving active transport. The resulting relations are simple and easy to use, and address an important need in the membrane transport literature.

Keywords

Membrane pumps; Membrane cotransporters; Membrane potential; Momentum supply; Free energy; Mechano-electrochemical potential

Introduction

The objective of this study is to formulate governing equations for active transport across semi-permeable membranes within the framework of the theory of mixtures,^{6,9,33} which accounts for the various species of matter present in a system. In biological membranes, primary active transport is mediated by transmembrane pumps that are driven by a supply of energy generated from chemical reactions, such as the hydrolysis of adenosine triphosphate (ATP). Secondary active transport is mediated by transmembrane carriers that derive free energy from the mechano-electrochemical (MEC) gradient of solutes.

Mass transport phenomena have been well described within the framework of continuum mechanics.^{5,19} The theory of mixtures^{6,9,33} has been particularly well suited for the generalized description of biological tissues as deformable porous media^{22,27} whose interstitial fluid may include solute species.^{15,17,24,26} When applied to passive membrane transport, mixture theory has been shown to reproduce,^{4,14} the classical framework initially derived from the phenomenological equations of nonequilibrium thermodynamics.^{19,20}

The classical field of nonequilibrium thermodynamics describes biological transport in terms of conjugate force–flux pairs. Active processes mediated by motor proteins (pumps,

exchangers, and transporters) are incorporated into these force-flux pairs with the addition of a driving force for the active flux; in the case of ATP-driven active transport, this driving force is the affinity¹¹ of the phosphorylation reaction.¹³ The objective of this study is to provide an alternative approach to this classical framework, using the more modern framework of mixture theory.

To achieve this objective, it is necessary to consider the role of chemical reactions in the theory of mixtures^{2,7,8} and the resulting thermodynamic constraints regarding the free energy available for primary and secondary active transport. At the completion of this process, it can be shown that a simple jump condition may be formulated for the MEC potential difference of solutes and solvent across a membrane, to account for free energy gains or losses resulting from active and passive transport. The simplicity of this formulation should facilitate the analysis of membrane transport under general conditions, providing a straightforward presentation of cellular transport mechanisms. The formulation identifies momentum supply terms for passive and active transport processes, but does not commit to a specific form of constitutive relations for these terms, which need to be guided by experimental investigations.

To illustrate the application of active transmembrane transport to a familiar problem, a solution is provided for the intracellular concentration of Na^+ , K^+ , and Cl^- in an idealized cell whose plasma membrane has leak channels for these ions as well as sodium–potassium pumps (Na^+/K^+ -ATPase).

Model Formulation

Conservation of Linear Momentum

This study uses the framework of mixture theory³³ specialized to the case where each mixture constituent is intrinsically incompressible.^{9,27} The mixture includes a porous solid matrix ($\alpha = s$), a solvent ($\alpha = w$), and multiple solute species ($\alpha \neq s, w$) that may be electrically charged or neutral; it is assumed that electroneutrality is satisfied at every point in the continuum.¹ The mixture as a whole remains compressible, since the fluid species (solvent and solutes) may enter or leave the porous solid matrix, leading to an increase or decrease in pore volume. The notation adopted here follows closely that of our recent study on reactive mixtures.²

Mixture theory offers a very general framework that encompasses the classical fields of solid mechanics, fluid mechanics and transport. A fundamental concept in this theory is that the various constituents of a mixture may exchange mass, momentum, energy, and entropy. These supplies to each constituent are internal to the mixture and their summation over all mixture constituents reduces to zero.

Once the dependence of the Helmholtz free energy of each constituent on specific state variables has been proposed in a constitutive formulation, it becomes possible to separate constituent stresses and momentum supplies into conservative and dissipative parts. Only dissipative parts remain in the reduced Clausius–Duhem inequality.^{2,6,33} For fluid constituents ($\alpha \neq s$), the conservative parts of the stress tensor and the momentum supply combine in the equation of conservation of linear momentum to produce the MEC potential $\tilde{\mu}^\alpha$ for that constituent. This MEC potential represents the sum of free energy potentials in

¹In a strict sense, enforcing electroneutrality in a membrane implies that it cannot act as a capacitor. Therefore, the equations presented in this paper are not sufficiently general to model that part of the transient response of action potentials resulting from charge accumulation in the membrane.

the constituent arising from the fluid pressure, the electric potential in the mixture, and the chemical potential for that constituent,

$$\tilde{\mu}^\alpha = \frac{p}{\rho_T^\alpha} + \frac{z^\alpha}{M^\alpha} F_c \psi + \mu^\alpha, \quad (1)$$

where ρ_T^α is the true density (mass of α per volume of α in the mixture), z^α is the charge number, and M^α is the molecular weight of fluid constituent α ; F_c is Faraday's constant, and ψ is the electric potential in the mixture. $\mu^\alpha = \partial \Psi / \partial \rho^\alpha$ is the chemical potential of constituent α , where Ψ is the mixture Helmholtz free energy per volume (in the current configuration) and ρ^α is the apparent density (mass of α per volume of the mixture in the current configuration). In mixture theory, μ^α and $\tilde{\mu}^\alpha$ have units of energy per mass of constituent α , which is the more convenient choice; $M^\alpha \mu^\alpha$ represents the MEC potential in units of energy per mole of constituent α , more commonly used in chemistry and classical nonequilibrium thermodynamics. The appearance of the term p/ρ_T^α in Eq. (1) is the consequence of assuming that each constituent is intrinsically incompressible; it follows that the chemical potential μ^α is not a function of pressure under this assumption.

Under isothermal conditions, when neglecting inertia forces and dissipative stresses (such as stresses arising from the fluid's viscosity), the equation of conservation of linear momentum (Newton's second law of motion) reduces to

$$-\rho^\alpha \nabla \tilde{\mu}^\alpha + \rho^\alpha \mathbf{b}^\alpha + \widehat{\mathbf{p}}_d^\alpha = \mathbf{0} \quad \alpha \neq s. \quad (2)$$

In this expression, $\mathbf{b}^\alpha$ is the external body force acting on constituent α , and $\widehat{\mathbf{p}}_d^\alpha$ is the dissipative part of the momentum exchanged with constituent α as a result of its interactions with all other constituents. The dependence of μ^α on the apparent densities (or equivalently, the concentrations) of the constituents, and on other variables (such as the strain in the solid matrix) needs to be provided by a constitutive relation for the Helmholtz free energy Ψ .²

Though an equivalent relation may be written for the solid constituent, it is more convenient to provide the equation of conservation of linear momentum for the entire mixture, which reduces to

$$\nabla \cdot \mathbf{T} + \rho \mathbf{b} = \mathbf{0} \quad (3)$$

when neglecting inertia forces. Here, $\rho = \sum_\alpha \rho^\alpha$ is the mixture density, $\mathbf{b} = (\sum_\alpha \rho^\alpha \mathbf{b}^\alpha)/\rho$ is the external body force on the mixture, and

$$\mathbf{T} = -p\mathbf{I} + \mathbf{T}^e \quad (4)$$

is the mixture stress, with $\mathbf{T}^e$ representing the stress resulting from strains in the solid constituent; $\mathbf{I}$ is the identity tensor. The dependence of the stress tensor $\mathbf{T}^e$ on the strain (and other state variables such as the concentrations of solutes) needs to be provided by a constitutive relation.

The dissipative part of the momentum exchange, $\widehat{\mathbf{p}}_d^\alpha$, represents a critical term in our analysis of passive and active transport mechanisms in a mixture. It is a vector representing an internal body force and, since the mean response of a heterogeneous mixture must obey the ordinary equations of a pure substance,³³ it must satisfy

$$\sum_{\alpha} \widehat{\mathbf{p}}_d^\alpha + \widehat{\rho}^\alpha \mathbf{u}^\alpha = \mathbf{0}, \quad (5)$$

where $\widehat{\rho}^\alpha$ is the density of mass supply to constituent α from chemical reactions (having units of mass per volume, per time). The diffusion velocity $\mathbf{u}^\alpha = \mathbf{v}^\alpha - \mathbf{v}$ is the relative velocity between constituent α and the mixture, where the mixture velocity is given by $\mathbf{v} = (\sum_{\alpha} \rho^\alpha \mathbf{v}^\alpha) / \rho$.

Conservation of Mass

The equation of conservation of mass for each constituent α is given by

$$\frac{\partial \rho^\alpha}{\partial t} + \nabla \cdot (\rho^\alpha \mathbf{v}^\alpha) = \widehat{\rho}^\alpha \quad (6)$$

and the principle of mixtures requires that

$$\sum_{\alpha} \widehat{\rho}^\alpha = 0 \quad (7)$$

to enforce the conservation of mass between reactants and products. Therefore, the formulation of a suitable constitutive relation for $\widehat{\mathbf{p}}_d^\alpha$ must satisfy the constraints of Eqs. (5) and (7). For a saturated mixture of intrinsically incompressible constituents, it can be shown that the conservation of mass for the mixture reduces to

$$\nabla \cdot \left(\sum_{\alpha} \phi^\alpha \mathbf{v}^\alpha \right) = \sum_{\alpha} \frac{\widehat{\rho}^\alpha}{\rho_T}, \quad (8)$$

where $\phi^\alpha = \rho^\alpha / \rho_T$ is the volume fraction of each constituent.

Momentum Exchange

The classical formulation of a constitutive relation describing the passive transport mechanisms in a mixture has assumed that $\widehat{\mathbf{p}}_d^\alpha$ depends linearly on the relative velocities between constituent α and all other constituents in the mixture. This formulation is the basis for the phenomenological equations of irreversible thermodynamics.¹⁹ This type of constitutive relation leads to the familiar relations known as Darcy's law, for permeability of the solvent in a porous solid matrix, and Fick's law, for the diffusivity of a solute in a solvent. In mixture theory, the dependence of the frictional response on the relative velocities between constituents is predicated by the need to satisfy the second law of thermodynamics for arbitrary motions; this formulation is also generalized to account for

active transport mechanisms, while recognizing that such active transport may be fueled by chemical reactions. Based on our recent study,² which first introduced the contribution of chemical reactions to this momentum supply, it is proposed that this generalization take the form

$$\widehat{\mathbf{p}}_d^\alpha = \widehat{\mathbf{p}}_a^\alpha - \frac{1}{\rho^\alpha} \mathbf{f}^\alpha \cdot \mathbf{m}^\alpha + \sum_{\beta \neq s} \frac{1}{\rho^\beta} \left(\mathbf{f}^{\alpha\beta} - \frac{\rho^\alpha}{\rho} \widehat{\rho}^\beta \mathbf{I} \right) \cdot \mathbf{m}^\beta \quad \alpha \neq s, \quad (9)$$

where

$$\mathbf{m}^\alpha = \rho^\alpha (\mathbf{v}^\alpha - \mathbf{v}^s) \quad \alpha \neq s \quad (10)$$

is the mass flux of constituent α relative to the solid matrix; $\mathbf{f}^{\alpha\beta}$ are second-order *diffusive drag tensors* representing frictional interactions between constituents α and β , which must be positive semi-definite and satisfy $\mathbf{f}^{\beta\alpha} = \mathbf{f}^{\alpha\beta}$ (see “Thermodynamic Constraints” section); and

$$\mathbf{f}^\alpha = \sum_{\beta} \mathbf{f}^{\alpha\beta}, \quad (11)$$

where it is understood that $\mathbf{f}^{\alpha\beta} = 0$ when $\beta = \alpha$. In general, $\mathbf{f}^{\alpha\beta}$ is not constant but may vary with temperature, constituent densities, solid matrix strain, magnitude of relative velocities, and possibly other variables; the specific dependence of $\mathbf{f}^{\alpha\beta}$ on these state variables may be proposed in the form of constitutive relations validated from experiments. For example, the diffusive drag tensors may be related to the more familiar diffusivity and permeability tensors.^{2,15,17,24}

The vector $\widehat{\mathbf{p}}_a^\alpha$ represents the momentum exchange with constituent α resulting from active transport (primary or secondary). Equation (9), which generalizes the classical formulation with the addition of $\widehat{\mathbf{p}}_a^\alpha$ and the term involving $\widehat{\rho}^\beta$, only commits a general constitutive form for the passive frictional interactions between constituents, keeping the active transport term $\widehat{\mathbf{p}}_a^\alpha$ in a generic form. Recognizing the need for introducing the active transport exchange $\widehat{\mathbf{p}}_a^\alpha$ in the dissipative part of the momentum supply represents a fundamental element of this study.

Using this constitutive relation for $\widehat{\mathbf{p}}_d^\alpha$, the constraint of Eq. (5) now reduces to

$$\sum_{\alpha} \widehat{\mathbf{p}}_a^\alpha = \mathbf{0}. \quad (12)$$

This constraint implies that these active internal momentum exchanges must cancel out. For example, if the porous solid matrix carries molecular motors that can pump solutes through it, the momentum imparted by the molecular motors to the solutes generates an equal and opposite momentum supply to the solid matrix.

Membrane Transport

These vector equations, obtained from the conservation of linear momentum, are valid for a general continuum. They can be specialized to membranes by projecting these vector quantities along the unit normal to the membrane surface, and taking a suitable limit as the three-dimensional continuum is collapsed onto that surface. Substituting Eq. (9) into Eq. (2) produces

$$-\nabla \mu^{\alpha} + \mathbf{b}^{\alpha} - \frac{1}{(\rho^{\alpha})^2} \mathbf{f}^{\alpha} \cdot \mathbf{m}^{\alpha} + \frac{1}{\rho^{\alpha}} \sum_{\beta \neq s} \frac{1}{\rho^{\beta}} \left(\mathbf{f}^{\alpha\beta} - \frac{\rho^{\alpha}}{\rho} \widehat{\rho}^{\beta} \mathbf{I} \right) \cdot \mathbf{m}^{\beta} + \frac{1}{\rho^{\alpha}} \widehat{\mathbf{p}}_a^{\alpha} = 0 \quad \alpha \neq s \quad (13)$$

Consider a membrane whose unit outward normal is given by the unit vector $\mathbf{n}$. Also assume for simplicity that the membrane only allows transport normal to it (negligible transverse solute diffusion or solvent permeation within the membrane), such that

$$\begin{aligned} \mathbf{f}^{\alpha} &= f_{nn}^{\alpha} \mathbf{n} \otimes \mathbf{n} \\ \mathbf{f}^{\alpha\beta} &= f_{nn}^{\alpha\beta} \mathbf{n} \otimes \mathbf{n} \end{aligned} \quad (14)$$

where $\otimes$ represents the dyadic product of vectors. To evaluate the jump condition on the MEC potential across the membrane, Eq. (13) may be projected along $\mathbf{n}$ to yield

$$-\frac{d\mu^{\alpha}}{dr} + \mathbf{n} \cdot \mathbf{b}^{\alpha} - \frac{f_{nn}^{\alpha}}{(\rho^{\alpha})^2} m_n^{\alpha} + \sum_{\beta \neq s} \left(\frac{f_{nn}^{\alpha\beta}}{\rho^{\alpha} \rho^{\beta}} - \frac{1}{\rho^{\beta}} \widehat{\rho}^{\beta} \right) m_n^{\beta} + \frac{1}{\rho^{\alpha}} \widehat{\mathbf{p}}_a^{\alpha} = 0 \quad \alpha \neq s, \quad (15)$$

where r is the coordinate along the normal to the membrane, $m_n^{\alpha} = \mathbf{m}^{\alpha} \cdot \mathbf{n}$, and $\widehat{\mathbf{p}}_a^{\alpha} = \widehat{\mathbf{p}}_a^{\alpha} \cdot \mathbf{n}$.

The equation of conservation of mass, Eq. (6), may be similarly evaluated across the thickness h of the membrane, producing

$$\Delta m_n^{\alpha} = - \int_0^h \widehat{\rho}^{\alpha} dr = - \bar{\rho}^{\alpha}, \quad (16)$$

where Δm_n^{α} represents the difference in the mass flux normal to the membrane, between the internal and external compartments, and $\bar{\rho}^{\alpha}$ represents the area density of mass supply due to chemical reactions occurring on the membrane, which add ($\bar{\rho}^{\alpha} > 0$) or remove ($\bar{\rho}^{\alpha} < 0$) mass from constituent α as it traverses the membrane.

Equations (15) and (16) are more general than needed for the study of active transport of solutes across biological membranes, since they allow for chemical reactions to occur on the membrane, that alter the mass flux of solutes as they transport across it. Therefore, we may reduce the scope of these equations by considering that α refers only to membrane-permeant solvent and solutes that are *not* involved as reactants or products in chemical reactions on the membrane ($\bar{\rho}^{\alpha} = 0$). In this case, the jump condition on the conservation of mass, Eq. (16), stipulates that m_n^{α} is continuous across the membrane,¹² implying that it does not suffer a jump discontinuity across this interface. Furthermore, make the simplifying assumption

that solutes β involved in chemical reactions that produce the free energy needed to fuel active processes have a negligible transmembrane flux ($m_n^\beta \approx 0$ when $\hat{\rho}^\beta \neq 0$, implying that the chemical reaction, such as ATP hydrolysis, takes place on one side of the membrane). Finally, assume that the external body forces $\mathbf{b}^\alpha$ (such as gravity) do not suffer a jump across the membrane. With these assumptions, Eq. (15) may be integrated along r to yield

$$\Delta\tilde{\mu}^\alpha = \left(\int_0^h \frac{f_{nn}^\alpha}{(\rho^\alpha)^2} dr \right) m_n^\alpha - \sum_{\beta \neq s} \left(\int_0^h \frac{f_{nn}^{\alpha\beta}}{\rho^\alpha \rho^\beta} dr \right) m_n^\beta - \left(\int_0^h \frac{1}{\rho^\alpha} \widehat{p}_a^\alpha dr \right) \quad \alpha \neq s, \quad (17)$$

where $\Delta\tilde{\mu}^\alpha$ represents the difference in the MEC potential $\tilde{\mu}^\alpha$ between the internal and external compartments separated by the membrane, and α, β represent mixture constituents (solvent or solute) not involved as reactants or products in chemical reactions on the membrane. The terms appearing in parentheses on the right-hand-side are material functions whose dependence on solvent and solute content, solid deformation, flux, etc., may be described by constitutive relations.

To simplify the analysis, it is assumed that for sufficiently dilute solutions the diffusive drag coefficients between the various solutes sharing a common pathway may be neglected ($f^{\alpha\beta} \approx 0$ when $\alpha, \beta, \neq s, w$). Define the following set of material functions,

$$\begin{aligned} \frac{1}{N_p} &= \int_0^h \left(\frac{\rho_T^w}{\rho^w} \right)^2 f_{nn}^w dr & \widehat{W} &= \int_0^h \frac{\rho_T^w}{\rho^w} \widehat{p}_a^w dr \\ \frac{1}{P_0^\alpha} &= \frac{1}{R\theta} \int_0^h \frac{\rho_T^w}{\rho^w} \frac{M^\alpha}{\rho^\alpha} f_{nn}^{w\alpha} dr & \widehat{S}^\alpha &= \frac{1}{R\theta} \int_0^h \frac{M^\alpha}{\rho^\alpha} \widehat{p}_a^\alpha dr \\ \frac{1}{Q^\alpha} &= \frac{1}{R\theta} \int_0^h \left(\frac{M^\alpha}{\rho^\alpha} \right)^2 f_{nn}^\alpha dr & & \alpha \neq s, w \end{aligned} \quad (18)$$

and let $\mathbf{w} = \mathbf{m}^w / \rho_T^w$ represent the volume flux of solvent and $\mathbf{j}^\alpha = \mathbf{m}^\alpha / M^\alpha$ the molar flux of solutes, such that $w_n = \mathbf{w} \cdot \mathbf{n}$ and $j_n^\alpha = \mathbf{j}^\alpha \cdot \mathbf{n}$ are their respective components normal to the membrane. Using these fluxes, Eq. (17) now yields the desired jump conditions on the solvent and solute MEC potential,

$$\begin{aligned} \rho_T^w \Delta\tilde{\mu}^w &= \frac{1}{N_p} w_n - \widehat{W} - R\theta \sum_{\beta \neq s, w} \frac{1}{P_0^\beta} j_n^\beta & \alpha \neq s, w \\ \frac{M^\alpha}{R\theta} \Delta\tilde{\mu}^\alpha &= \frac{1}{Q^\alpha} j_n^\alpha - \frac{1}{P_0^\alpha} w_n - \widehat{S}^\alpha \end{aligned} \quad (19)$$

where $\widehat{W}$ and $\widehat{S}^\alpha$ ($\alpha \neq s, w$) are measures of the momentum supply to the solvent and solutes, respectively, from active transport processes across the membrane²; note that $\widehat{W}$ has units of force per area (or energy per volume), whereas $\widehat{S}^\alpha$ is a dimensionless quantity.³ Equation (19) represents a fundamental result of this study. Under equilibrium conditions ($w_n = 0$, $j_n^\alpha = 0$, $\alpha \neq s, w$), in the absence of active transport ($\widehat{W} = 0$, $\widehat{S}^\alpha = 0$), these relations reduce to the well-known condition $\Delta\tilde{\mu}^\alpha = 0$ ($\alpha \neq s$).

²Active solvent transport is not known to occur in biological membranes. However, in human-made systems, water pumps or air fans, placed across thin walls or membrane-like partitioning structures, are pervasive.

³However, $\widehat{W} / \rho_T^w$ and $R\theta \widehat{S}^\alpha / M^\alpha$ share the units of energy per mass.

The relations of Eq. (19) can be inverted to yield

$$w_n = L_p \left[\rho_T^w \Delta \tilde{\mu}^w + \widehat{W} + R\theta \sum_{\beta \neq s, w} \frac{Q^\beta}{P_0^\beta} \left(\frac{M^\beta}{R\theta} \Delta \tilde{\mu}^\beta + \widehat{S}^\beta \right) \right] \quad \alpha \neq s, w$$

$$j_n^\alpha = Q^\alpha \left(\frac{M^\alpha}{R\theta} \Delta \tilde{\mu}^\alpha + \frac{1}{P_0^\alpha} w_n + \widehat{S}^\alpha \right) \quad (20)$$

where

$$L_p = \left(\frac{1}{N_p} - R\theta \sum_{\beta \neq s, w} \frac{Q^\beta}{(P_0^\beta)^2} \right)^{-1} \quad (21)$$

is the effective membrane hydraulic permeability, accounting for the contribution of solutes $\alpha \neq s, w$. Both L_p and Q^α may be functions of the solute concentrations in the membrane. Note that the sign convention adopted for these expressions implies that the fluxes (w_n and j_n^α) and momentum supplies for active transport ($\widehat{W}$ and $\widehat{S}^\alpha$) are positive when directed outward.

Example 1—In the case of ideal dilute mixtures, the constitutive relations for the mechano-electrochemical potentials of the solvent and solutes are given by

$$\tilde{\mu}^w = \mu_0^w(\theta) + \frac{1}{\rho_T^w} \left(p - p_0 - R\theta \sum_{\beta \neq s, w} c^\beta \right) \quad \alpha \neq s, w$$

$$\tilde{\mu}^\alpha = \mu_0^\alpha(\theta) + \frac{1}{M^\alpha} \left(z^\alpha F_c (\psi - \psi_0) + R\theta \ln \frac{c^\alpha}{\kappa^\alpha c_0^\alpha} \right) \quad (22)$$

where K^α is the solubility of solute α .^{1,26,4} $\mu_0^\alpha(\theta)$ represents the chemical potential, p_0 the fluid pressure, ψ_0 the electric potential, and c_0^α the solute concentration, in the respective standard states of the solvent and solute; the standard state is invariant by definition. It follows that the jumps in the MEC potential across the membrane are given by

$$\rho_T^w \Delta \tilde{\mu}^w = \Delta p - R\theta \sum_{\beta \neq s, w} \Delta c^\beta \quad \alpha \neq s, w$$

$$\frac{M^\alpha}{R\theta} \Delta \tilde{\mu}^\alpha = \frac{z^\alpha F_c}{R\theta} \Delta \psi - \ln \frac{\kappa_i^\alpha c_e^\alpha}{\kappa_e^\alpha c_i^\alpha} \quad (23)$$

where $\Delta f = f_i - f_e$ for any variable f , where f_i represents the value in the inner compartment enclosed by the membrane, and f_e is the corresponding value in the compartment external to the membrane. Substituting these relations into Eq. (20) yields

⁴The solubility of a solute in a mixture containing a porous solid, as defined here, is a measure of the volume exclusion of that solute from some of the pores of the solid matrix due to steric effects and short-range electrostatic interactions. It represents the fraction of the pore volume which is accessible to that solute. It is a dimensionless number in the range $0 \leq \kappa^\alpha \leq 1$. (It does not account for long-range electrostatic interactions, such as those arising from the fixed charge density of the solid matrix and the resulting Donnan electric potential.)

$$w_n = L_p \left[\Delta p - R\theta \sum_{\beta \neq s, w} \Delta c^\beta + \widehat{W} + R\theta \sum_{\beta \neq s, w} \frac{Q^\beta}{P_0^\beta} \left(\frac{z^\beta F_c}{R\theta} \Delta \psi - \ln \frac{\kappa_i^\beta c_e^\beta}{\kappa_e^\beta c_i^\beta} + \widehat{S}^\beta \right) \right] \quad \alpha \neq s, w$$

$$j_n^\alpha = Q^\alpha \left(\frac{z^\alpha F_c}{R\theta} \Delta \psi - \ln \frac{\kappa_i^\alpha c_e^\alpha}{\kappa_e^\alpha c_i^\alpha} + \frac{1}{P_0^\alpha} w_n + \widehat{S}^\alpha \right) \quad (24)$$

To better understand the relation between Q^α , P_0^α , and the more familiar membrane solute permeability P^α and Staverman reflection coefficient σ^α , recognize that a Taylor series expansion yields

$$\ln \frac{\kappa_i^\beta c_e^\beta}{\kappa_e^\beta c_i^\beta} \approx \frac{1}{\bar{c}^\alpha} \left(\frac{c_e^\alpha}{\kappa_e^\alpha} - \frac{c_i^\alpha}{\kappa_i^\alpha} \right)$$

in the limit when the right-hand-side is much smaller than unity, where $\bar{c}^\alpha$ is a measure of the mean value of the concentration of solute α across the membrane (such as

$\bar{c}^\alpha = (c_i^\alpha + c_e^\alpha)/2$). Using this approximation in the above expression for j_n^α , and reducing the resulting relation to the special case where $\kappa_i^\alpha = \kappa_e^\alpha = 1$, $\widehat{S}^\alpha = 0$ and $\Delta \psi = 0$ yields

$$j_n^\alpha = \frac{Q^\alpha}{\bar{c}^\alpha} \Delta c^\alpha + \frac{Q^\alpha}{P_0^\alpha} w_n$$

Comparing this expression to the conventional relation from the Kedem–Katchalsky equations, $j_n^\alpha = P^\alpha \Delta c^\alpha + (1 - \sigma^\alpha) \bar{c}^\alpha w_n$,^{19,20} shows that

$$P^\alpha \equiv \frac{Q^\alpha}{\bar{c}^\alpha}$$

$$\sigma^\alpha \equiv 1 - \frac{P^\alpha}{P_0^\alpha} \quad (25)$$

Therefore $Q^\alpha = \bar{c}^\alpha P^\alpha$ and $P_0^\alpha = P^\alpha / (1 - \sigma^\alpha)$ are parameters that can be related to the more familiar solute permeability P^α and Staverman reflection coefficient σ^α . P_0^α may be interpreted as the permeability of the solute across the shared solute-solvent pathway in the limit when interference from the membrane solid matrix reduces to zero.⁴

Example 2—Consider the same ideal mixture described in Example 1. When solute α is charged ($z^\alpha \neq 0$), we may define its Nernst potential across the membrane as

$$V^\alpha \equiv \frac{R\theta}{z^\alpha F_c} \ln \frac{\kappa_i^\beta c_e^\beta}{\kappa_e^\beta c_i^\beta} \quad (26)$$

This relation may be combined with Eq. (23) to produce

$$\frac{M^\alpha}{R\theta} \Delta\mu \stackrel{\sim}{=} \frac{z^\alpha F_c}{R\theta} (\Delta\psi - V^\alpha) \quad (27)$$

Similarly, the electrical current density I_e^α for solute α across the membrane may be defined as

$$I_e^\alpha \equiv z^\alpha F_c j_n^\alpha \quad (28)$$

Substituting these relations into the expression for j_n^α in Eq. (20) yields

$$I_e^\alpha = z^\alpha F_c Q^\alpha \left[\frac{z^\alpha F_c}{R\theta} (\Delta\psi - V^\alpha) + \frac{1}{P_0^\alpha} w_n + \widehat{S}^\alpha \right] \quad (29)$$

In the special case when there is no solvent flux ($w_n = 0$) or pumping of the solute across the membrane ($\widehat{S}^\alpha = 0$), we recover the conventional relation for ohmic conductance,

$I_e^\alpha = g^\alpha (\Delta\psi - V^\alpha)$, where g^α is the membrane electrical conductance to solute α . Thus, it follows that

$$g^\alpha \equiv \frac{(z^\alpha F_c)^2}{R\theta} Q^\alpha = \frac{(z^\alpha F_c)^2}{R\theta} \bar{c}^\alpha P^\alpha \quad (30)$$

In general, neither g^α nor P^α need be constant.^{28,35} However, if P^α is assumed constant for a particular problem, the above relation suggests that g^α is simply proportional to $\bar{c}^\alpha$.

Equation (29) also shows that the electrical current density $z^\alpha F_c Q^\alpha \widehat{S}^\alpha$ represents a current source for solute α across the membrane, contributed by the membrane pump. Therefore, the continuum mixture approach is consistent with the classical treatment of membrane pumps as current sources in an equivalent electric circuit.^{28,35}

Thermodynamic Constraints

According to the second law of thermodynamics, under isothermal conditions, if viscous stresses in the solid, solvent, and solutes are neglected,² the Clausius-Duhem inequality reduces to

$$\sum_\alpha \widehat{\mathbf{p}}_d^\alpha \cdot \mathbf{u}^\alpha + \widehat{\rho}^\alpha \left(\bar{\mu}^\alpha + \frac{1}{2} \mathbf{u}^\alpha \cdot \mathbf{u}^\alpha \right) \leq 0, \quad (31)$$

where $\bar{\mu}^\alpha = p/\rho_t^\alpha + \mu^\alpha$ is the mechano-chemical potential of constituent α (in units of energy per mass of constituent α). Substituting the constitutive relation of Eq. (9) into this inequality and using the relation of Eq. (7) produces

$$\sum_{\alpha} \widehat{\mathbf{p}}_a^{\alpha} \cdot \mathbf{u}^{\alpha} + \widehat{\rho}^{\alpha} \left(\widehat{\mu}^{\alpha} + \frac{1}{2} \mathbf{u}^{\alpha} \cdot \mathbf{u}^{\alpha} \right) - \frac{1}{2} \sum_{\beta} (\mathbf{u}^{\beta} - \mathbf{u}^{\alpha}) \cdot \mathbf{f}^{\alpha\beta} \cdot (\mathbf{u}^{\beta} - \mathbf{u}^{\alpha}) \leq 0 \quad (32)$$

To better understand this expression, consider each term in isolation. In the absence of chemical reactions ($\widehat{\rho}^{\alpha} = 0$) and active transport ($\widehat{\mathbf{p}}_a^{\alpha} = 0$), the last term must be negative for arbitrary relative motions $\mathbf{u}^{\beta} - \mathbf{u}^{\alpha}$ of the constituents in order to obey the second law of thermodynamics. Thus, mathematically, $\mathbf{f}^{\alpha\beta}$ must be positive semi-definite, and satisfy $\mathbf{f}^{\beta\alpha} = \mathbf{f}^{\alpha\beta}$ (this is mixture theory's equivalent formulation of Onsager's reciprocal relations,^{30,31} showing that the latter relations are rooted in the second law of thermodynamics).⁵

Consequently, even in the more general case ($\widehat{\rho}^{\alpha} \neq 0$, $\widehat{\mathbf{p}}_a^{\alpha} \neq 0$), the last term on the left-hand-side of Eq. (32) is always negative, representing dissipation of free energy (or equivalently, entropy production) due to the diffusive drag between the various mixture constituents.

In the absence of active transport ($\widehat{\mathbf{p}}_a^{\alpha} \neq 0$), when the diffusive drag between various constituents is neglected ($\mathbf{f}^{\alpha\beta} = \mathbf{0}$), only the second term in Eq. (32) remains. The term

$\widehat{\mu}^{\alpha} + \frac{1}{2} \mathbf{u}^{\alpha} \cdot \mathbf{u}^{\alpha}$ in this expression is a measure of the mechano-chemical potential and diffusion free energy of constituent α per unit mass.⁶ Thus, under isothermal conditions, the second law of thermodynamics stipulates that chemical reactions can proceed spontaneously only if they lead to a net decrease in the potential and diffusion free energy of the mixture. Unlike the conventional presentation in introductory chemistry textbooks, stipulating that the net change in total Gibbs energy from the start to the end of a spontaneous reaction at constant temperature and pressure must be negative ($\Delta G < 0$), Eq. (32) is a continuum formulation in local form and holds at any instant in time and space. Furthermore, since the mechano-chemical potential $\widehat{\mu}^{\alpha}$ includes the pressure p in this mixture of intrinsically incompressible constituents, this thermodynamic constraint is not limited to processes at constant pressure (a conventional constraint when using the concept of Gibbs energy).

Finally, consider the first term in (32), which is the sum over all mixture constituents of the free energy supply $\widehat{\mathbf{p}}_a^{\alpha} \cdot \mathbf{u}^{\alpha}$ to constituent α , resulting from active transport processes. In the absence of diffusive drag ($\mathbf{f}^{\alpha\beta} = \mathbf{0}$) and chemical reactions ($\widehat{\rho}^{\alpha} = 0$), the analysis would reduce to secondary active transport only, and the second law of thermodynamics stipulates that the net supply of this free energy over all constituents involved in secondary active transport, $\sum_{\alpha} \widehat{\mathbf{p}}_a^{\alpha} \cdot \mathbf{u}^{\alpha}$, cannot be positive. If chemical reactions are occurring ($\widehat{\rho}^{\alpha} \neq 0$), as applicable to primary active transport, the second law indicates that the net free energy

⁵As also explained by Nye,²⁹ since the dissipative term $(\mathbf{u}^{\beta} - \mathbf{u}^{\alpha}) \cdot \mathbf{f}^{\alpha\beta} \cdot (\mathbf{u}^{\beta} - \mathbf{u}^{\alpha})$ has a quadratic form that only involves the symmetric part of the tensor $\mathbf{f}^{\alpha\beta}$, experimental measurements of frictional interactions between constituents (such as measurements of diffusivity and permeability) can only yield the symmetric part of $\mathbf{f}^{\alpha\beta}$; its anti-symmetric part can never be measured. Consequently, it may be assumed that $\mathbf{f}^{\alpha\beta}$ is symmetric with no loss of generality. This is the fundamental argument behind the symmetry of the diffusivity and permeability tensors.

⁶The *diffusion free energy* per unit mass of solute α , $\frac{1}{2} \mathbf{u}^{\alpha} \cdot \mathbf{u}^{\alpha}$, emerges from the theory of mixtures and is probably not familiar to a general audience. It expresses the free energy of mixture constituents resulting from their motion relative to the barycentric mixture velocity $\mathbf{v}$. The diffusion free energy of a mixture reduces to zero when all mixture constituents move at the same velocity ($\mathbf{u}^{\alpha} = \mathbf{0}$). It

is not the same as the kinetic energy per unit mass of constituent α , $\frac{1}{2} \mathbf{v}^{\alpha} \cdot \mathbf{v}^{\alpha}$, which reduces to zero only when $\mathbf{v}^{\alpha} = \mathbf{0}$. In most biological applications, the diffusion free energy is negligible in comparison to $\widehat{\mu}^{\alpha}$ due to the relatively small magnitude of the diffusion velocities $\mathbf{u}^{\alpha}$.

supply to primary active transport processes cannot exceed the net free energy supply from

chemical reactions, $\sum_{\alpha} \widehat{\rho}^{\alpha} \left(\widehat{\mu}^{\alpha} + \frac{1}{2} \mathbf{u}^{\alpha} \cdot \mathbf{u}^{\alpha} \right)$.

Since the diffusion velocity may be written as $\mathbf{u}^{\alpha} = \mathbf{v}^{\alpha} - \mathbf{v}^s + \mathbf{u}^s$, and in light of the constraint of Eq. (12), it follows that the net free energy supply resulting from active transport processes may also be written as $\sum_{\alpha} \widehat{\mathbf{p}}_a^{\alpha} \cdot \mathbf{u}^{\alpha} = \sum_{\alpha \neq s} \left(\widehat{\mathbf{p}}_a^{\alpha} / \rho^{\alpha} \right) \cdot \mathbf{m}^{\alpha}$, where $\mathbf{m}^{\alpha}$ is given in Eq. (10). The net free energy supply in active transport processes may now be expressed for the membrane, under the simplifying assumptions adopted in the above treatment: Assume that there is negligible transverse solute diffusion or solvent permeation within the membrane $\mathbf{w}^{\alpha} = w_n^{\alpha} \mathbf{n}$; and consider that solutes transporting across the membrane are not involved in chemical reactions ($\widehat{\rho}^{\alpha} = 0$), so that w_n^{α} is continuous across the membrane, whereas those that are involved in chemical reactions ($\widehat{\rho}^{\gamma} \neq 0$) have negligible transmembrane transport ($w_n^{\gamma} \approx 0$); then

$$\begin{aligned} \Lambda &\equiv \sum_{\alpha} \int_0^h \widehat{\mathbf{p}}_a^{\alpha} \cdot \mathbf{u}^{\alpha} dr \\ &= \sum_{\alpha \neq s} \left(\int_0^h \frac{\widehat{\mathbf{p}}_a^{\alpha}}{\rho^{\alpha}} dr \right) m_n^{\alpha} \\ &= \widehat{W} w_n + R\theta \sum_{\alpha \neq s, w} \widehat{S}^{\alpha} j_n^{\alpha} \end{aligned} \quad (33)$$

where the constituents appearing explicitly in this relation (solvent and solutes $\alpha \neq s, w$) satisfy $\widehat{\rho}^{\alpha} = 0$. The mass fluxes m_n^{α} of solvent and solutes have been substituted with the related membrane volume flux for the solvent, w_n , and molar fluxes for the solutes, j_n^{α} .

The net energy supply from chemical reactions may be represented by

$$\Gamma \equiv \sum_{\gamma \neq s, w} \int_0^h \widehat{\rho}^{\gamma} \left(\widehat{\mu}^{\gamma} + \frac{1}{2} \mathbf{u}^{\gamma} \cdot \mathbf{u}^{\gamma} \right) dr \quad (34)$$

Using (14) and the simplifying assumptions regarding the diffusive drag adopted above, it can be shown that

$$\begin{aligned} \Pi &\equiv \int_0^h -\frac{1}{2} \sum_{\alpha} \sum_{\beta} (\mathbf{u}^{\beta} - \mathbf{u}^{\alpha}) \cdot \mathbf{f}^{\alpha\beta} \cdot (\mathbf{u}^{\beta} - \mathbf{u}^{\alpha}) dr \\ &= -\frac{1}{N_p} (w_n)^2 - R\theta \sum_{\beta \neq s, w} \frac{1}{Q^{\beta}} (j_n^{\beta})^2 + 2R\theta w_n \sum_{\beta \neq s, w} \frac{1}{P_0^{\beta}} j_n^{\beta}, \end{aligned} \quad (35)$$

where Π represents the net free energy supply resulting from frictional interactions of the solvent and solutes with the membrane. Combining these relations produces the entropy inequality for a membrane,

$$\Lambda + \Gamma + \Pi \leq 0 \quad (36)$$

This relation summarizes the basic concept that there cannot be a net gain in free energy during transmembrane transport.

Enforcing this entropy inequality requires careful consideration. For example, in passive transport mechanisms ($\hat{W} = 0$, $\hat{S}^\alpha = 0$, implying $\Lambda = 0$) and in the absence of chemical reactions ($\hat{\rho}^\gamma = 0$, implying $\Gamma = 0$), the entropy inequality reduces to $\Pi \leq 0$, implying that frictional interactions dissipate free energy; this condition is satisfied if and only if the diffusive drag coefficients $\mathbf{f}^{\alpha\beta}$ are positive-definite. For membranes, this translates into the requirement that $f_{nn}^{\alpha\beta} \geq 0$ and, based on the relations of Eq. (18), these constraints also imply that $N_p \geq 0$, $P_0^\alpha \geq 0$, and $Q^\alpha \geq 0$. More generally, the inequality of Eq. (36) places a constraint on free energy exchanges in primary and secondary active transport mechanisms.

Stoichiometric Constraints

Additional stoichiometric constraints may be imposed in cases where active transmembrane transport occurs via carrier molecules, which translocate multiple solutes across the membrane in a prescribed ratio (see p. 518 of Weiss35),

$$\frac{1}{\nu^\alpha} j_n^\alpha = \dots = \frac{1}{\nu^\beta} j_n^\beta \quad (37)$$

This relation applies to all the solutes transporting across that carrier molecule. (In case the solvent is also transported, its molar flux is given by $j_n^w = \rho_T^w w_n / M^w$.) ν^α are stoichiometric coefficients for active transport, representing the number of molecules of constituent α translocated across the carrier molecule within each complete cycle; we may choose a positive ν^α for outward flux, and a negative value for inward flux.

Example 3—Consider the Na^+/K^+ -ATPase pump, which has been described to pump three Na^+ ions out for every two K^+ ions pumped in. This stoichiometric constraint may be summarized as

$$\frac{1}{3} j_n^{\text{Na}} = -\frac{1}{2} j_n^{\text{K}} \quad (38)$$

According to this relation, j_n^{Na} and j_n^{K} have opposite signs, consistent with their transport in opposite directions during pumping.

Constitutive Relations for Active Transport Momentum Supply

Constitutive equations that relate the supply terms $\hat{W}$ and $\hat{S}^\alpha$ to various parameters such as solute concentrations, membrane strain, and temperature, are needed to complete the set of governing equations for a particular analysis. As usual, the formulation of constitutive equations is guided by experiments, and constrained by the second law of thermodynamics. In this study, no specific constitutive relation is proposed for these supply terms, as this topic deserves a separate treatment.

Application to Cell Biophysics

Multiple Pathways

Solvent and solutes may transport across a biological membrane via multiple pathways. For example, Na^+ may transport actively across the Na^+/K^+ -ATPase pump, and passively across Na^+ leak channels. Similarly, water may transport across the plasma membrane as well as aquaporin channels. For a particular constituent α , each pathway may exhibit its own distinct passive and, when present, active transport properties, that regulate its flux according to Eq. (20). However, for that constituent, the jump $\Delta\mu^\alpha$ in MEC potential remains the same for all pathways, under the continuum modeling assumption that these multiple pathways co-exist within an elemental region of the membrane. The net transmembrane flux w_n or j_n^α is given as the sum of fluxes across all pathways for that constituent. If a particular pathway is denoted by the subscript π , it follows that

$$\begin{aligned} w_n &= \sum_{\pi} w_{\pi} \\ j_n^\alpha &= \sum_{\pi} j_{\pi}^\alpha \end{aligned} \quad (39)$$

Using the relations of Eq. (20), it can be shown that membrane properties equivalent to the net transport over multiple pathways are given by

$$\begin{aligned} L_p &= \sum_{\pi} L_{p\pi} & Q^\alpha &= \sum_{\pi} Q_{\pi}^\alpha \\ \widehat{W} &= \frac{1}{L_p} \sum_{\pi} L_{p\pi} \widehat{W}_{\pi} & \widehat{S}^\alpha &= \frac{1}{Q^\alpha} \sum_{\pi} Q_{\pi}^\alpha \widehat{S}_{\pi}^\alpha \end{aligned} \quad (40)$$

For the transmembrane transport of solutes, it is often the case that a transporter or channel excludes the solvent and only allows the solute to pass through. In these selective pathways, the only nonzero frictional interaction is between the solute and the membrane (in effect, between the solute and the walls of the channel), thus $f^{\alpha s} \neq 0$ and $f^{\alpha w} = f^{\alpha\beta} = 0$ when $\alpha, \beta \neq s$,

w . Examining Eq. (18), it becomes evident that $(P_0^\alpha)^{-1} = 0$ for solvent-excluding solute pathways. According to Eq. (25), this condition also implies that Staverman's reflection coefficient reduces to unity in such cases, $\sigma^\alpha = 1$. This limiting condition for Staverman's reflection coefficient has not been generally recognized.

Example 4—Consider the transport of K^+ across a membrane that includes Na^+/K^+ -ATPase (the pump, denoted by a subscript p) and K^+ leak channels (denoted by a subscript l). The pump provides a momentum supply to the ion, but the leak channel does not. The leak channel does not allow water through, implying that $1/P_{l0}^{\text{K}} = 0$. Thus, the relations of Eq. (20) reduce to

$$j_p^{\text{K}} = Q_p^{\text{K}} \left(\frac{M^{\text{K}}}{R\theta} \Delta\mu^{\text{K}} + \widehat{S}_p^{\text{K}} \right) \quad (41)$$

in the case of the pump, and

$$j_1^K = Q_1^K \frac{M^K}{R\theta} \Delta\mu^{\sim K} \quad (42)$$

in the case of the leak channel. The net flux of potassium ion across the membrane is given by

$$\begin{aligned} j_n^K &= j_p^K + j_1^K \\ &= (Q_p^K + Q_1^K) \frac{M^K}{R\theta} \Delta\mu^{\sim K} + Q_p^K \widehat{S}_p^K \\ &\equiv Q^K \left(\frac{M^K}{R\theta} \Delta\mu^{\sim K} + \widehat{S}^K \right) \end{aligned} \quad (43)$$

where we used the relations of Eq. (40) while recognizing that the leak channel does not provide active transport, $\widehat{S}_1^K = 0$. Note that Q_p^K represents a measure of the frictional interactions between the potassium ion and the pump (the Na^+/K^+ -ATPase complex), whereas Q_1^K is a measure of the ion's frictional interactions with the leak channel. This example illustrates the fact that active transporters exhibit frictional properties.

Cell Homeostasis

Consider a simplified model of a cell consisting of a homogeneous protoplasm and membrane where the dominant intracellular and extracellular ion species are Na^+ , Cl^- , and K^+ . The membrane has leak channels for all these ions, as well as sodium–potassium pumps. No other active primary or secondary transport mechanisms are assumed to be present. Furthermore, since only Na^+ and K^+ are being pumped, it follows that $\dot{W} = 0$ and $\dot{S}^{\text{Cl}} = 0$. Under homeostasis, there is no net transport of solvent or solutes across the membrane, so that the fluxes satisfy $w_n = 0$, $j_n^\alpha = 0$ ($\alpha = \text{Na}, \text{K}, \text{Cl}$). Substituting these relations into Eq. (19) and using the relation of Eq. (27) for ions produces the following relations,

$$\begin{aligned} \widehat{S}^{\text{Na}} &= \frac{F_s}{R\theta} (V^{\text{Na}} - \Delta\psi) \\ \widehat{S}^{\text{K}} &= \frac{F_s}{R\theta} (V^{\text{K}} - \Delta\psi) \\ 0 &= \frac{F_s}{R\theta} (V^{\text{Cl}} - \Delta\psi) \end{aligned} \quad (44)$$

From the last of these relations, it follows that the cell resting membrane potential is equal to the Nernst potential of Cl^- ,

$$\Delta\psi = V^{\text{Cl}} \quad (45)$$

This theoretical prediction is consistent with experimental findings in some cells, such as human red blood cells^{18,25} and rabbit chondrocytes.³⁴ The first two relations of Eq. (44) may be used to calculate the momentum supplies to Na^+ and K^+ from active pumping, based on experimental measurements of the membrane and Nernst potentials. Such measurements may be used to formulate constitutive relations for $\widehat{S}^{\text{Na}}$ and $\widehat{S}^{\text{K}}$.

A more detailed analysis of this problem, where transport across each pathway (pump and leak channel) is examined, may yield additional insight. For example, it may be noted that

the momentum supply terms $\widehat{S}^i$ ($i = \text{Na}, \text{K}$) may be expressed in terms of the respective conductances in the pump and leak channel according to the general expression of Eq. (40),

$$\widehat{S}^i = \frac{Q_p^i \widehat{S}_p^i}{Q_p^i + Q_l^i} = \frac{g_p^i \widehat{S}_p^i}{g_p^i + g_l^i} \quad i = \text{Na}, \text{K} \quad (46)$$

where Eq. (30) was used to substitute g^i for Q^i . Furthermore, the flux in each pathway is given by

$$j_p^i = Q_p^i \left[\frac{z^i F_c}{R\theta} (\Delta\psi - V^i) + \widehat{S}_p^i \right] = -j_l^i = Q_l^i \frac{z^i F_c}{R\theta} (V^i - \Delta\psi) \quad (47)$$

Using these results, the stoichiometry of the pump, given by $2j_p^{\text{Na}} + 3j_p^{\text{K}} = 0$ in accordance with Eq. (38), produces the following constraint on the leak channel conductances for sodium and potassium,

$$2Q_l^{\text{Na}}(V^{\text{Na}} - \Delta\psi) + 3Q_l^{\text{K}}(V^{\text{K}} - \Delta\psi) = 0 \quad (48)$$

Experimental verification of this constraint may be used to validate the pump stoichiometry, under the assumption that the main membrane transport mechanisms are limited to those included in this simple model of the cell. That the pump stoichiometry places a constraint on the leak channel conductances is a consequence of the conservation of mass constraint under homeostasis, $j_n^i = j_p^i + j_l^i = 0$, which relates the pump and leak fluxes as per Eq. (47).

If additional membrane transporters are included, the finding that $\Delta\psi = V^{\text{Cl}}$ may not necessarily persist. For example, if the membrane includes sodium–potassium–chloride cotransporters (NKCC), it may no longer be assumed that $\widehat{S}^{\text{Cl}} = 0$, since momentum may now be supplied to chloride ions in the NKCC. In this case, under homeostasis, the jump conditions produce

$$\begin{aligned} \widehat{S}^{\text{Na}} &= \frac{Q_p^{\text{Na}} \widehat{S}_p^{\text{Na}} + Q_c^{\text{Na}} \widehat{S}_c^{\text{Na}}}{Q_p^{\text{Na}} + Q_c^{\text{Na}} + Q_l^{\text{Na}}} = \frac{F_c}{R\theta} (V^{\text{Na}} - \Delta\psi) \\ \widehat{S}^{\text{K}} &= \frac{Q_p^{\text{K}} \widehat{S}_p^{\text{K}} + Q_c^{\text{K}} \widehat{S}_c^{\text{K}}}{Q_p^{\text{K}} + Q_c^{\text{K}} + Q_l^{\text{K}}} = \frac{F_c}{R\theta} (V^{\text{K}} - \Delta\psi) \\ \widehat{S}^{\text{Cl}} &= \frac{Q_c^{\text{Cl}} \widehat{S}_c^{\text{Cl}}}{Q_c^{\text{Cl}} + Q_l^{\text{Cl}}} = -\frac{F_c}{R\theta} (V^{\text{Cl}} - \Delta\psi) \end{aligned} \quad (49)$$

where the subscript c refers to the NKCC pathway. These relations reduce to the simpler model of Eq. (44) in the limit when the NKCC pathway is shut ($Q_c^\alpha = 0$, $\alpha = \text{Na}, \text{K}, \text{Cl}$). Here again, experimental measurements of the membrane and Nernst potentials may be used to estimate the net momentum supplies $\widehat{S}^\alpha$ to all ions. By varying intracellular or extracellular ion concentrations, a functional dependence of $\widehat{S}^\alpha$ on these concentrations may be determined experimentally to motivate specific forms of constitutive relations.

In this more general model of the cell membrane, stoichiometric constraints of Na^+/K^+ -ATPase $j_p^{\text{Na}}/3 = -j_p^{\text{K}}/2$ and NKCC $j_p^{\text{Na}} = j_p^{\text{K}} = j_c^{\text{Cl}}/2$ apply to the respective fluxes through

these transporters. They combine with the homeostasis condition ($j_n^\alpha = j_p^\alpha + j_c^\alpha + j_l^\alpha = 0$ for all ions) to place constraints on the conductances across the various pathways.

Minimum Pumping Energy Expenditure

In the analysis of cell biophysics, it may be of interest to estimate the minimum magnitude of the energy Γ supplied from chemical reactions, needed to pump ions across a leaking membrane, under homeostatic conditions. It might seem at first that the answer to this question may be given by the entropy inequality as applied to the membrane, Eq. (36), since this relation includes the free energy Λ imparted to solutes during active transport, the free energy Γ supplied from chemical reactions, and the free energy Π dissipated by frictional mechanisms. However, the entropy inequality does not provide the desired answer directly, as illustrated in the following example.

Example 5—Consider the simple model of a cell presented in “Cell Homeostasis” section, with leak channels for Na^+ , K^+ and Cl^- , and sodium–potassium pumps. Substituting the expressions for the pump and leak channel fluxes from Eq. (47) into the general expressions for Λ in Eq. (33) and Π in Eq. (35) produces

$$\Lambda = -\Pi = R\theta \sum_{i=\text{Na,K}} Q_i^l \left(1 + \frac{Q_i^l}{Q_p^k} \right) \left[\frac{F_c}{R\theta} (V^i - V^{\text{Cl}}) \right]^2 \quad (50)$$

Thus, $\Lambda + \Pi = 0$, informing us that under homeostasis the free energy imparted to sodium and potassium ions by the pump must balance the loss of free energy resulting from frictional interactions in the pump and leak channels. The entropy inequality thus reduces to $\Gamma \leq 0$.

Upon closer examination of this example, the outcome should not be surprising. Indeed, the entropy inequality only provides an algebraic upper bound on Γ (or equivalently, a lower bound on its magnitude) for any process that produces zero net fluxes under homeostasis. The simplest such process is one where the membrane is sealed to prevent any active or passive transport, thus requiring zero minimum free energy from chemical reactions to maintain zero flux. The entropy inequality simply informs us about this possibility, and otherwise places a constraint on any constitutive assumption we might advance with regard to the actual chemical energy requirement for pumping.

A more sensible approach is to consider that chemical reactions should at least produce enough free energy to account for the net effect of active transport processes,

$$\Lambda + \Gamma \leq 0 \quad (51)$$

Since Π has been formulated such that $\Pi \leq 0$ for any process (as long as $\mathbf{f}^{\alpha\beta}$'s are positive semi-definite, see Eq. (35)), this constitutive assumption never violates the general entropy inequality of Eq. (36). The limiting case where $\Lambda + \Gamma = 0$ corresponds to ideally efficient transporters that can convert all of the chemical free energy into active transport.

Example 6—In Example 5, the free energy supply required to pump solutes in the simplified cell model is the negative of the free energy dissipated from frictional interactions, $\Lambda = -\Pi$. The expression of Eq. (50) in that example may be rewritten in the equivalent form of an electrical circuit. For example, starting from the relation for Π in Eq.

(35), letting $w_n = 0$, performing the summation over pump and leak channel pathways, and enforcing the constraint $j_n^i = j_p^i + j_l^i = 0$ produces

$$\Lambda = R\theta \sum_{i=\text{Na}, \text{K}} \left(\frac{1}{Q_l^i} + \frac{1}{Q_p^i} \right) (j_l^i)^2 \quad (52)$$

Substituting the relations of Eqs. (29) and (30) into this expression produces

$$\Lambda = \sum_{i=\text{Na}, \text{K}} \left(\frac{1}{g_l^i} + \frac{1}{g_p^i} \right) (I_l^i)^2 \quad (53)$$

which has the standard form for ohmic heating. The equivalence of this expression with that of Example 5 follows from the ohmic conductance relation for leak channels,

$$I_l^i = g_l^i (V^{Cl} - V^i) \quad i=\text{Na}, \text{K} \quad (54)$$

Λ has units of power per area and needs to be multiplied by the area of the cell membrane to provide the pumping power requirements for an entire cell.

In the case of membranes with secondary active transporters only ($\Gamma = 0$), the constraint of Eq. (49) reduces to $\Lambda \leq 0$, implying that the free energy gained by solutes transporting against their MEC gradient cannot exceed the free energy donated by solutes transporting down their MEC gradient.

Example 7—Consider a Na^+ /glucose cotransporter, which cotransports one Na^+ ion with one mole of glucose into the cell, thus

$$j_c^{\text{Na}} = j_c^{\text{Glc}} \quad (55)$$

Typically, the extracellular concentration of Na^+ is higher than the intracellular concentration, while the opposite is generally true for glucose, producing negative (inward) fluxes for both species. According to Eq. (24), given that this symporter does not allow solvent transport, it follows that

$$\widehat{S}_c^{\text{Glc}} = \frac{1}{Q_c^{\text{Glc}}} j_c^{\text{Glc}} + \ln \frac{c_e^{\text{Glc}}}{c_i^{\text{Glc}}} \quad (56)$$

(assuming $\kappa_i^{\text{Glc}} = \kappa_e^{\text{Glc}} = 1$). Therefore, the conditions $j_c^{\text{Glc}} < 0$ and $c_e^{\text{Glc}} < c_i^{\text{Glc}}$ imply that $\widehat{S}_c^{\text{Glc}}$ is negative (directed inward), since momentum must be imparted to the glucose to push it into the cell against its MEC gradient. Notwithstanding other transporters or channels in the cell membrane, according to Eq. (33) the net free energy exchange in the cotransporter is

$$\Lambda_c = \widehat{S}_c^{\text{Na}} j_c^{\text{Na}} + \widehat{S}_c^{\text{Glc}} j_c^{\text{Glc}} = (\widehat{S}_c^{\text{Na}} + \widehat{S}_c^{\text{Glc}}) j_c^{\text{Na}} \quad (57)$$

The conditions $j_c^{\text{Na}} < 0$ and $\widehat{S}_c^{\text{Glc}} < 0$, and the constitutive assumption $\Lambda_c \leq 0$ imply that

$$\widehat{S}_c^{\text{Na}} \geq -\widehat{S}_c^{\text{Glc}} > 0 \quad (58)$$

Thus, $\widehat{S}_c^{\text{Na}}$ is positive (pointing outward) such that momentum is drawn away from the sodium ion as it enters the cell down its MEC gradient. The limiting condition $\widehat{S}_c^{\text{Na}} + \widehat{S}_c^{\text{Glc}} = 0$ corresponds to an ideal Na^+ /glucose cotransporter ($\Lambda_c = 0$).

Discussion

The objective of this study was to formulate jump conditions for the MEC potential of solvent and solutes across a membrane, which are valid under non-equilibrium conditions and account for active transport. Though the derivation of these conditions is rather involved, the final relations given in Eqs. (19) and (20) are remarkably simple. The formulation of these simple relations addresses an important need in the membrane transport literature, making it possible to introduce nonequilibrium and active transport processes at the most elementary level of analysis. An illustration of the application of these equations to cell homeostasis demonstrates that the fundamental concept of active membrane transport may be described very easily within this framework.

The framework of mixture theory was adopted for this formulation for a number of reasons, as partly outlined in the introduction. As shown in the above analysis, mixture theory also presents a number of additional advantages. These include the lack of a need to identify conjugate force-flux pairs *a priori*, a clear distinction between relations representing general axioms of conservation and those representing constitutive assumptions, the application of the second law of thermodynamics to prove Onsager's reciprocal relations, and most importantly, the intuitive formulation of active transport processes in the momentum supply term for each constituent. Mixture theory may also appeal to the biomedical engineering community because it encompasses the classical fields of fluid and solid mechanics, and may be extended to the analysis of growth processes in biological tissues.^{2,3,10,16,23}

A number of material functions are introduced in the presentation of these relations, as listed in Eq. (18). Those functions which govern passive transport processes (N_p , P_0^α and Q^α) are related to the familiar membrane hydraulic permeability L_p , membrane solute permeability P^α and Staverman reflection coefficient σ^α according to Eqs. (21) and (25); for charged solutes, Q^α may also be related to the membrane conductance g^α according to Eq. (30). Therefore, these material functions may be measured experimentally using standard methodologies, such as osmotic loading of cells or patch clamping techniques. The material functions which govern active transport processes ($\widehat{W}$ and $\widehat{S}^\alpha$) may be determined by measuring the resting membrane potential and internal solute concentrations, for various external bathing environments, using Eq. (19) with $w_n = 0$ and $j_n^\alpha = 0$, and suitable constitutive relations for the MEC potentials such as those of ideal solutions (Eqs. 22 and 23). Thus, the material functions appearing in this framework are well defined and observable.

The expressions of Eqs. (19) and (20) are more general than prior related presentations, though, as expected, they reduce to prior formulations for active transport that employ electrical circuit equivalents, where ion pumping is described using current sources.^{28,35} They are also consistent with the treatment of ATP-mediated active transport as a driving force representing the affinity¹¹ of the phosphorylation reaction, as described by Friedman.¹³ As formulated here, these jump conditions do not commit to a particular form for constitutive relations of the momentum supplies $\hat{W}$ and $\hat{S}^\alpha$; therefore, they retain a general form with regard to these supply terms. A presentation of the constraints imposed by the second law of thermodynamics provides guidance for the formulation of such constitutive relations as may be needed. Similarly, constitutive relations may be proposed for the remaining material functions in Eq. (18), which govern passive transport mechanisms.

This presentation should also help clarify the inherent limitation of classical, approximate relations reported in the cell biophysics literature. For example, textbook presentations^{21,32} have shown that the reversal potential of a membrane may be derived from the ohmic conductance relation $I_c^\alpha = g^\alpha (\Delta\psi - V^\alpha)$, by enforcing that the net current density reduce to zero, $I_c = \sum_\alpha I_c^\alpha = 0$, such that

$$\Delta\psi = \frac{\sum_\alpha g^\alpha V^\alpha}{\sum_\alpha g^\alpha} \quad (59)$$

Examining Eq. (29), it is evident that the above relation is not valid in the presence of active transport processes since it does not account for the momentum supplied to solutes by primary or secondary transporters; therefore, it may not be used to evaluate the resting membrane potential of cells. The correct relation is given by

$$\Delta\psi = \frac{\sum_\alpha (g^\alpha V^\alpha - z^\alpha F_c Q^\alpha \hat{S}^\alpha)}{\sum_\alpha g^\alpha} \quad (60)$$

by recognizing that $w_n = 0$ under homeostasis. Consequently, the active momentum supply terms $\hat{S}^\alpha$ are needed in order to evaluate the membrane potential $\Delta\psi$ from the zero current density condition.

When V^α and $\hat{S}^\alpha$ are known for a given solute, Eq. (60) is not needed since Eq. (29) may be used directly to evaluate the resting membrane potential $\Delta\psi$, by letting $j_n^\alpha = 0$ and $w_n = 0$ as illustrated in “Cell Homeostasis” section. In the case when there is no primary or secondary active transport of Cl^- , the membrane potential is simply given by $\Delta\psi = V^{\text{Cl}^-}$ as shown in Eq. (45). More generally, if all ions are involved in some form of active transport, evaluation of the membrane potential requires knowledge of at least one of the momentum supply terms $\hat{S}^\alpha$ (such as $\hat{S}^{\text{Cl}^-}$), along with the Nernst potential of all the ions, as illustrated in the example leading to Eq. (49). In a strict sense, knowledge of membrane conductances (g^α or Q^α) is not required to determine $\Delta\psi$, consistent with the fundamental understanding that conductances govern the transient response in transport processes, not the equilibrium response.

Clearly, the approach presented in this study also represents an alternative to the well-known Goldman (or Goldman–Hodgkin–Katz) equation, which proposes a relation for the reversal potential of a membrane that depends on the relative ratio of the membrane permeabilities of the various ions. By the arguments presented here, the GHK equation is not strictly valid

when active transport is taking place, since it does not account for the momentum supplied by transporters to the various ions. In effect, this study reinforces more modern concepts for the analysis of cell membrane resting potential via the introduction of momentum supply terms for active transport.

The availability of the relations of Eqs. (19) and (20) makes it possible to model cell biophysics under a wide variety of conditions. Models of cell membranes that include specific primary and secondary transporters may be formulated and analyzed under homeostatic and transient conditions, as illustrated briefly in “Cell Homeostasis” section. In particular, mechanisms of cell volume regulation may thus be investigated. Future studies will address various applications of these relations to problems related to cell mechanics. Furthermore, constitutive relations will be formulated that relate $\hat{S}^\alpha$ to intracellular and extracellular concentrations of solutes involved in active primary and secondary transport, as well as other governing parameters.

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Nomenclature

α, β	Mixture constituent
Γ	Net free energy supply from chemical reactions
θ	Absolute temperature
κ^α	Solubility
Λ	Net free energy supply from active transport
μ^α	Chemical potential
$\bar{\mu}^\alpha$	Mechano-chemical potential
$\tilde{\mu}^\alpha$	Mechano-electrochemical potential
ν^α	Stoichiometric coefficient
Π	Net free energy supply from frictional drag
ρ, ρ^α	Apparent density
ρ_T^α	True density
$\hat{\rho}^\alpha$	Volume density of mass supply
$\bar{\rho}^\alpha$	Area density of mass supply
σ^α	Staverman reflection coefficient
φ^α	Volume fraction
ψ	Electric potential
Ψ	Mixture free energy density
b, b^α	External body force
c^α	Solute concentration
$f^{\alpha\beta}, f^\alpha$	Diffusive drag tensor

F_c	Faraday constant
G	Gibbs energy
g^α	Ion membrane electric conductance
I	Identity tensor
J_e^α	Electric current density
j^α	Solute molar flux relative to solid
L_p	Membrane solution permeability
M^α	Molecular weight
m^α	Mass flux relative to solid
n	Membrane outward unit normal
N_p	Membrane solvent permeability
p	Interstitial fluid pressure
P_0^α, P^α	Solute membrane permeability
$\widehat{\mathbf{p}}_d^\alpha$	Dissipative part of momentum supply
$\widehat{\mathbf{p}}_a^\alpha$	Momentum supply from active transport
Q^α	Solute membrane molar flux coefficient
R	Universal gas constant
$\hat{S}^\alpha$	Solute active transport momentum supply coefficient
T	Mixture stress
T^e	Effective stress in solid
u^α	Diffusion velocity
v, v^α	Absolute velocity
V^α	Nernst potential
w	Solvent volume flux relative to solid
$\dot{W}$	Solvent active transport momentum supply energy density
z^α	Charge number

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