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Stability analysis of several non-dilute multiple solute transport equations

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Abstract Recently a new solute and solvent transmembrane cellular transport model accounting for non-dilute solute concentrations was introduced. This model depends on a second or third order polynomial expansion in mole fraction of Gibbs energy for solutes and solvents along with a mixing term that depends only on single solute data. This model is applicable to cells in so called semi-dilute anisosmotic conditions. The extents of these conditions are not immediately clear from within the theory. Therefore, in order to provide an estimate of the upper concentration bound of this model we rederive the original model in the practical molality form, apply a natural extension of the model to an arbitrary number of solutes, and provide concrete bounds on the maximal concentrations where the model may be stable, and thus likely physiologically relevant. Moreover, we apply a similar stability analysis for a simpler, and more classic model based on similar Gibbs energy. The results show that the classical model has an asymptotically stable rest point for all parameter values, whereas the new model does in fact become unstable at very high solute concentrations. This instability, however, occurs at concentrations that are most likely well beyond the intended applicability of the model.

Keywords Mathematical biology · Cellular mass transport · Non-dilute osmolality

Applied molecular biology, biochemistry, and biophysics depend on accurate estimates of the cellular state in the presence of anisosmotic extracellular conditions. Models describing the behavior of cells exposed to extracellular permeating and non permeating solutes have been proposed and analyzed for nearly a century (see [6] for a concise and recent review). Applications of these models range from pharmicokinetics

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to cryobiology to endocrinology and physiology, but the majority of applications are well within the concentration range that could be considered "dilute," and as such, do not require sophisticated modeling of chemical potential or osmolality. In the field of cryobiology, however, this is not the case. Because the unfrozen portion of the extracellular milieu is tied to its phase diagram, multi-molar concentrations are nearly always encountered upon cooling. Additionally, because the inhibition of deleterious intracellular ice is usually proportional to the concentration of permeating cryoprotective agents—usually low molecular weight polyols such as glycerol or 1–2, propane diol—cells are often exposed to multimolal concentrations of these polyols above the melting point.

In fact, two important challenges in the development of a cryopreservation protocol require an accurate mass transport model appropriate for the requisite multi-solute and multi-molar conditions: the maintainance of cell volumes between pre-defined volume or concentration limits, and the prediction of the intracellular viscosity during cooling below the solution melting point.

With this in mind, recently Elmoazzen et al. derived a new nondilute solute transport equation by combining the polynomial expansion of the Gibbs energy in mole fraction [6] with their previous work defining solute mixing terms which only depend on data derived from binary solutions [5]. To facilitate applications in a wide range of biological contexts where the specific mixing terms for each set of solutes might be challenging to obtain, Elliott et al. defined specific mixing rules to allow the determination of the Gibbs energy solely from binary solution data, and showed that this approach yields a good approximation of the osmolality and chemical potentials in solutions of biological interest.

The degree of non-ideality accounted for in this formalism is not immediately obvious from within the theory. Additionally it is unclear what effects an arbitrary number of solutes might have on the model. Elliott et al. claim heuristically that their formalism should not be applied for concentrations beyond those measured in the binary systems [5], i.e. those used to determine the model coefficients. One fairly straightforward approach to test both of these questions is to analyze the dynamical behavior of these models. If some combination of parameters yields unlikely dynamics, either there are unexpected phenomena that are modeled by the equations, or the model is invalid in this region of the parameter space and either result may be of considerable interest. Additionally, model stability is a component of controllability, and in many cases one wishes to control (optimally if possible) the cellular state. This type of analysis was performed on a similar but simpler ideal-dilute system Benson et al. and global stability was shown [1]. Therefore, for the non-ideal model the implicit assumption in membrane mass transfer is that there is a stable equilibrium. Below we investigate the effects of the model parameters on the stability at the equilibrium.

1 Osmotic transport equations

1.1 Chemical potentials

Elliott et al. use Landau and Lifshitz solution theory when deriving the chemical potential in terms of molality [5,10], but Elmoazzen et al. [6] derive the mass transport

equations in terms of mole fraction. It is often more natural for biologists and simpler mathematically to work in terms of molality instead of mole fraction, therefore, following Landau and Lifshitz, the $n-1$ solute Gibbs energy is given by

$$\Phi(T,P,N) = N_1\mu_0 + \sum_{i=2}^{n} N_i kT \ln\frac{N_i}{eN_1} + \sum_{i=2}^{n} N_i\psi_i + \frac{1}{2N_1}\sum_{i,j=2}^{n}\beta_{ij}N_iN_j, \tag{1}$$

where $N=(N_1,\ldots,N_n)$ is a vector of moles of solutes, and ψ_i and $\beta_{ij}=\beta_{ji}$ are functions of temperature and pressure. We define the chemical potential $\mu_i = \partial G/\partial N_i$, and scaled molality $m_i = N_i/N_1$ differing from the true molality $M_i = m_i/\nu_s$ where ν_s is the molecular weight of the solvent. Therefore we have

$$\begin{aligned}\mu_1 &= \mu_0 - kT\sum_{i=2}^{n}\frac{N_i}{N_1} - \frac{1}{2N_1^2}\sum_{i,j=2}^{n}\beta_{ij}N_iN_j \\ &= \mu_0 - kT\left(\sum_{i=2}^{n} m_i + (kT)^{-1}\sum_{i,j=2}^{n}\beta_{ij}m_im_j\right).\end{aligned} \tag{2}$$

By similar differentiation for $i\neq 1$,

$$\begin{aligned}\mu_i &= kT\ln(N_i/N_1) + \psi_i + \frac{1}{2N_1}\sum_{j=1}^{n}\beta_{ij}N_j \\ &= kT(\ln m_i + \psi_i^* + (kT)^{-1}\sum_{j=1}^{n}\beta_{ij}m_j),\end{aligned} \tag{3}$$

with $\psi_i^* = \psi_i/kT$. In this case, Elliott et al. define $\beta_{ij}/kT = (B_i + B_j)$ where B_i are the second osmotic virial coefficients determined in the presence of the ith solute and water alone. Thus in this context we have

$$\mu_1 = \mu_0 - kT\left(\sum_{i=2}^{n} m_i + \frac{1}{2}\sum_{i,j=2}^{n}(B_i+B_j)m_im_j\right), \tag{4}$$

$$\mu_i = kT\left(\ln m_i + \psi_i^* + \sum_{j=1}^{n}(B_i+B_j)m_j\right). \tag{5}$$

1.2 Transport models

As mentioned above, modeling of osmotically driven solute and solvent transmembrane flux has been undertaken since the 1930s [7]. The ordinary differential equation based on Fick's law of diffusion governing the flux of water has essentially remained unchanged since its inception:

$$\frac{dN_1}{dt} = -L_p AkT(\pi^e - \pi^i), \tag{6}$$

where $\pi = (\mu_1 - \mu_0)/kT$, superscripts e and i indicate extra- and intra-cellular properties, respectively, A is the constant surface area, and L_p is the membrane hydraulic conductivity. The solute flux equations are similar, and depend on the governing theory:

$$\frac{dN_i}{dt} = b_i(\mu_i^e - \mu_i^i), \tag{7}$$

for the Fick's model.

A more recent thermodynamic formalism applied in this area is Statistical Rate Theory [4]. This approach applied by Elliott et al. to transmembrane water flux is

$$\frac{dN_i}{dt} = b_i \sinh(\mu_i^e - \mu_i^i), \tag{8}$$

where b_i is a positive constant [4]. We will not analyze the irreversible thermodynamical approach proposed by Kedem and Ketchalsky [8].

Finally, the classical ordinary differential equation governing the solute flux utilizes a linear approximation of μ_i when $i \neq 1$, and is simply a rate constant times the difference of extra- and intra-cellular molality:

$$\frac{dN_i}{dt} = b_i(m_i^e - m_i^i). \tag{9}$$

Though Elmoazzen et al. argue that this is thermodynamically incorrect [6], it is commonly employed in the literature [9] so we will treat this as a special case in our analysis. In fact, we will generalize Eq. 9 to the form

$$\frac{dN_i}{dt} = b_i(\bar{\mu}_i^e - \bar{\mu}_i^i), \tag{10}$$

where $\bar{\mu}_i$ is the chemical potential of the solute that has no mixing terms, e.g. $\bar{\mu}_i = \sum_{j=1}^{\infty} a_j m_i^j$, with a_j constants, or more concisely,

$$\frac{\partial \mu_j^i(m(N))}{\partial m_k} = 0,$$

for $j \neq k$.

Therefore we have three analogous nonlinear systems of first order ordinary differential systems:

$$\dot{N} = b\, G(N, B), \tag{11}$$
$$\dot{N} = b \sinh(G(N, B)), \tag{12}$$
$$\dot{N} = b\, \bar{G}(N, B), \tag{13}$$

where $G(N, B) = (G_1, \ldots, G_n)^T = (\pi^i - \pi^e, \mu_2^e - \mu_2^i, \ldots, \mu_n^e - \mu_n^i)^T$, $\bar{G}(N, B) = (\pi^i - \pi^e, \bar{\mu}_2^e - \bar{\mu}_2^i, \ldots, \bar{\mu}_n^e - \bar{\mu}_n^i)^T$, $B = (B_1, \ldots, B_n)^T$, $b = (b_1, \ldots, b_n)^T$, and

$$\sinh(G) := \begin{pmatrix} \sinh(G_1(N, B)) \\ \vdots \\ \sinh(G_n(N, B))) \end{pmatrix}^T,$$

a slight abuse of notation. Without loss of generality we may set $b_1 = 1$.

2 Stability

We now are interested in the stability of systems (11)–(13) evaluated at the rest point $N = N^*$. found by examining the spectrum of the linearized system. If the real part of the eigenvalues of the linearized system are all negative, the system is stable, if not, the system is unstable (c.f. [3,12]). Moreover, we show below that this rest point exists except for when $M_1^{ext} = 0$.

2.1 Classical case

In the classical case, we have

$$\frac{\partial \mu_j^i(m(N))}{\partial m_k} = 0,$$

for $j \neq k$. This is a classical condition (e.g. where $\mu_k(m) = -\lambda_k m_k$, see [5,9]) where we only need physically relevant monotonicity conditions for g and μ.

Theorem 1 *Suppose x is governed by system (13), $M_1 \neq 0$, and $\partial \mu_j / \partial m_j > 0$ for $j = 2, \ldots, n$. Then there exists an asymptotically stable rest point N^*.*

Proof Suppose there is a rest point $N = N^*$ with $N_1^* < \infty$. We have the partial derivatives

$$\frac{\partial G_1}{\partial N_1} = \sum_{i=1}^{n} m_i + 2 \sum_{i,j=1}^{n} (B_i + B_j) m_i m_j, \tag{14}$$

and for $i > 1$,

$$\frac{\partial G_1}{\partial N_i} = 1 + \sum_{j=1}^{n} (B_i + B_j) m_j. \tag{15}$$

Also,

$$\begin{aligned}
\frac{\partial G_k}{\partial N_1} &= -b_k \sum_{j=1}^{n} \frac{\partial \mu_k^i(m(N))}{\partial m_j} \frac{\partial m_j}{\partial N_1} \\
&= b_k \frac{1}{N_1} \sum_{j=1}^{n} m_j(N) \frac{\partial \mu_k^i(m(N))}{\partial m_j} \quad \text{since } m_j = N_j/N_1 \\
&= b_k \frac{1}{N_1} m_i(N) \frac{\partial \mu_k^i(N)}{\partial m_k},
\end{aligned}$$

since $\partial \mu_k / \partial m_j = 0$ for $j \neq k$, and

$$\begin{aligned}
\frac{\partial G_k}{\partial N_i} &= -b_k \frac{\partial \mu_k^i(m(N))}{\partial N_i} \\
&= -b_k \frac{\partial \mu_k^i(m(N))}{\partial m_i} \frac{\partial m_j}{\partial N_i} \\
&= -b_k \frac{1}{N_1} \frac{\partial \mu_k^i(m(N))}{\partial m_i} \quad \text{since } m_j = N_j/N_1 \\
&= -\frac{1}{N_1} d_k.
\end{aligned}$$

Thus

$$\frac{\partial G_k}{\partial N_1} = -m_k \frac{\partial G_k}{\partial N_i} = \frac{1}{N_1} m_k(N^*) d_k.$$

Then the Jacobian $L := \{\partial G_i / \partial N_j\}_{i,j}$ is

$$L = \frac{1}{N_1} \begin{pmatrix} -r_1 & r_2 & \cdots & \cdots & r_n \\ m_2 d_2 & -d_2 & 0 & \cdots & 0 \\ \vdots & 0 & \ddots & \vdots & \vdots \\ m_n d_n & 0 & \cdots & 0 & -d_n \end{pmatrix}, \tag{16}$$

where

$$r_1 = \sum_{i=1}^{n} m_i + 2\sum_{i,j=1}^{n}(B_i + B_j)m_i m_j, \tag{17}$$

$$r_i = 1 + \sum_{j=1}^{n}(B_i + B_j)m_j, \tag{18}$$

We define a diagonal matrix $D = \mathrm{diag}(1, \sqrt{r_2/a_2}, \ldots, \sqrt{r_n/a_n})$ and note that $L^* = DLD^{-1}$ is symmetric with the form

$$L^S = \begin{pmatrix} -r_1 & \sqrt{\eta_2} & \cdots & \sqrt{\eta_n} \\ \sqrt{\eta_2} & -d_2 & 0 & \cdots \\ \vdots & 0 & \ddots & 0 \\ \sqrt{\eta_n} & 0 & \cdots & -d_n \end{pmatrix}, \tag{19}$$

where $\eta_i = r_i d_i m_i$.

If L^S is negative definite, then our system is asymptotically stable. It remains to look at the leading principal minors.

Since $m_i > 0$ and $r_j > 0$, we have $r_1 > 0$, then we note that the ith principal minor is given by the formula

$$\begin{aligned} L_i^S &= -d_i L_{i-1}^S + m_i(N^*)r_i \prod_{j=2}^{i}(-d_j) \\ &= -\left(r_1 + \sum_{j=2}^{i}(-1)^{j+1} m_j(N^*)r_j\right)\prod_{j=2}^{i}(-d_j) \\ &= (-1)^i\left(r_1 + \sum_{j=2}^{i}(-1)^{j+1} m_j(N^*)r_j\right)\prod_{j=2}^{i} d_j. \end{aligned}$$

Therefore, if $\left(r_1 + \sum_{j=2}^{i}(-1)^{j+1} m_j(N^*)r_j\right)\prod_{j=2}^{i} d_j > 0$ for $i = 2, \ldots, n$, we are done. But note that

$$\begin{aligned} r_1 + \sum_{j=2}^{i}(-1)^{j+1} m_j(N^*)r_j &> \sum_{j=1}^{n} m_i(N^*)r_j \\ &\quad + \sum_{j=2}^{i}(-1)^{j+1} m_j(N^*)r_j \\ &> \sum_{j=1}^{i} m_j r_j(1 + (-1)^i) \\ &> 0, \end{aligned}$$

for each $i = 2, \ldots, n$, which by our hypothesis is positive.

Finally, we claim that by the inverse function theorem the rest point exists and Jacobian is negative definite. First, suppose that N_1^* exists. Then we may apply the inverse function theorem to determine the N_i^* for $i \neq 1$. Without loss of generality, we may assume that $M_i = 0$ and thus $N_i^* = 0$ for $i \neq 1$. Then $\dot{N}_1 = 0$ if $N_{np}/N_1 = M_1$. Since $N_{np} \neq 0$, if $M_1 = 0$ there is no rest point. □

2.2 New systems

Suppose $N = N^*$ is the rest point of both systems (11) and (12) (to be proved below), and now note that, in fact, both systems have the same Jacobian at $N = N^*$. Namely, note that

$$\begin{aligned} \frac{d}{dx}\sinh(f(x))\,\big|_{f(x)=0} &= \cosh(f(x))\frac{df}{dx}\,\big|_{f(x)=0} \\ &= \frac{df(x)}{dx}\,\big|_{f(x)=0}\,. \end{aligned}$$

Therefore the local stability of these two formalisms is the same.

What we are interested in is whether there exist m or B such that the system is unstable. As shown above, there are no such m or B for the classical system, but we will show below that such m do exist for the newer systems. We are interested in whether this instability occurs in a region for which the newer systems are applicable. If so, this would either indicate that there are new phenomena described by the system or that the system is inappropriate for use in this region. Either result would be of interest for further study.

We have the main result:

Theorem 2 *If*

$$\min m_i > \left(\left(n\sum_{i=2}^{n} B_i^2\right)^{1/2} - \sum_{i=2}^{n} B_i\right)^{-1} \tag{20}$$

systems (11) *and* (12) *are unstable at their rest points.*

Inequality (20) implies that if any B_i corresponding to a permeating solute is large, then the system will be unstable for small m_i. Choosing two of the most common components of cryopreservation media, 1,2 Propane Diol and DMSO, with measured virial coefficients $B_{\mathrm{PG}} = 0.0399$, $B_{\mathrm{DMSO}} = 0.0843$, respectively [6], a system modeling the transport of the two would be unstable if $\min_i m_i > 81$, a value well beyond the intended applicability of the model.

Proof The Jacobian is defined by the partial derivatives of G and using the definitions from above, $\frac{\partial G_1}{\partial N_1} = -\frac{1}{N_1} r_1$, $\frac{\partial G_1}{\partial N_i} = \frac{\partial G_i}{\partial N_1} = \frac{1}{N_1} r_i$,

$$\frac{\partial G_i}{\partial N_i} = -\frac{1}{N_1}\left(\frac{1}{m_i} + 2B_i\right),$$
$$\frac{\partial G_i}{\partial N_j} = -\frac{1}{N_1}\left(B_i + B_j\right).$$

and so

$$J = \frac{1}{N_1}\Lambda A, \tag{21}$$

where Λ is a diagonal $n \times n$ matrix with diagonal entries b, and

$$A = \begin{pmatrix} -r_1 & r_2 & \dots & r_n \\ r_2 & & & \\ \vdots & & -A_1 & \\ r_n & & & \end{pmatrix},$$

where

$$A_1 = \{B_i + B_j + \delta_{ij} m_i^{-1}\}_{i,j=2}^{n} \tag{22}$$
$$:= A_1^* + D(m). \tag{23}$$

Let $\bar{r} = (r_2, \dots, r_n)^T$ and note that the matrix A is symmetric. We may multiply on the right and left of ΛA by $\Lambda^{-1/2} > 0$ to yield $\Lambda^{1/2} A \Lambda^{1/2}$ which is negative definite if and only if $-A > 0$.

$-A > 0$ holds if and only if $A_1 > 0$ and $A_1 + \bar{r} r_1 \bar{r}^T > 0$, and we have two possibilities to check.

A_1 is a real-symmetric matrix and thus has smallest eigenvalue $\lambda_n(A_1) > 0$. We apply a standard inequality for eigenvalues of sums of Hermitian matrices [2][1] and thus $\lambda_n(A_1) - \lambda_n(A_1^*) \le \lambda_1(D) = (\min_i m_i)^{-1}$. For $n > 2$ the rank of $A_1^* \le 2$, and it can be shown that the nonzero eigenvalues are $\sum_{i=2}^n B_i \pm (n \sum_{i=2}^n B_i^2)^{1/2}$. Thus, $\lambda_n(A_1^*) = \sum_{i=2}^n B_i - (n \sum_{i=2}^n B_i^2)^{1/2}$, and

$$\lambda_n(A_1) \le (\min_i m_i)^{-1} + \sum_{i=2}^{n} B_i - \left(n \sum_{i=2}^{n} B_i^2\right)^{1/2}.$$

Thus if $\min_i m_i > (-\sum_{i=2}^n B_i + (n \sum_{i=2}^n B_i^2)^{1/2})^{-1}$, $\lambda_n(A_1) < 0$.

[1] Namely, let $\lambda(X) = (\lambda_1(X), \dots, \lambda_n(X)$ be the vector of eigenvalues of a Hermitian matrix X with $\lambda_1(X) \ge \dots \ge \lambda_n(X)$. Then if $A = B + C$, with B and C Hermitian, then for $i = 1, \dots, n$, $\lambda_n(C) \le \lambda_i(A) - \lambda_i(B) \le \lambda_1(C)$.

Next, suppose hypothesis (20) is true and assume $C := A_1 + \bar{r} r_1 \bar{r}^T > 0$. Because C is real-symmetric, $C > 0$ if and only if $\lambda_n(C) > 0$. Applying the same theorem as above,

$$\begin{aligned}\lambda_n(C) &< \lambda_n(A_1) + \lambda_1(\bar{r} c r_1 \bar{r}^T) \\ &= r_1 \sum_{i=2}^{n} r_i^2 + \lambda_n(A_1) \\ &\leq r_1 \sum_{i=2}^{n} r_i^2 + \lambda_1(D) + \lambda_n(A_1^*).\end{aligned}$$

But $\lambda_1(D) + \lambda_n(A_1^*) < 0$ only if (20) holds, and we are done.

Finally, we note that, similar to the previous proof, the existence of a stable rest point is guaranteed by the inverse function theorem if (20) is not satisfied and if $m_1^{ext} \neq 0$. □

3 Discussion and conclusions

In this manuscript we have analyzed the local stability of three solute-solvent osmotic transmembrane flux models with an arbitrary number of solutes. In the classical case, we have found local stability for all combinations of parameters. In both modern models, we showed first that their stability is at least locally identical, and that there is a simple function of the parameters that bounds the instability region. This region is bounded well away from the intended applicability of the models used.

Because the stability shown in this manuscript is local stability, the behavior away from the rest point is unclear. Because cells are often exposed to high concentrations of solutes in a step function, further study would investigate the region of stability. We expect that this region would depend on B in a similar fashion. A Lyapunov function for the system would be ideal, but such functions for n dimensional nonlinear systems are difficult to find. However, there have been some recent developments in algebraic geometry which may be promising [11].

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