Ussing's flux ratio theorem for nonlinear diffusive transport with chemical interactions

A. J. Bracken A. McNabb * M. Suzuki †

The Department of Mathematics, The University of Queensland,
Brisbane, QLD.4072 Australia.

1 Introduction

Ussing's flux ratio theorem (1978) reflects a reciprocal relationship behavior between the unidirectional fluxes in asymmetric steady diffusion-convection in a membrane slab. This surprising result has led to many subsequent studies in a wide range of applications, in particular involving linear models of time dependent problems in biology and physiology. Ussing's theorem and its extensions are inherently linear in character. It is of considerable interest to ask to what extent these results apply, if at all, in situations involving, for example, nonlinear reaction.

A physiologically interesting situation has been considered by Weisiger *et al.* (1989, 1991, 1992) and by McNabb *et al.* (1990,1991) who studied the role of albumin in the transport of ligands across aqueous diffusion barriers in a liver membrane slab. The results are that there exist reciprocal relationships between unidirectional fluxes in the steady state, although albumin is chemically interacting in a nonlinear way of the diffusion processes. However, the results do not hold in general at early times. Since this type of study first started, it has been speculated about when and how the Ussing's flux ratio theorem fails in a general diffusion-convection-reaction system.

In this paper we discuss the validity of Ussing-type theorems in time-dependent situations, and consider the limiting time behavior of a general nonlinear diffusion system with interaction.

2 Transport equations for a diffusion-reaction system

The model assumed here is of one-dimensional diffusion of ligand across two unstirred layers. The first ($0 < x < \delta_1$) is an aqueous solution of ingredients at concentration $u(x,t), b(x,t)$ and $a(x,t)$ of unbound ligand, ligand-protein bound complex and protein(albumin). The slab is bounded by an aqueous equilibrium solution at concentrations u_0, b_0 and a_0, respectively, of these ingredients at $x \geq \delta_1$ and a lipid-water interface at $x = 0$. The second ($-\delta_2 < x < 0$) is a solution of ligand in lipid(decane) at concentration $w(x,t)$ and bounded by a well stirred decane solution in the region at $x \leq -\delta_2$ and the decane-water interface at $x = -\delta_2$. These decane solutions are assumed to be insoluble for protein and ligand-protein complex. The decane-water interface at $x = 0$ is also assumed to have permeability partition ratio $\alpha w(0_-, t) = u(0_+, t)$ of ligand between decane layer and aqueous layer for the absence of protein, where α is a positive constant.

The concentrations $u(x,t), b(x,t)$ and $a(x,t)$ in the unstirred and well stirred aqueous layers $x > 0$ are governed by following mass conservation equations of diffusion-reaction

$$\left.\begin{array}{rcrcl} u_t(x,t) & - & D_u u_{xx}(x,t) & = & -\rho(x,t) \\ b_t(x,t) & - & D_b b_{xx}(x,t) & = & \rho(x,t) \\ a_t(x,t) & - & D_a a_{xx}(x,t) & = & -\rho(x,t) \end{array}\right\} \quad (x > 0, \quad t > 0). \tag{2.1}$$

*Permanent address: Mathematical Physics Research Institute, 107 Dittmer Drive, Palmerston North, New Zealand.
†Corresponding to be sent.

where $\rho(x,t)$ is a general chemical interaction term for the net rate of ligand-protein complex production per unit time in unit volume, given by

$$\rho(x,t) = k_1 u(x,t) a(x,t) - k_2 b(x,t) \qquad (x > 0, \quad t > 0). \tag{2.2}$$

where k_1 and k_2 are association and disassociation constants respectively.

The concentrations in the unstirred and well stirred decane layers $x < 0$ are governed by

$$w_t(x,t) - D_w w_{xx}(x,t) = 0 \qquad (x < 0, \quad t > 0). \tag{2.3}$$

The flux continuity and permeability conditions are

$$\alpha w(0_-,t) = u(0_+,t) \qquad \text{(where } \alpha \text{ is a positive constant.)} \tag{2.4}$$

$$D_w w_x(0_-,t) = D_u u_x(0_+,t) \tag{2.5}$$

$$b_x(0_+,t) = a_x(0_+,t) = 0 \tag{2.6}$$

The initial conditions of interest for the study of Weisiger *et al.* (1989) are $w(x,0) = 0$ for $-\delta_2 < x < 0$, $u(x,0) = b(x,0) = 0$ and $a(x,0) = b_0 + a_0$ for $0 < x < \delta_1$. This generates a flux

$$j(-\delta_2,t) = -D_w w_x(-\delta_2,t) \tag{2.7}$$

of ligands out of the unstirred decane layer at $x = -\delta_2$ at time t. Denote by $u^*(x,t)$, $b^*(x,t)$, $a^*(x,t)$, $w^*(x,t)$ and $j^*(x,t)$ which the quantities corresponding to $u(x,t)$, $b(x,t)$, $a(x,t)$ and $j(x,t)$ for a second complementary solution which satisfies to the same initial condition as before but boundary conditions $u^*(\delta_1,t) = b^*(\delta_1,t) = 0$ and $a^*(\delta_1,t) = b_0 + a_0$ in the well-stirred layer $x \geq \delta_1$, and $w^*(-\delta_2,t) = u_0$ in the second well-stirred decane layer $x \leq \delta_2$. This generates a flux

$$j^*(\delta_1,t) = -D_u u_x^*(\delta_1,t) - D_b b_x^*(\delta_1,t) \tag{2.8}$$

through $x = \delta_1$ at time t. The flux ratio of interest is $R(t) = \dfrac{-j(-\delta_2,t)}{j^*(\delta_1,t)}$.

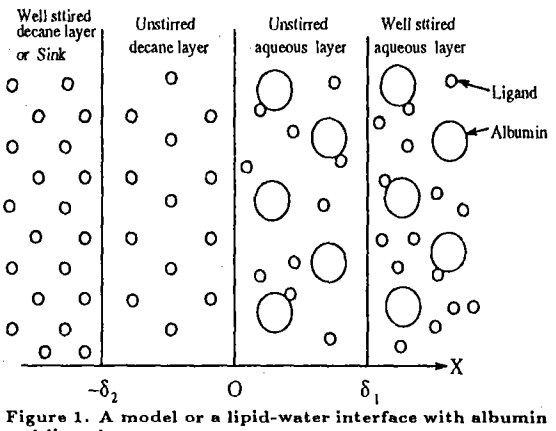

Figure 1. A model or a lipid-water interface with albumin and ligands.

3 Initial behavior of flux ratio

To investigate the time-dependent behavior, it is a good idea to make the transport equations (2.1) – (2.8) dimensionless by introducing

$$t = \frac{\delta_1^2}{D_u}\tau, \qquad x = \delta_1 \sigma$$

$$u(x,t) = u_0 U(\sigma,\tau), \qquad b(x,t) = b_0 B(\sigma,\tau) \tag{3.1}$$

$$a(x,t) = a_0 A(\sigma,\tau), \qquad \alpha w(x,t) = u_0 W(\sigma,\tau)$$

so that

$$\left.\begin{aligned} U_\tau(\sigma,\tau) - U_{\sigma\sigma}(\sigma,\tau) &= -\epsilon R(\sigma,\tau) \\ B_\tau(\sigma,\tau) - \triangle_2 B_{\sigma\sigma}(\sigma,\tau) &= \lambda_2 \epsilon R(\sigma,\tau) \\ A_\tau(\sigma,\tau) - \triangle_3 A_{\sigma\sigma}(\sigma,\tau) &= -\lambda_3 \epsilon R(\sigma,\tau) \\ R(\sigma,\tau) = U(\sigma,\tau)A(\sigma,\tau) &- \nu B(\sigma,\tau) \end{aligned}\right\} \qquad (\sigma > 0, \quad \tau > 0) \tag{3.2}$$

$$W_\tau(\sigma,\tau) - \triangle_4 W_{\sigma\sigma}(\sigma,\tau) = 0 \qquad (\sigma < 0, \quad \tau > 0)$$

Table 1: Variables and functions.

τ	-Dimensionless time for real time t
$\hat{x}$	-Dimensionless space variable $\hat{x} = x\delta$ for real space x
δ_1, δ_2	-Length scales for unstirred solution layer and unstirred decane layer
δ_d	-Dimensionless position: $\delta_2 = \delta_d\delta_1$
u, b, a, d	-Concentratioin for unbound ligand, bound ligand-protein, free protein in the solution and unbound ligand in the decane respectively
U, B, A, W	-Dimensionless concentrations: $u = u^0U$, $b = b^0B$, $a = a^0A$, $w = \alpha u^0W$
j, j^*	-Tracer fluxes
J, J^*	-Dimensionless tracer fluxes
D_u, D_b, D_a, D_w	-Diffusion constants for unbound ligand, bound ligand-protein and unbound protein in the solution and unbound ligand in the decane respectively
k_1	-Binding constant for ligand - protein
k_2	-Unbinding constant for ligand-protein complex
ϵ	-Dimensional binding constant: $D_u\epsilon = \delta_1^2 k_1 a_0$
ν	-Dimensionless unbounding constant
$\Delta_2, \Delta_3, \Delta_4$	-Dimensionless diffusion constans $D_b = \Delta_2 D_u$, $D_a = \Delta_3 D_u$, $D_d = \Delta_4 D_u$ respectively
$\lambda_2, \lambda_3, \lambda_4$	-Concentration ratio at equilibrium state $\lambda_2 u^0 = b^0$, $\lambda_3 u^0 = a^0$, $\lambda_4 u^0 = d^0$ respectively

where the definitions of the dimensonless parameters ϵ, μ, λ_2, λ_3, λ_4, Δ_2, Δ_3, Δ_4 are given in Table 1. The initial conditions become

$$\begin{aligned} &U(\sigma,0) = B(\sigma,0) = 0, \quad A(\sigma,0) = 1 \quad (\sigma > 0) \\ &W(\sigma,0) = 0 \qquad\qquad\qquad\qquad\quad (\sigma < 0) \end{aligned} \tag{3.3}$$

The boundary conditions in this dimensionless form are,

$$\begin{aligned} &U(1,\tau) = B(1,\tau) = A(1,\tau) = 1 \\ &U(0,\tau) = W(0,\tau), \quad \Delta_4 W_\sigma(0,\tau) = U_\sigma(0,\tau), \quad B_\sigma(0,\tau) = A_\sigma(0,\tau) = 0 \quad (\tau > 0) \\ &S(-\delta_d,\tau) = 0, \end{aligned} \tag{3.4}$$

This generates the nondimensional flux at $\sigma = -\delta_d$

$$J(-\delta_d,\tau) = -\frac{\Delta_4}{\lambda_4} W_\sigma(-\delta_d,\tau), \qquad (\tau > 0). \tag{3.5}$$

The second complementary solution corresponds to the same initial conditions, but with the boundary conditions,

$$\begin{aligned} &U^*(1,\tau) = B^*(1,\tau) = 0, \qquad A^*(1,\tau) = 1 \\ &W^*(-\delta_d,\tau) = 1 \end{aligned} \qquad (\tau > 0), \tag{3.6}$$

This generates the nondimensional flux at $\sigma = 1$

$$J^*(1,\tau) = -U_\sigma^*(1,\tau) - \frac{\Delta_2}{\lambda_2} B_\sigma^*(1,\tau), \qquad (\tau > 0). \tag{3.7}$$

When the time τ is very small, $U(\sigma,\tau)$ differs from 1 significantly only in small neighborhood near $\sigma = 1$. Similarly, $W^*(\sigma,\tau)$ differs from 1 in small neighborhood near $\sigma = -\delta_d$. Suppose that a typical interaction time $\frac{1}{k_1 a_0}$ is larger compared with a typical diffusion time $\frac{\delta_1^2}{D_u}$ for unbound ligand, then, the nondimensional variavle

$$\epsilon = \frac{\delta_1^2 k_1 a_0}{D_u} << 1 \tag{3.8}$$

and far scaled time $\tau = O(1)$, and interacting effects will be small compared with diffusion effects. Then we expand $U(\sigma,\tau)$, $B(\sigma,\tau)$, $A(\sigma,\tau)$ $W(\sigma,\tau)$ as asymptotic series in ϵ so that

$$\begin{aligned} U(\sigma,\tau) &= U^{(0)} + \epsilon U^{(1)} + \epsilon^2 U^{(2)} + \\ B(\sigma,\tau) &= B^{(0)} + \epsilon B^{(1)} + \epsilon^2 B^{(2)} + \\ A(\sigma,\tau) &= A^{(0)} + \epsilon A^{(1)} + \epsilon^2 A^{(2)} + \\ W(\sigma,\tau) &= W^{(0)} + \epsilon W^{(1)} + \epsilon^2 W^{(2)} + , \end{aligned} \tag{3.9}$$

and we find that the zeroth order terms of equations (3.2) have no chemical reaction term and are linear,

$$
\begin{aligned}
U_\tau^{(0)}(\sigma,\tau) &- U_{\sigma\sigma}^{(0)}(\sigma,\tau) &= 0\\
B_\tau^{(0)}(\sigma,\tau) &- \triangle_2 B_{\sigma\sigma}^{(0)}(\sigma,\tau) &= 0\\
A_\tau^{(0)}(\sigma,\tau) &- \triangle_3 A_{\sigma\sigma}^{(0)}(\sigma,\tau) &= 0\\
W_\tau^{(0)}(\sigma,\tau) &- \triangle_4 W_{\sigma\sigma}^{(0)}(\sigma,\tau) &= 0
\end{aligned}
\tag{3.10}
$$

If $\overline{U}(\sigma,s)$, $\overline{B}(\sigma,s)$ and $\overline{W}(\sigma,s)$ denote the Laplace transforms of these dependent variables,

$$
\overline{U}(\sigma,s)=\int_0^\infty e^{(-s\tau)}U(\sigma,y)dy,\qquad \overline{B}(\sigma,s)=\int_0^\infty e^{(-s\tau)}B(\sigma,y)dy,
$$
$$
\overline{W}(\sigma,s)=\int_0^\infty e^{(-s\tau)}W(\sigma,y)dy
\tag{3.11}
$$

the zeroth order equations (3.10) give

$$
s\overline{U}^{(0)}(\sigma,s)-\overline{U}_{\sigma\sigma}^{(0)}(\sigma,s)=0,\qquad s\overline{B}^{(0)}(\sigma,s)-\triangle_2\overline{B}_{\sigma\sigma}^{(0)}(\sigma,s)=0
$$
$$
s\overline{W}^{(0)}(\sigma,s)-\triangle_2\overline{W}_{\sigma\sigma}^{(0)}(\sigma,s)=0.
\tag{3.12}
$$

Designate by $U(\sigma,\tau)$, $B(\sigma,\tau)$, $W(\sigma,\tau)$ the solutions of the problem (3.2), (3.4) satisfying the zero initial condition (3.3) and by $U^*(\sigma,\tau)$, $B^*(\sigma,\tau)$, $A^*(\sigma,\tau)$ the solutions of the same problem but with (3.6) boundary condition. Then, trivially

$$
\begin{aligned}
0=E=\ & \int_{-\delta_d}^{0_-}\frac{1}{\lambda_4}(\overline{W}^{(0)}s\overline{W}^{*(0)}-s\overline{W}^{(0)}\overline{W}^{*(0)})d\sigma\\
&+\int_{0_+}^{1}\left\{(\overline{U}^{(0)}s\overline{U}^{*(0)}-s\overline{U}^{(0)}\overline{U}^{*(0)})+\frac{1}{\lambda_2}(\overline{B}^{(0)}s\overline{B}^{*(0)}-s\overline{B}^{(0)}\overline{B}^{*(0)})\right\}d\sigma.
\end{aligned}
\tag{3.13}
$$

It is noted that $B^{(0)}$ and $B^{*(0)}$ are zero, because the initial condition for $B(\sigma,\tau)$ for $0<x<1$ is zero, and there exists no chemical interaction in the zero-th order to create bound complex. On the other hand, integration by parts leads from equation (3.13) into

$$
\begin{aligned}
E=\ & -\left[\overline{W}^{(0)}\overline{J}^{*(0)}-\overline{J}^{(0)}\overline{W}^{*(0)}\right]_{-\delta_d}^{0_-}-\int_{-\delta_d}^{0_-}\frac{1}{\lambda_4}\left\{\overline{W}_\sigma^{(0)}\triangle_4\overline{W}_\sigma^{*(0)}-\triangle_4\overline{W}_\sigma^{(0)}\overline{W}_\sigma^{*(0)}\right\}d\sigma\\
&-\left[\overline{U}^{(0)}\overline{J}^{*(0)}-\overline{J}^{(0)}\overline{U}^{*(0)}\right]_{0_+}^{1}-\int_{0_+}^{1}\left\{\overline{U}_\sigma^{(0)}\overline{U}_\sigma^{*(0)}-\overline{U}_\sigma^{(0)}\overline{U}_\sigma^{*(0)}\right\}d\sigma,
\end{aligned}
\tag{3.14}
$$

and both integrals vanish. Therefore,

$$
\begin{aligned}
E=\ & -\overline{W}^{(0)}(0_-)\overline{J}^{*(0)}(0_-)+\overline{W}^{(0)}(-\delta_d)\overline{J}^{*(0)}(-\delta_d)+\overline{J}^{(0)}(0_-)\overline{W}^{*(0)}(0_-)-\overline{J}^{(0)}(-\delta_d)\overline{W}^{*(0)}(-\delta_d)\\
&-\overline{U}^{(0)}(1)\overline{J}^{*(0)}(1)+\overline{U}^{(0)}(0_+)\overline{J}^{*(0)}(0_+)+\overline{J}^{(0)}(1)\overline{U}^{*(0)}(1)-\overline{J}^{(0)}(0_+)\overline{U}^{*(0)}(0_+)
\end{aligned}
\tag{3.15}
$$

From (3.4) (3.6), now the nondimensional boundary conditions at $\sigma=0$ give

$$
\begin{aligned}
\overline{W}^{(0)}(0_-,s) &= \overline{U}^{(0)}(0_+,s), & \overline{W}^{*(0)}(0_-,s) &= \overline{U}^{*(0)}(0_+,s),\\
\overline{J}^{(0)}(0_-,s) &= \overline{J}^{(0)}(0_+,s), & \overline{J}^{*(0)}(0_-,s) &= \overline{J}^{*(0)}(0_+,s),
\end{aligned}
\tag{3.16}
$$

and so

$$
E=-\overline{J}^{(0)}(-\delta_d)\overline{W}^{*(0)}(-\delta_d)-\overline{U}^{(0)}(1)\overline{J}^{*(0)}(1)=0.
\tag{3.17}
$$

Thus we obtain

$$\frac{-J^{(0)}(-\delta_d,\tau)}{J^{*(0)}(1,\tau)} = \frac{U^{(0)}(1,\tau)}{W^{*(0)}(-\delta_d,\tau)}. \tag{3.18}$$

In terms of the original variables, this result is

$$\frac{1}{\alpha^2}\frac{-j^{(0)}(-\delta_2,t)}{j^{*(0)}(\delta_1,t)} = \frac{u^{(0)}(\delta_1,t)}{w^{*(0)}(-\delta_2,t)}, \tag{3.19}$$

where α is the permeability constant.
We see that the flux ratio is independent of time in the early stages, despite the nonlinear chemical interacts.

4 Infinite time analysis

Numerical results suggests that in this nonlinear system the Ussing's flux ratio theorem fails for larger times. To avoid investigating time dependent behavior of the nonlinear system with all its difficulties, we consider instead infinite time steady state analysis.

The flux of tracers are measured of $u(x,t)$ $b(x,t)$ and $w(x,t)$. Equation of ligands and ligand-protein complex (2.1)(2.2)(2.3) can be rewritten as follows,

$$\begin{aligned} (u_t(x,t)+b_t(x,t)) - (D_u u_{xx}(x,t) + D_b b_{xx}(x,t)) = 0 \qquad & (x>0, \quad t>0) \\ W_t(x,t) - D_w w_{xx}(x,t) = 0 \qquad & (x<0, \quad t>0). \end{aligned} \tag{4.1}$$

Now cale the independent variables as

$$t = \frac{1}{k_1 a_0}\tau \qquad x = \delta_1 \sigma \tag{4.2}$$

with the same nondimensional variables $U(\sigma,\tau)$, $B(\sigma,\tau)$ and $W(\sigma,\tau)$ as before. Then, we obtain

$$\begin{aligned} \epsilon\left(U_\tau(\sigma,\tau)+\frac{1}{\lambda_1}B_\tau(\sigma,\tau)\right) - \left(U_{\sigma\sigma}(\sigma,\tau)+\frac{\triangle_2}{\lambda_2}B_{\sigma\sigma}(\sigma,\tau)\right) = 0 \qquad & (\sigma>0, \quad \tau>0) \\ \frac{\epsilon}{\lambda_2}W_\tau(\sigma,\tau) - \left(\frac{\triangle_4}{\lambda_4}W_{\sigma\sigma}(\sigma,\tau)\right) = 0 \qquad & (\sigma>0, \quad \tau>0), \end{aligned} \tag{4.3}$$

with ϵ as before. Then, we can consider the limit of large times again regarding ϵ as a small variable. and expanding $U(\sigma,\tau)$, $B(\sigma,\tau)$ and $W(\sigma,\tau)$ as asymptotic series in ϵ, the zeroth order term of the equations (4.3) are time -independent,

$$\begin{aligned} U^{(0)}_{\sigma\sigma}(\sigma) + \frac{\triangle_2}{\lambda_2}B^{(0)}_{\sigma\sigma}(\sigma) = 0 \qquad & (\sigma>0) \\ \frac{\triangle_2}{\lambda_2}W^{(0)}_{\sigma\sigma}(\sigma) = 0 \qquad & (\sigma<0) \end{aligned} \tag{4.4}$$

On the other hand, the dimensionless fluxes of interests are

$$J(-\delta_d) = -\frac{\triangle_4}{\lambda_4}W_{\sigma\sigma}(-\delta_d) \tag{4.5}$$

$$J^*(1) = -\left(U_\sigma(1)+\frac{\triangle_2}{\lambda_2}B_\sigma(1)\right) \tag{4.6}$$

which correspond to the boundary conditions (3.4) and (3.6) respectively. Then these fluxes at infinite time can be obtained by a simple calculation involving linear functions of x,

$$J^{(0)}(\delta_d) = -\frac{U^{(0)}(\delta_1)+\frac{\triangle_2}{\lambda_2}(B^{(0)}(\delta_1)-B^{(0)}(0))}{\delta_1+\delta_2} \tag{4.7}$$

$$J^{*(0)}(1) = \frac{\frac{\triangle_4}{\lambda_4}W^{(0)}(-\delta_2)+\frac{\triangle_2}{\lambda_2}B^{(0)}(0)}{\delta_1+\delta_2} \tag{4.8}$$

The ratio of nondimensional fluxes at the infinite time is therefore,

$$\frac{J^{(0)}(-\delta_2)}{J^{*(0)}(1)} = -\frac{U^{(0)}(1) + \frac{\Delta_2}{\lambda_2}(B^{(0)}(1) - B^{(0)}(0))}{\frac{\Delta_4}{\lambda_4}W^{(0)}(-\delta_d) + \frac{\Delta_4}{\lambda_2}B^{(0)}(0)}. \tag{4.9}$$

In terms of the original variables, the result is

$$\frac{1}{\alpha^2}\frac{j^{(0)}(-\delta_2)}{j^{*(0)}(\delta_1)} = -\frac{D_u u^{(0)}(\delta_1) + D_b b^{(0)}(\delta_1) - D_b b^{(0)}(0)}{D_w w^{(0)}(-\delta_2) + D_b B^{(0)}(0)}, \tag{4.10}$$

which is quite different from the flux ratio at the early stage.

5 Discussion

As a result of this limiting time analysis, we see that the flux ratios at the early time stage and infinite time stage are in general different. There is no parameter available which may be adjusted to match the flux ratios between the limiting times, which are therefore different except in special circumstances. (*i.e.* that chemical reactions in the unstirred solution is fast enough to diffuse like in an equilibrium state, or that there are no chemical reaction in the system.) Thus the conclusion is that a general nonlinear diffusion reaction model with chemical interaction fails Ussing's flux ratio theorem for larger times, while a constant flux ratio indicates the reciprocal relationship behavior in the early stages, before the nonlinearities come into play.

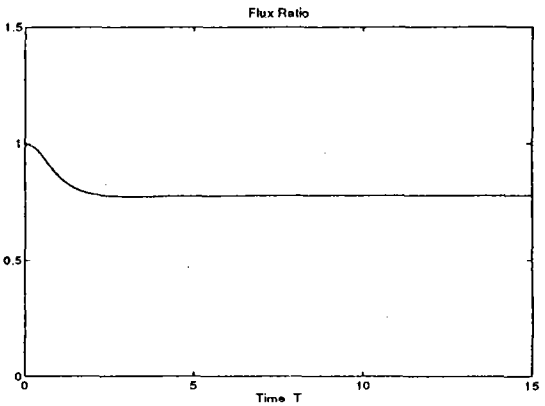

Figure 2. Flux ratio $R(t)$ for a nonlinear diffusion reaction system with chemical interaction.

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