Concentration-dependent Unloading as a Necessary Assumption for a Closed Form Mathematical Model of Osmotically Driven Pressure Flow in Phloem

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ABSTRACT

Previous attempts to model steady state Münch pressure flow in phloem (Christy and Ferrier [1973]. Plant Physiol. 52: 531-538; and Ferrier et al. [1974]. Plant Physiol. 54: 589-600) lack sufficient equations, and results were produced which do not represent correct mathematical solutions. Additional equations for the present closed form model were derived by assuming that unloading of a given solute is dependent upon the concentration of that solute in the sieve tube elements. Examples of linear and enzymic type unloading mechanisms are given, although other concentration-dependent mechanisms could be substituted. A method for a numerical solution is outlined, and proof of convergence is presented along with some representative data and the speed of computer calculations. The model provides the minimal set of equations for describing the Münch pressure flow hypothesis as it might operate in plants.

Recent attempts to develop closed form mathematical models of the Münch (11, 12) pressure flow hypothesis of phloem translocation (modified to include osmotic fluxes of water along the path region) have met with problems heretofore unresolved. From the equations of Horwitz (8), it can be seen that one must calculate the profiles of at least four dependent variables along the sieve tube, namely, concentration of solutes (C), pressure (P), water flux through peripheral membranes (Jw), and the axial velocity of phloem sap (v or Jo). The relationships of these variables to the sieve tube as a whole and to individual sieve tube elements are illustrated in Figures 1 and 2, respectively. The irreversible thermodynamic models of Christy and Ferrier (2) and of Tyree et al. (18) do not provide unique, closed form solutions because each has at least one more variable than independent equations. A mathematical inconsistency of this type can be expected to produce aberrant behavior in the predictions of the model. As stated by Tyree et al. (18):

"...we are forced to conclude that several steady state solutions are possible which will give the same specific mass transfer rate for sucrose depending on the choice of r at s = 0. This point may have been overlooked by Christy and Ferrier (1) (our ref. 2) but it is difficult to tell because they made no definitive statement about it. The exact solution of the profiles of C, P, v, and Jw under any given loading and unloading conditions depends on the previous history of the system; i.e., it depends upon how the steady state transport is approached. The previous history of the system out of steady state is in some way specified by the choice of C(0). This point can be proved by examining equation 2; J does not depend on C, but as C drops, v increases to keep J constant (see equation 12) (our ref. 12)."

As no unique steady state mathematical solution could be found, the above models were used to approximate steady state sieve tube transport by a judicious choice of initial concentrations and convergence criteria, in a quasi-time-dependent iteration procedure. It is unlikely that such inconsistencies occur in real phloem, and thus, another equation must exist which would provide a unique, closed form solution at a given loading rate.

The above problem did not arise in the mechanical analogue model of Eschrich et al. (4). In this system, a given, initial amount of sugar was placed within an open or closed tubular, semipermeable membrane. In the absence of continuous loading and/or unloading of solute, their mathematical model correctly predicted the velocity of the solute front, the lack of equilibrium in the open tube, and the equilibrium condition when the front reached the end of the closed tube. Therefore, the necessary additional equations for a closed form mathematical model of steady state phloem translocation may lie with the assumptions regarding the loading and/or unloading of solutes. These considerations can best be illustrated by examining the existing equations as they apply to all of the individual elements of a sieve tube.

REVIEW OF GENERAL THEORY

The standard water potential terminology (9, 15, 16) will be used throughout to avoid thermodynamically inconsistent expressions such as negative pressures. Specifically,

\[ \psi_w = \psi_p + \psi_m \]

where \( \psi_w \) is water potential, \( \psi_p \) is pressure potential, and \( \psi_m \) is matric potential (usually ignored), and the modified term \( \psi_m \) is osmotic potential. (The subscript \( \pi \) is used here to avoid confusion between the subscript zero, often used to represent the outside water potential, and the subscript "o" for osmotic potential.) Water potentials of the xylem-apoplast continuum will be denoted by the subscript \( x \), and those of the sieve tube elements as \( i \). The flux of water through the peripheral membrane (\( J_{w} \), see Table 1 for units) of the \( i \)th sieve element would be a function of the water potential difference, and the hydraulic conductivity (\( L_p \)) and reflection coefficient (\( \sigma \)) of membranes, i.e.

\[ J_w = L_p (\psi_{w,i} - \psi_{m,i} + \sigma \psi_m) \]

where \( i = 1, 2, 3, \ldots n \).

The first element (\( i = 1 \)) has no open sieve plate nor flux of...
phloem sap through its initial end (Fig. 2). However, for all remaining elements, the volume flux or axial velocity of phloem sap into the element \( J_{in} \) is a function of the pressure gradient and the net solution conductivity \( L_{n-1} \) of the previous element i.e.,

\[
J_{in} = L_{n-1}(\Psi_{p-1} - \Psi_p)
\]

where \( i = 2, 3, \ldots n \), and the reflection coefficient of the sieve plate \( \sigma = 0 \). The problems of acceleration and deceleration of phloem sap in the vicinity of the sieve plate have been reviewed previously (8, 18).

Water must be conserved in each element, thus, in the initial element \( (i = 1) \)

\[
Jw,A_p = Jw(1 - \bar{V}_c)A_n
\]

in the intervening elements \( (i = 2, 3, \ldots n - 1) \)

\[
Jw,((1 - \bar{V}_c)A_n) + Jw,A_p = Jw(1 - \bar{V}_c)A_n
\]

and in the terminal element \( (i = n) \)

\[
Jw,((1 - \bar{V}_c)A_n) - Jw,A_p = -(Q)_n
\]

where \( C_i \) is the concentration of solute, \( \bar{V}_c \) is the partial molar volume of the solute in solution, \( A_n \) is the cross-sectional area of the element lumen, and \( A_p \) is the area of peripheral membrane.

Likewise, sugar must be conserved in the initial element \( (i = 1) \)

\[
(Q)_1 = Jw,C_iA_n
\]

in the intervening elements \( (i = 2, 3, \ldots n - 1) \)

\[
Jw,C_iA_n, + (Q)_i = Jw,C_iA_n
\]

and in the terminal element \( (i = n) \)

\[
Jw,C_iA_n, = -(Q)_n
\]

where \( (Q)_i \) is the rate at which sugar is loaded or unloaded in each element.

**Problem of Insufficient Equations.** For the present, let us treat all values of \( \sigma, \Psi_{w1}, L_p, A_p, L_n, A_n, \) and \( (Q)_i \) as constants with the stipulation that sugar be conserved at steady state for the whole sieve tube.

\[
\sum(Q)_i = 0
\]

It is important to note that as long as all values of \( (Q)_i \) are assigned constants, equation 6 degenerates to an arbitrary identity, i.e. \( 0 = 0 \). Also note that the equation describing the conservation of water for the entire sieve tube, i.e.

\[
\sum(Jw,A_p) = 0
\]

is a redundant form of equations 4a, 4b, and 4c combined.

In the initial element, there now remain four variables, \( C_1, \Psi_{wu}, Jw_1 \), and \( Jw_1 \) (Fig. 2) and only three independent equations, i.e. equations 2, 4a, and 5a. In each intervening element, there would be four variables, \( C_i, \Psi_{wi}, Jw_i \) and \( Jw_i \) and four independent equations, i.e. 2, 3, 4b, and 5b. In the terminal element, there would be only three variables, \( C_n, \Psi_{wn} \) and \( Jw_n \), and three corresponding equations, i.e. 2, 3, and 4c. The inapplicability of
beets, thus, sections, each 0.24 zone unloading concentration-dependent unloading with number of elements.

There are 50 sections, each 0.24 cm long, and has a linearly tapered cross-sectional area (as indicated). The path zone consists of 40 untapered sections, and the unloading zone consists of 40 sections with negative taper.

Mathematically, it would be possible to create a new independent equation by assuming that any of the variables in the initial element is a specific function of the loading rate ($Q_i$). However, for reasons discussed below, it is more reasonable physiologically to assume that the unloading rate ($Q_i$) varies as a function of $C_i$. Although this creates a new variable and introduces an additional dependent equation (i.e. $-Q_i = f(C_i)$), it also restores equation 5c to its independent status. There are now 4n variables and 4n-2 independent equations. Elements other than the terminal element may also be treated as unloading elements, since each would have one additional variable ($Q_{ik}$), and one additional equation. Thus, a model with concentration-dependent unloading will retain its closed form solution regardless of the number of elements or the number of additional variables each with its corresponding independent equation. Furthermore, such models have the capability of predicting the patterns of unloading as a function of $C_i$ and the efficiency and distribution of any proposed (concentration-dependent) unloading mechanisms.

**LOADING AND UNLOADING ASSUMPTIONS**

It is generally agreed that solutes are loaded into the phloem, if not actually into the sieve tubes, against a concentration gradient by some form of active transport (see reviews 3, 6, and 13). The rate of loading would thus be a function of the solute concentration in mesophyll cells, specialized loading cells, and/or companion cells, and a function of their metabolic energy level (e.g. ATP/ADP ratio). Except for possible feedback effects, the loading rate may be relatively independent of solute concentrations within the sieve tube elements. For purposes of comparison with previous models the loading rate ($Q_i$) for each element in the source region will be set as a constant ($a_i$) with a positive value which indicates loading inward to the sieve tube.

$$Q_i = a_i$$

This would approximate the condition where photosynthesis and loading were uniform along the source region. In the future, it would be more interesting and realistic to include concentration-dependent loading and appropriate feedback terms to describe the interaction of phloem translocation with the rate of photosynthesis (e.g. 7).

With respect to the concepts of unloading, Horwitz (8) assumed that the unloading rate ($Q_i$) for the $i$th sieve element might be proportional to a diffusion coefficient ($a_i$), the area of peripheral membrane ($A_{ip}$), and the difference in concentration across the membrane.

$$Q_i = a_i A_{ip} C_i$$

Such a diffusive mechanism implies that the reflection coefficient of the membranes is less than one. Although we may eventually deal with this implication, it is useful for the present to propose a more general unloading mechanism defined only as being linearly dependent upon the concentration of solute within a sieve element, i.e.

$$Q_i = a_i C_i$$

where $a_i$ is an unloading constant, and by convention, has a negative value. Concentration-dependent unloading has also been discussed previously by Tyree (17), and a formulation similar to equation 10 was suggested by MacRobbie (10) in a discussion of activated mass flow.

If solutes move through peripheral membranes by passive and/or active enzyme carriers (e.g. see Bevers [1]), then other
kinetic formulations must be used. In each case, these kinetic formulations are likely to be concentration-dependent. For example, the traditional enzyme kinetics (Briggs-Haldane in 1928 as reviewed by Reiner [14]) may be applied to the unloading of solutes in the form

\[
(Q_u) = \frac{K_2 \epsilon A_P C_i}{(K_1 + K_2)/K_1 + C_i}
\]  

(11)

where \(K_2\) is the enzyme turnover constant, \(\epsilon\) is the enzyme concentration per unit area of membrane, \(A_P\) is the area of peripheral membrane, and \(K_1\) and \(K_1\) and \(K_1\) are rate constants of the forward and back reactions (14). Again, for purposes of simplicity on a per cell basis, and for consistency with current enzyme kinetic notation, we may modify equation 11 to the following

\[
(Q_u) = -\frac{V_{\text{max}} C_i}{K_m + C_i}
\]  

(12)

where \(V_{\text{max}}\) is the maximum unloading rate per element, and \(K_m\) is the concentration which causes \(1/2 V_{\text{max}}\). Note that the negative value of the right hand term indicates outward loading. Variations in the value of \(V_{\text{max}}\) can be considered equivalent to variations in the amount of unloading enzyme per cell. More complex carrier kinetics (i.e. including product inhibition, substrate cooperativity) could also be substituted if warranted by experimental findings.

We must recognize at this point that any loading or unloading mechanism may be sensitive to the effects of physiological parameters besides solution concentration. For example, one could hypothesize loading or unloading mechanisms whose efficiency was affected by pressure and/or water potential (e.g. \(V_{\text{max}} = f(P)\), or \(\alpha = f(\Psi_{\text{tot}})\)). Even so, the concentration of reactants plays an inescapable role in the rate of any known chemical or diffusive process. Thus, the treatment of unloading as being concentration-dependent is not only mathematically necessary, but biophysically reasonable.

RESULTS

Using the procedure outlined in the Appendix, a FORTRAN-G program was written for an IBM-360-65 computer. The program treats an isolated sieve tube in contact with an infinite source of water at a water potential of \(-3\) bars, with a fixed loading rate, and an infinite sink for unloading of solutes. The dimensions and properties of the sieve tube are described in Table I, and are essentially the same as those chosen by Christy and Ferrier (2). Depending upon the chosen values for the unloading constants, the numerical steady state values of \((Q_u), \; C_i, \; J_{\text{tot}}, \; \Psi_{\text{tot}}, \; J_{\text{pl}}\) and all of the necessary sums and derivatives for all 120 sections can be calculated (and printed) in 3 to 8 sec of computer time. This might be reduced further by such simplifications as assuming a nontapered sieve tube or reducing the number of elements. In any case, the program allows numerous, inexpensive analyses of the interaction of many parameters. (A copy of the present computer code or more recent developments is available from the authors.)

Convergence Tests of the Model with Concentration-dependent Unloading. We may examine the curve which leads to numerical solutions of the model with various unloading efficiencies by plotting the algebraic sum of the water fluxes \(\Sigma(J_{\mu A_P})\) as a function of specifically chosen values for solute concentration in the first element \(C_i\). At any point where the function equals zero, all equations are simultaneously satisfied. A closed form, steady state model exists only if the function curve crosses through the abscissa at a unique value for \(C_i\) as shown in Figure 3, A and B (and consequently, a unique value for all other dependent variables).

![Figure 3](https://www.plantphysiol.org)
Convergence of the model, using various values for the linear unloading constant (see equation 10) or the enzymic unloading expression (equation 12), attests to its closed form nature (Fig. 3, A and B). Attempts to extend the function curves for the enzymic unloading model further below the zero point (i.e. by higher values of $C_1$) lead to discontinuities since extension of $(Q_0)$, from equation 12 beyond the value of $V_{\text{max}}$ leads mathematically to negative values of solute concentration. Likewise, choosing inadequate values for $V_{\text{max}}$ and/or $K_{\text{m}}$ (from equation 12) at a given loading rate will cause the model not to converge (except by allowing negative values for solute concentration in one or more elements).

**Convergence Test of a Model with Non-Concentration-dependent Unloading.** By arbitrarily setting $(Q_0)_i = -(Q_0)_i$ and disregarding the inapplicability of equations A-5 and A-7 to the terminal element (see “Appendix”), we can calculate values of the total water flux $\Sigma (J_{\text{w}} A_{\text{p}})$ as a function of values of $C_1$ as seen in Figure 3C. (Note: applying equations A-5 and A-7 to the terminal element is equivalent to assuming it to have an open sieve plate.) Because of the shallow slope of the function curve as it approaches the zero line (possibly asymptotically), the actual value obtained for $C_1$ is influenced by the convergence criteria (in our case, Absolute $[\Sigma (J_{\text{w}} A_{\text{p}})] / [\Sigma \text{Absolute} (J_{\text{w}} A_{\text{p}})] \leq 1.0 \times 10^{-5}$). With this criterion and starting with an initially low value for $C_1$, the computer model iterated to a value of 0.5212 molar or 17.84% sucrose. This value for $C_1$ and the resulting values of all other variables were nearly the same as those in Figure 4 of Christy and Ferrier (2). As the value of $C_1$ is increased, the $\Sigma (J_{\text{w}} A_{\text{p}})$ remains essentially zero. Thus, in their model, there are an infinite number of apparent solutions since any value for $C_1$ above that minimum seems to fit the convergence criteria. On the other hand, with the concentration-dependent models, an initial estimate $C_1$ above or below the convergence point would cause the model to iterate down or up, respectively, to the true value of $C_1 \pm$ the convergence criteria (Fig. 3, A and B).

**Consequences of Concentration-dependent Unloading.** Each element in the unloading zone was assigned the same value for its unloading constant, thus giving the zone a uniform potential unloading capacity. However, because unloading is concentration-dependent, the profile of unloading will not necessarily be uniform. With a large value for the unloading constant (e.g. $\alpha_0 = -10 \times 10^3 \alpha_1$), most of the solute is unloaded near the top of the unloading zone (Fig. 4A). This profile is rather unrealistic for many plants where unloading rates might be expected to increase toward the tip of roots or other meristematic regions. The profile of unloading can be forced into a more rectangular or uniform distribution (Fig. 4A) by reducing the efficiency of the unloading mechanism (by decreasing the absolute numerical value of the constant e.g. $\alpha_0 = -1.25 \times 10^2 \alpha_1$). Any attempt to force an exactly rectangular unloading pattern by further reductions in efficiency would lead to absurdly high concentrations of solutes in the sieve tube. The pattern of unloading can also be altered by assigning a different distribution of the unloading mechanism. For example, we might increase the efficiency of unloading toward the terminal end of the sieve tube and/or unload into a series of finite capacity sinks, each with an appropriate utilization rate and feedback effects.

Other consequences of the various values for the linear unloading constant can be seen in Figure 4B and C. As the absolute value of the unloading constant is decreased, the average solute concentration is increased as is the average pressure (not shown). At first glance, this might seem inconsistent with the lower velocities (Fig. 4C) which are required to maintain the rate of sucrose $(\Sigma Q_i)$ is the same in all cases, thus, there is an inverse relationship between the velocity and the concentration. The peculiar shape of these velocity curves results in part from the tapered sieve tube.

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**Figure 4.** A: Profiles of loading $(Q_0)$ and unloading $(Q_u)$ in the appropriate regions of the sieve tube at specified values for the linear, concentration dependent unloading mechanism $(\alpha_0)$; B: profiles of sucrose concentration along the axis of the sieve tube; C: profiles of the axial velocity of the solution along the sieve tube. Note that the total loading velocity $(J_{\text{w}} A_{\text{p}})$ is the same in all cases, thus, there is an inverse relationship between the velocity and the concentration. The peculiar shape of these velocity curves results in part from the tapered sieve tube.
same mass transfer rate. However, the decreased velocities result from the decreased gradient of concentration (Fig. 4B) and pressure (not shown) along the axis of the sieve tube. Note that the profiles of concentration and velocity do not correlate in a simple, obvious way; this results in part from the fact that the sieve tube is tapered in both the loading and unloading regions (Table I).

**Enzymic Unloading Mechanism.** The unloading profiles generated by an enzymatic unload mechanism differ primarily when the value of $V_{\text{max}}$ is large. In this case, most of the solute is unloaded before reaching the terminal end of the region. Attempts to force the enzymic unloading profile into a rectangular pattern by lowering the value $V_{\text{max}}$ (or raising the value of $K_m$) fail because one or more elements would be required to unload at a rate greater than $V_{\text{max}}$. With the present set of dimensions and properties (Table I), the minimum value for $V_{\text{max}} = -1.5a_0$ (e.g. using the value $V_{\text{max}} = -1.4$) caused the model not to converge. Thus, as seen in Figure 3B, the enzymic unloading mechanism allows the model to converge at several values of $V_{\text{max}}$, but over a much narrower range than that of the linear unloader.

**Other Modifications of the Model.** We have presented only those results which are necessary to demonstrate that the model is indeed closed form, and would apparently be so with any concentration-dependent unloading mechanism. We have determined that with little change in the existing mathematics or computer codes, it will be possible to incorporate other concepts such as loading mechanisms which are controlled by the concentration of solutes outside the sieve tube, feedback or back-leakage terms in both the loading and unloading zones, concentration-dependent viscosity, and others. (These results are being incorporated in papers being prepared for publication.) These and other modifications will make it possible to link the phloem model with models of sources (leaves) and sinks (roots, seeds, meristems).

**DISCUSSION**

The present model, to a great extent, incorporates the same rationale and equations as those of previous workers (2, 4, 8, 18). By adding an independent equation for concentration-dependent unloading, we have defined a minimal set of equations necessary for a mathematically consistent expression of the Münch hypothesis as it might operate in plants. The model correctly treats the sieve tube as having closed initial and terminal ends. It also has the ability to predict possible patterns of unloading.

All of the current models (2, 5, 18), including the present one, are somewhat unrealistic because they treat the phloem as an isolated sieve tube with infinite sources and sinks of solutes and water. They lack the metabolic requirements, P-protein, regulatory mechanisms, and other constraints which undoubtedly operate in real phloem. The model may be corrected or further developed as the nature of these parameters is exposed by experimental plant physiologists. In its present form, the model can retain its closed form nature while incorporating these parameters in the form of new terms or independent equations to be solved simultaneously with the existing mathematical expressions. As a result, the model will provide a mathematically consistent analysis of the possible interactions and implications of each suggested hypothesis, and may reveal previously unsuspected interactions or lead to new hypotheses which might be tested experimentally.

**APPENDIX**

**METHOD OF SOLUTION**

Numerical solutions were obtained using the following sequence of equations and the constants and variables shown in Table I. Most of the values are those used by Christy and Ferrier (2), and the term $(V_{\text{max}})$ is usually left expanded as $-(C_{\text{RT}})$.

**Calculation Sequence for the Initial Cell in the Loading Zone.** Concentration is determined as:

$$C_i = \text{an initial estimated value, then a series of values estimated by a Newton-Raphson iteration until a unique value is found which gives a numerical solution (simultaneously) to all of the following equations such that water is conserved in a steady state flux (i.e. see equation 7)}$$

Using the convergence criterion $\text{Absolute} [\Sigma (J_i A_i)] / \text{Absolute} (J_i A_i) \approx 1 \times 10^{-4}$.

Then, velocity is determined from equation 5a

$$J_i = \frac{(Q_i)}{C_i A_i} \quad (A-1)$$

then, water flux from equation 4a

$$J_w = \frac{J_i (1 - \frac{V_i}{C_i} A_i)}{A_i} \quad (A-2)$$

then, pressure from equation 2

$$\Psi_p = -\frac{J_w}{L_p} - \frac{\Psi_{wr} + C_i RT}{L_p} \quad (A-3)$$

**Calculations for the Remaining Elements in the Loading Zone.** First, pressure potential is calculated from equation 3

$$\Psi_p = -\frac{J_m}{L_m} + \frac{\Psi_{wr} + C_i RT}{L_m} \quad (A-4)$$

then, concentration as

$$C_i = \text{that value at each estimated value } C_i \text{ which satisfies the following equation (derived by combination of equations 2, 4b, and 5b)}$$

$$C_i (L_p A_i RT) + C_i (J_{(m), A_i}) + J_{(p)} (\Psi_{wr} - \Psi_p) + V_i (Q_i) - (J_m, C_i A_i_{m, i} + (Q_i)) = 0 \quad (A-5)$$

then, water flux from equation 2

$$J_m = L_m (\Psi_{wr} - \Psi_p + C_i RT) \quad (A-6)$$

then, velocity from equations 4b and 5b

$$J_w = \frac{J_m A_{m, i} + J_{(p)} A_{p, i} + V_i (Q_i)}{A_{m, i}} \quad (A-7)$$

noting the $\Sigma (J_i A_i)$ and $\Sigma (Q)$ and the sums of their absolute values are accumulated at each element.

**Calculations for Elements in the Path Zone.** The same sequence of equations is used as in the loading zone with the exception that $(Q)$ equals zero.

**Calculations for Elements in the Unloading Zone** (except one or more cells at the terminus). The same sequence of equations as those in the loading zone except that $(Q_i)$ must be calculated by an equation such as 10 or 12 and then substituted for $(Q_i)$ in equations A-5 and A-7; in the case of equation A-5, this is accomplished simultaneously with an internal Newton-Raphson iteration.

**Calculations for the Terminal Cell in the Unloading Zone.** One may first calculate pressure potential using equation A-4, then a value of concentration $(C_i)$ based on the appropriate kinetic expression for the unloading mechanism, such as the linear unloader (combining equations 5c and 10)

$$C_n = \frac{J_{(n), C_i A_i}}{\alpha_n} \quad (A-8)$$

or respectively, the enzymatic unloader (combining equations 5c and 12)

$$C_n = \frac{J_{(n), C_i A_i}}{V_{\text{max}} + \frac{J_{(n), C_i A_i}}{V_{\text{max}}}} \quad (A-9)$$

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or any other concentration-dependent mechanism; then \( (J_w) \) by equation A-6.

**LITERATURE CITED**