Journal of Mathematical Chemistry 2(1988)171 – 196

THE ROLE OF THE REFERENCE STATE IN NONLINEAR KINETIC MODELS: NETWORK THERMODYNAMICS LEADS TO A LINEAR AND RECIPROCAL COORDINATE SYSTEM FAR FROM EQUILIBRIUM

D.C. MIKULECKY

Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Box 551, MCV Station, Richmond, Virginia 23298-0001, USA

and

F. SAUER

Max Planck Institute for Biophysics, Kennedy Allee, D 6000 Frankfurt am Main, FRG

Received 25 June 1987
(in final form 19 October 1987)

Abstract

Nonlinear systems can not be totally determined by the conjugate pairs of through and across variables routinely used in nonequilibrium thermodynamics and physical systems theory [1,2]. Once a reference state is specified, this problem is solved, but the constitutive relations now are reference state dependent. In this work, network thermodynamics, as developed by Peusner [3,4], is applied to nonlinear kinetic networks. The unification with the Hill, King-Altman approaches is further refined using the reference state idea of Sauer [1,2]. This results in a new, formal "thermokinetic" coordinate system which preserves both linearity and reciprocity far from equilibrium. This work also identifies two distinct classes of networks, namely "mechanistic" and "thermodynamic". It will be shown that Peusner's approach [3–8, 24–28] provides a smooth transition between them as the mechanistic model approaches the near equilibrium (Onsager) domain.

1. Introduction

Sauer developed a nonlinear theory of nonequilibrium thermodynamics [1,2] and clearly stated the need to define reference states in order to completely determine such systems. The reference state is a specification of an extra set of constraints beyond the specification of chemical potential or concentration differences. For example, a subset of concentrations may be held fixed in addition to the concentration differences. The clarity which the reference state provides in potential experi-

mental design is often overlooked. The reference state also plays a natural role in Peusner's network thermodynamic models of kinetic systems [3–6]. This work is a systematic development of Peusner networks, using the reference state concept to construct linear networks (networks with constant resistors) for a given reference state. Since the choice of reference state is equivalent to the choice of variables to be held constant in any experimental design, the implication for the design and interpreation of experiments is an important one.

The need for the explicit identification of reference states is ubiquitous in nonlinear systems. Some simple examples illustrate this point.

THE NEED FOR REFERENCE STATES: A SIMPLE KINETIC EXAMPLE

Consider the simple first-order chemical reaction:

$$A \rightleftharpoons B.$$

The flow of reaction J_r is measured either by the disappearance of A or the appearance of B:

$$J_r = -\mathrm{d}A/\mathrm{d}t = \mathrm{d}B/\mathrm{d}t.$$

Kinetically, this can be related to the concentrations of A and B in a well stirred region:

$$J_r = K_{AB} A - K_{BA} B,$$

where A and B are the concentrations. It should be clear that two quantities are necessary to determine J_r given that the rate constants K_{AB} and K_{BA} are truly constant and known: namely A and B. Thus

$$J_r = J_r (A, B).$$

Notice that knowing only differences of the concentrations or ratios does not suffice. Consider two experiments such that $A_1 - B_1 = A_2 - B_2$, for example. Then the reaction flows are

$$J_{r1} = K_{AB} A_1 - K_{BA} B_1 \quad \text{and} \quad J_{r2} = K_{AB} A_2 - K_{BA} B_2.$$

When does

$$J_{r1} = J_{r2}?$$

The condition is

$$K_{AB} A_1 - K_{BA} B_1 = K_{AB} A_2 - K_{BA} B_2 .$$

Although we also have required that

$$A_1 - B_1 = A_2 - B_2 ,$$

we now have only two equations and four variables. This hardly determines the system. The same argument can be made when we fix the ratios of A and B rather than the differences. If we require that

$$A_1 / B_1 = A_2 / B_2 ,$$

the system will still not be determined.

THE REFERENCE STATE

Let us now further define the system by specifying a reference state. We require that, in addition to either of the above conditions, $B_1 = B_2 = B$ (some "clamped" concentration of B where only A can vary). Now we have for $J_{r1} = J_{r2}$ that

$$K_{AB} A_1 - K_{BA} B = K_{AB} A_2 - K_{BA} B$$

or that

$$A_1 = A_2 !$$

This example may seem trivial, but it is extremely important to realize what is the minimal amount of information necessary to determine the system.

NONEQUILIBRIUM THERMODYNAMICS

The reaction kinetics according to Onsager's nonequilibrium thermodynamics [9–12] would be formulated according to the following transformations:

$$J_r = K_{AB} A - K_{BA} B .$$

At equilibrium, $J_r = 0$, and

$$A^\star / B^\star = K_{BA} / K_{AB} .$$

Defining the deviation from the equilibrium concentrations as follows:

$$\alpha = A - A^\star \quad \text{and} \quad \beta = B - B^\star ,$$

the reaction rate can now be expressed as:

$$J_r = K_{AB} A^\star + K_{AB} \alpha - K_{BA} B^\star - K_{BA} \beta$$

$$= K_{AB} \alpha - K_{BA} \beta = K_{AB} \alpha (1 - K_{BA} \beta / K_{AB} \alpha).$$

It should be noted that *in general* two quantities, in this case both α and the ratio β/α, need to be specified to determine the flow of reaction.

THE CHEMICAL AFFINITY

The chemical affinity for the reaction is

$$A = \mu_A - \mu_B = \mu_A^0 + RT \ln A - \mu_B^0 - RT \ln B$$

$$= \mu_A^0 + RT \ln (\alpha + A^\star) - \mu_B^0 - RT \ln (\beta + B^\star)$$

$$= \mu_A^0 - \mu_B^0 + RT [\ln A^\star (1 + [\alpha/A^\star]) - \ln B^\star (1 + [\beta/B^\star])].$$

Also at equilibrium,

$$\mu_A^\star = \mu_B^\star$$

$$\mu_A^0 + RT \ln A^\star = \mu_B^0 + RT \ln B^\star$$

$$\mu_A^0 - \mu_B^0 = RT \ln [B^\star/A^\star].$$

Therefore,

$$A = \mu_A^0 - \mu_B^0 - RT \ln [B^\star/A^\star] + RT [\ln (1 + [\alpha/A^\star])$$

$$- \ln (1 + [\beta/B^\star]),$$

and since the first three terms sum to zero,

$$A = RT [\ln (1 + [\alpha/A^\star]) - \ln (1 + [\beta/B^\star])].$$

THE NEAR EQUILIBRIUM APPROXIMATION

Near equilibrium $\alpha \ll A^\star$ and $\beta \ll B^\star$, and using the fact that $\ln(1 + x) \approx x$ under these conditions, we have that

$$A \cong RT([\alpha/A^{\star}] - [\beta/B^{\star}])$$

$$= (RT\alpha/A^{\star})(1 - [\beta/\alpha]\,[A^{\star}/B^{\star}])$$

$$= (RT\alpha/A^{\star})(1 - [\beta/\alpha]\,[K_{BA}/K_{AB}]).$$

Using the expression for the reaction rate J_r,

$$A \cong [RT/(A^{\star}K_{AB})] \cdot J_r \quad \text{or} \quad J_r \cong (A^{\star}K_{AB}/RT) \cdot A.$$

Near equilibrium, the reaction flow is approximately determined by the affinity alone, which is a function of the concentration ratio. No reference state is necessary. Thus, in the linear, near equilibrium region the reference state is not used in nonequilibrium thermodynamics. There is danger here! The ability to ignore the reference state rest heavily on the near equilibrium assumption, independently from the use of the *same* near equilibrium assumption to write a linear phenomenological equation $J_r = LA$. What they have in common is *only* the near equilibrium assumption; they are not causally related. When formulated as networks, these kinetic systems are linear far from equilibrium with respect to other driving forces. Only in the near equilibrium domain do the network driving forces (sources) asymptotically approach the thermodynamic driving forces. This will be shown in detail in this work.

THE HILL DIAGRAM METHOD

Hill's diagram method [15,16] has been very fruitful for formulating nonlinear reaction-diffusion systems and relating the more general (and complete) kinetic description to the nonequilibrium thermodynamics of Onsager near equilibrium [9,10]. Once again, care must be used to confine this translation to the near equilibrium region. The most common nonlinear reaction the method is used to formulate is the binding of a ligand to a carrier:

$$A_i + C_i = X_i,$$

where A_i is the ligand concentration on side i of the membrane ($i = L$ or R), C_i is the carrier concentration on that side, and X_i is the carrier-ligand complex concentration on that side. By requiring steady-state conditions to be met, A_i can be considered constant and the kinetics can be made pseudo-first-order.

The actual kinetics are indeed not first order:

$$J_r = K_f A_i C_i - K_b X_i,$$

where K_f and K_b are the second- and first-order rate constants for the forward (bind-

ing) reaction and the backward (dissociation of the complex), respectively. For fixed values of A_i, the reaction assumes a pseudo-first-order form:

$$J_r = K_{cx} C_i - K_b X_i ,$$

where

$$K_{cx} = A_i K_f .$$

Next, by a set of manipulations involving the principle of detailed balance, the fluxes can be identified with the steady-state (clamped) ligand concentrations through relationships like the following:

$$\Pi_i^+ / \Pi_i^- = \exp(X_i / kT) = J_i^+ / J_i^- ,$$

where Π_i's are one-way cycle fluxes expressed as products of rate constants around closed loops in the system, X_i is the driving force for net flow in cycle i, and J_i's are the one-way cycle fluxes, expressed as fluxes usually are (see refs. [15,16] for details). Near equilibrium, $J_i^+ = J_i^- = J_i^\star$, so that the net flux around any cycle

$$J_i = J_i^+ - J_i^- = J_i^- [J_i^+ / J_i^- - 1] = J_i^- [\exp(X_i / kT) - 1] ,$$

or

$$J_i \cong J_i^\star / kT \cdot X_i .$$

Once again, near equilibrium the flows become functions of the forces alone, without need of a reference state. It should be clear that these diagrams represent systems built up from single kinetic steps so that the ligand concentrations can be varied independently in each bath and that, in general, the system is *highly* reference state dependent away from equilibrium. For a very simple example we take the simple carrier model, which has been well described by many methods. We will do Hill's analysis of this model to illustrate the necessity of using reference states to characterize the model.

THE SIMPLE CARRIER MODEL: HILL's METHOD OF ANALYSIS

The model to be discussed is shown in fig. 1. The binding reaction is obviously the one described above. For simplicity, we assume the movement of carrier and complex across the membrane is passive and that the binding reaction has the same kinetics on both sides. States are labeled as follows:

$$1 = X_1 , \quad 2 = C_1 , \quad 3 = C_r , \quad 4 = X_r .$$

The Hill diagram is the linear graph shown in fig. 2, and the rate constants are:

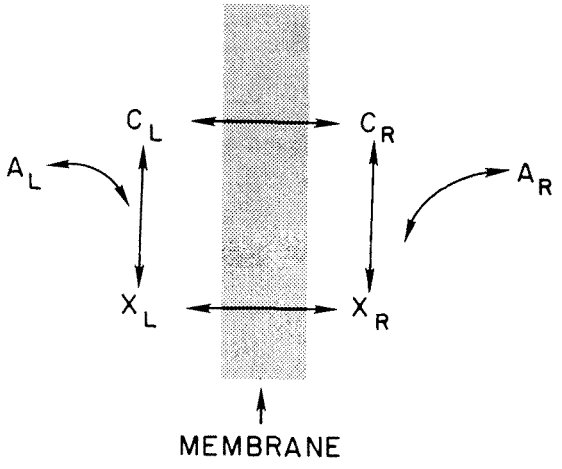

Fig. 1. The simple carrier model to describe the facilitated passive transport of a ligand A across a membrane.

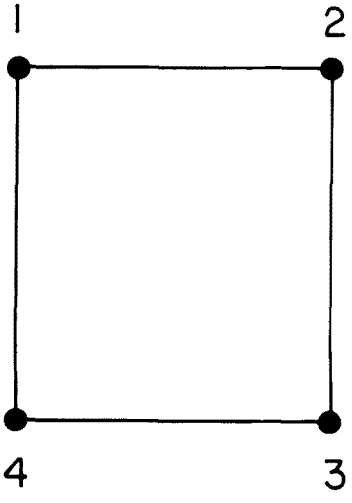

Fig. 2. Linear graph representation or Hill diagram of the simple carrier model in fig. 1.

$K_{12} = K_b$, the rate constant for the backward reaction;

$K_{21} = K_f A_1$, the rate constant for the forward (binding) reaction times ligand concentration in the left bath;

$K_{23} = K_{32} = P_c$, the permeability for the "naked" carrier crossing the membrane;

$K_{34} = K_f A_r$, $K_{43} = K_b$, and $K_{41} = K_{14} = P_x$, the permeability of the ligand-carrier complex, where A_1 and A_r are the ligand concentrations on either side of the membrane.

The fractional state populations $p(i)$, $i = 1, 2, 3, 4$, are found by Hill's method [15]:

$$N(1) = N \cdot p(1) = P_x K_f A_r P_c + P_x K_f A_r K_f A_1 + P_x P_c K_f A_1 + K_b P_c K_f A_1$$

$$N(2) = N \cdot p(2) = K_b P_x K_f A_r + K_b P_x P_c + K_b^2 P_c + P_x K_b P_c$$

$$N(3) = N \cdot p(3) = P_c K_b P_x + P_c K_b^2 + P_c K_b P_x + K_f A_1 P_x K_b$$

$$N(4) = N \cdot p(4) = P_x K_f A_1 P_c + P_x K_f A_1 K_f A_r + P_x P_c K_f A_r + K_f A_r P_c K_b .$$

Hill has developed a graph-based technique for obtaining the above expressions. For the graph in fig. 7, there are four distinct trees, each obtained by striking out one branch. For each state population there is one term per tree, obtained by directing the remaining branches toward that node and multiplying the corresponding rate constants together. For example, the first term in the first equation is for the tree obtained by striking out branch $1 \rightarrow 2$:

$$P_x K_f A_r P_c = K_{41} K_{34} K_{23} .$$

We have imposed the condition on the state populations so that the fractional state populations $[N(1) + N(2) + N(3) + N(4)] = N$ sum to unity:

$$p(1) + p(2) + p(3) + p(4) = [N(1) + N(2) + N(3) + N(4)]/N = 1.$$

The net flux of the ligand A across the system can be determined from

$$J_A = P_x \cdot [p(1) - p(4)],$$

or, after substitution

$$J_A = P(A_1 - A_r)/N$$

$$= P(A_1 - A_r)/\{R(A_1 + A_r) + SA_1 A_r + T\},$$

where

$$P = P_x P_c K_f K_b$$

$$R = 2K_f P_x P_c + K_f K_b (P_x + P_c)$$

$$S = 2P_x K_f^2$$

$$T = K_b [4P_x P_c + 2K_b P_c].$$

Clearly, this flow cannot be specified without a reference state! Merely fixing the difference $A_1 - A_r$ does not determine the flow. For example:

Let $(A_1 - A_r) = \delta$ and $A_r = 0$.

Then $A_1 = \delta$ and $J_A = P\delta/\{R\delta + T\}$.

Now let $(A_1 - A_r) = \delta$ and $A_r = \delta$.

Then $A_1 = 2\delta$, $A_1 + A_r = 3\delta$, and $A_1 A_r = 2\delta^2$

$$J_A' = P\delta/\{2R\delta + 2S\delta^2 + T\} \neq J_A!$$

Thus, the flow depends on the choice of reference state.

PEUSNER's NETWORKS

The use of network and graph theory in electronics has a history dating back to Kirchhoff [17]. It is completely unnecessary to point out the impact these ideas have had on the modern world (often called "the electronic age"). Network theory has already had a profound effect on macroscopic physics in the analysis and synthesis of electronic systems. Peusner's network thermodynamics generalizes these well-known results and makes them applicable to a much larger class of systems. Beyond this, the network thermodynamic approach has accomplished a number of important contributions to macroscopic physics, including (1) the discovery of a metric structure underlying thermodynamics; (2) a class of "thermokinetic potentials" which topologically connect spaces belonging to distinctly different physical processes; (3) the extension of the Kedem-Caplan energetics of nonequilibrium systems to a broader class of systems than those covered by the Onsager theory; (4) the generation of a unifying graph-theoretical approach to dynamic systems which can include flow graphs and the King-Altman and Hill approaches; (5) the proof of Onsager's reciprocity

for connected networks arising out of Kirchhoff's laws and Tellegen's theorem [3–8, 24–28].

Peusner introduced his network approach, applied it to a broad class of systems, and later made it public [3] at about the same time as Oster et al. introduced the bond-graph approach [18]. It is also possible to simulate very complicated living systems using SPICE and a variation on Peusner's ideas [19–23]. The unifying nature of graph-theoretical methods by using Peusner's approach was recently re-emphasized [5]. That work suggested that a number of alternative network representations of a mechanistic (kinetic) description of a system were available. It is now clear that, in fact, if the reference state is carefully chosen [1,2], there is a network representation of a system which has some very important properties. It is the purpose of the remainder of this work to spell out the interplay between the choice of reference state and network representation, as well as to show how mechanistic "thermokinetic" networks smoothly become isomorphic with the network thermodynamic description of Onsager systems near equilibrium, thus establishing the metric structure and other properties for them, as so clearly demonstrated in Peusner's work [3,4,8,24–27].

2. The creation of Peusner networks for thermokinetic systems

GENERAL COMMENTS

The Peusner approach has been described in detail in a number of places (see, for example, [3,4,7,8,24–28]), and need only be summarized here. The idea is very simple: to represent each kinetic step by the network thermodynamic structure shown in fig. 3.

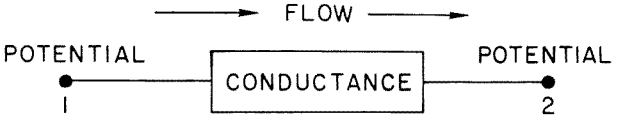

Fig. 3. The network thermodynamic structure representing a simple kinetic step. It is analogous to a resistor in electronics.

This structure is prototypical of dissipative elements of other types such as electrical resistance, diffusion resistance, hydraulic conductivity, etc. An object can be given a value which is either a conductance or a resistance, and has very specific relationships to the "through" (flow) and "across" (force) variables associated with it. The nodes are compartments and/or "pools" and represent points of connection with other network elements of the same kind or of other types. The element is a "branch" in a network, and as such can be defined in terms of the following network variables:

(1) Node potentials: These potentials are the key to the construction of connected network representations. Their difference constitute the *force across* the element (branch force).

(2) Branch flows: The response to the branch force. These flows go through the branch and constitute the movement of an amount of something (mass, volume, charge, mole number, etc.) from one point to another *through* the branches of the network, per unit time.

(3) The branch conductance or resistance defined by:

conductance = flow/force

resistance = force/flow.

Note that (1) and (2) constitute the operationally manipulative aspects of the system. They are either regulated or measured during an experiment. On the other hand, (3) is calculated from (1) and (2).

REPRESENTING A FIRST-ORDER KINETIC STEP AS A CONDUCTANCE

Now that we have defined a network element having characteristics analogous to electrical conductances, we wish to formulate kinetics in a way which fits this definition. We begin with the first-order kinetic step defined by

$$J_{12} = K_{12} C_1 - K_{21} C_2 .$$

Referring to fig. 3, the above symbols have the following meanings:

J_{12} – flow through the branch from node 1 to node 2 in units of amount of flowing substance per unit time (mole/sec, for example).

C_1 – concentration of substance flowing in some compartment or pool labeled 1.

C_2 – concentration of that substance in compartment or pool labeled 2 (note that the network nodes represent these compartments).

The units are the amount per unit volume (moles/liter, for example).

K_{12} – a rate constant for the transfer of material from compartment or pool 1 to compartment or pool 2 (liters/sec to be compatable with the above units).

K_{21} – a rate constant for the reverse transfer.

A simple rearrangement of the equation accomplishes what we want, namely that

$$J_{12} = K_{12}\,(C_1 - [K_{21}/K_{12}]\,C_2).$$

Now, by identifying the rate constant K_{12} as the conductance and the expression in parenthesis as the difference in two potentials which constitute the force across the branch, the network elements can be viewed as in fig. 4.

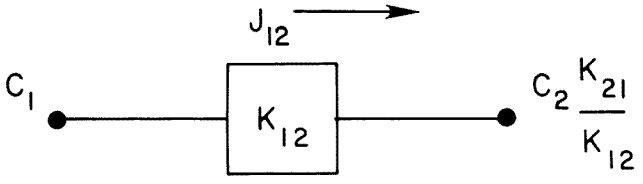

Fig. 4. The network thermodynamic structure for a first-order kinetic transition with its potential, conductance and flow explicitly noted.

A number of things have been accomplished in fig. 4. First of all, the transformation of the kinetic equation to a form which has a network branch complete with potentials, flow and conductance. This is the same basic network structure as those which represent Fick's law, Poiseulle's law and Ohm's law. A second, more subtle, result is the "scaling" of the second potential by the factor

$$K_{21}/K_{12} = (K_{eq})_{12} = \exp\,(\Delta G^0_{12}/RT).$$

This scaling is the key to creating complete, connected networks. To illustrate this point, we will examine a simple example.

THE THREE-STATE MODEL (GENERAL FORM)

The importance of the ideas introduced above manifests itself when we model the kinetics of enzyme mechanisms, carrier transport systems, etc. As a simple example, we will create a Peusner network for the example shown in fig. 5.

FIRST-ORDER VERSUS PSEUDO-FIRST-ORDER TRANSITIONS

The general scheme in fig. 5 treats each kinetic step as if it were first order. In reality, this would be the famous Onsager triangle reaction [9,10] where, for example, each of the states represents an isomer of the same chemical compound. Onsager used this example in his development of the reciprocity proof. The completely first-order system is capable of relaxing to equilibrium or being held in a stationary state by "clamping" one or more state populations (concentrations) at a nonequilibrium value. More interesting are the cases where at least some of the steps are pseudo-first order, representing second-order transitions with one reactant concentration held fixed:

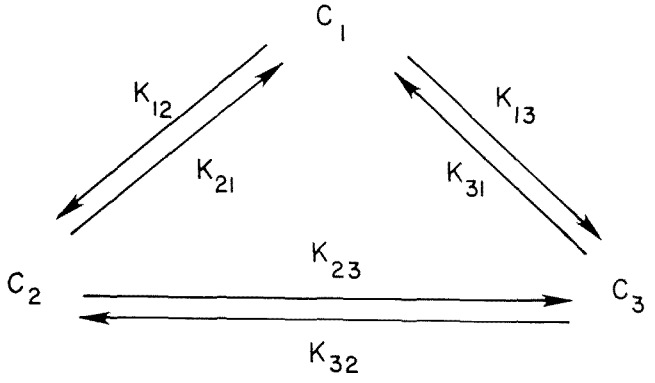

Fig. 5. A 3-state kinetic model. This is the general form, representing a number of different mechanisms, depending on the nature of the rate constants (see text for explanation).

$$A + X = Y.$$

The "flow" of this reaction in the forward direction is given by

$$J_{12} = \lambda_{12}[A][X].$$

If $[A]$ is held fixed, it can have this pseudo-first-order representation:

$$J_{12} = K_{12}[X],$$

where

$$K_{12} = \lambda_{12}[A].$$

Obviously, K_{12} has a new value each time the concentration of A is changed. What it important is the fact that this pseudo-first-order transition has the same *network* representation as the true first-order transition, if we remember that the conductance and/or the potentials may be parameterized by the concentration of A. With this idea in mind, we will create a Peusner model of the general 3-state model, and then look at a specific case with pseudo-first-order transitions. The first kinetic step is

$$J_{12} = K_{12}(C_1 - [K_{21}/K_{12}]\, C_2),$$

and has the network representation previously described (fig. 4). To turn the second kinetic step into a network branch, we notice that the first potential has already been defined by the first branch to be $C_2[K_{21}/K_{12}]$. Using this fact as a constraint, the second kinetic step is

$$J_{23} = (K_{23}K_{12}/K_{21}) \cdot (K_{21}C_2/K_{12} - K_{32}K_{21}C_3/[K_{23}K_{12}]).$$

Once more, it has the required form

$$\text{flow} = \text{conductance} \cdot (\text{potential } A - \text{potential } B),$$

and the partially complete network is shown in fig. 6.

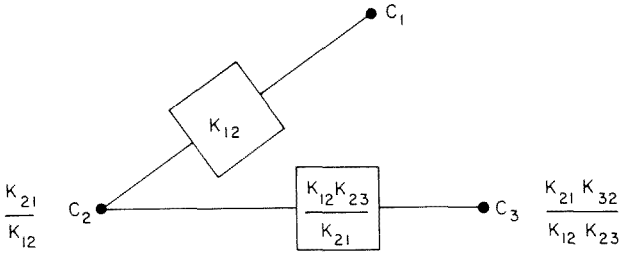

Fig. 6. Partial network for 3-state model: first two kinetic steps. Note scaling of the potentials.

The third and final kinetic step is treated in exactly the same manner. Using the previously defined potential as a constraint, the kinetic equation is

$$J_{31} = K_{31}K_{12}K_{23}/K_{21}K_{32}\,[K_{32}K_{21}C_3/(K_{23}K_{12}) - K_{13}K_{21}K_{32}C_1/(K_{31}K_{12}K_{23})].$$

The completed thermokinetic network is shown in fig. 7.

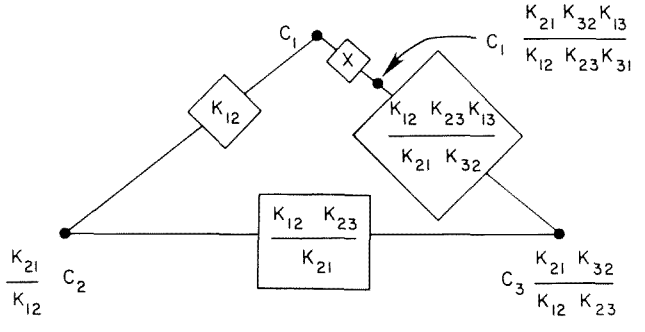

Fig. 7. The complete thermokinetic network for the general 3-state system.

THE PRINCIPLE OF DETAILED BALANCE

The system obeys the principle of detailed balance in the form:

$$K_{12}K_{23}K_{31}/K_{21}K_{13}K_{32} = K.$$

In the case of the Onsager triangle reaction, all the transitions are true first-order transitions, and the value of K must be unity in order for the principle of microscopic reversibility to be upheld. However, in the network the value of the source X is:

$$X = C_1 [1 - K_{13}K_{32}K_{21}/(K_{12}K_{23}K_{31})],$$

and in this case, $X = 0$. This constraint is equivalent to requiring that at equilibrium:

$$J_{12} = J_{23} = J_{31} = 0.$$

In other words, it is the requirement that each kinetic step go to equilibrium independently.

Since the source has the value zero for this case, it can omitted from the network and the network is purely passive. Without much further effort, it is easily demonstrated that this feature is at the root of Onsager's reciprocity proof for this system [3,4,7,8,24–28].

This point deserves further comment. Peusner's work repeatedly establishes a profound sameness between Onsager reciprocity in a system and the connectivity of its network representation. In this example, as well as others, this connectivity of a *passive*, purely resistive network arises out of the application of the principle of detailed balance; in other words, microscopic reversibility. The fact that the network is passive is central, since sources will, in general, destroy the reciprocity. In the examples that follow, the pseudo-first-order rate constants do not obey detailed balance, only the second-order rate constants contained within them do. Thus, the sources "disappear" only at equilibrium and in an arbitrarily small region around it.

It is also worth noting that $K = 1$ is equivalent to the fact that there is no net reaction taking place as the system completes a full cycle in the kinetic diagram.

The second, and generally more interesting set of examples, is that which contains systems having pseudo-first-order transitions. One simple example is the well-known Michaelis-Menten system:

(1) $E + S \rightleftharpoons EX$

(2) $ES \rightleftharpoons EP$

(3) $EP \rightleftharpoons E + P.$

Here, the enzyme E can exist in the three states E, ES and EP, and the three transitions are

$$J_{12} = \lambda_{12} \cdot E \cdot S - K_{12} \cdot ES = K_{12} \cdot E - K_{21} \cdot ES$$

$$J_{23} = K_{23} ES - K_{32} EP$$

$$J_{31} = K_{31} \cdot EP - \lambda_{13} \cdot E \cdot P = K_{31} \cdot EP - K_{13} E.$$

The complete network for this system can, in fact, be represented as shown in fig. 8.

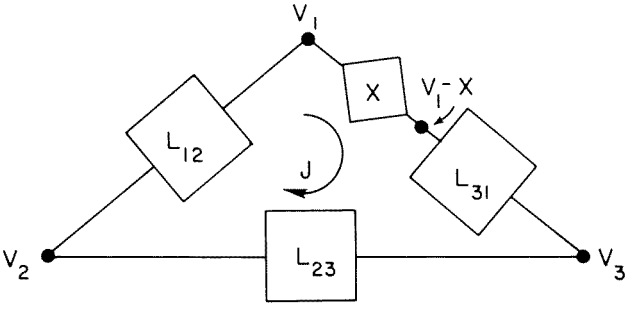

Fig. 8. The basic network for the Michaelis-Menton system (special case of the general case shown in fig. 5). This basic network represents three distinct networks, all valid representations of the system (see text for explanation).

The network shown in fig. 8 has a rather simple steady-state solution:

$$J = L_T X,$$

where $J = J_{12} = J_{23} = J_{31}$ in the steady state, X is the source value, and

$$1/L_T = 1/L_1 + 1/L_2 + 1/L_3.$$

Depending on the node used to start the construction of the network, three network representations are, in fact, possible. Table 1 summarizes the values of the conductances, potentials and sources for each. (Flows remain the same in every case.) Since each of the network representations has the same solution and each must represent the system, the following relations can easily be established. The three solutions are:

$$J = (\Pi_{ccw}/\epsilon_1)(C_1)(1 - \Pi_{cw}/\Pi_{ccw}),$$

$$J = (\Pi_{ccw}/\epsilon_2)(C_2)(1 - \Pi_{cw}/\Pi_{ccw}),$$

Table 1

Values of the network potentials, conductances and sources, depending on the starting node for the network construction (counter clockwise construction around loop)

Starting node	1	2	3
Potential at node 1, V_1	C_1	$C_1(K_{32}K_{13})/(K_{23}K_{31})$	$C_1(K_{13}/K_{31})$
Potential at node 2, V_2	$C_2(K_{21}/K_{12})$	C_2	$C_2(K_{21}K_{13})/K_{12}K_{31})$
Potential at node 3, V_3	$C_3(K_{21}K_{32})/K_{12}K_{23})$	$C_3(K_{32}/K_{23})$	C_3
Source value X	$C_1\Pi$	$C_2\Pi$	$C_3\Pi$
Conductance in branch 12, L_{12}	K_{12}	$K_{12}K_{23}K_{31}/(K_{32}K_{13})$	$K_{12}K_{31}/K_{13}$
Conductance in branch 23, L_{23}	$K_{23}K_{12}/K_{21}$	K_{23}	$K_{23}K_{31}K_{12}/(K_{13}K_{21})$
Conductance in branch 31, L_{31}	$K_{31}K_{12}K_{23}/(K_{32}K_{21})$	$K_{31}K_{23}/K_{32}$	K_{31}
Total conductance	Π_{ccw}/ϵ_1	Π_{ccw}/ϵ_2	Π_{ccw}/ϵ_3

$\Pi = (1 - \Pi_{cw}/\Pi_{ccw})$, $\Pi_{cw} = K_{13}K_{32}K_{21}$, $\Pi_{ccw} = K_{31}K_{23}K_{12}$.

$\epsilon_1 = K_{23}K_{31} + K_{21}K_{31} + K_{32}K_{21}$, $\epsilon_2 = K_{31}K_{12} + K_{32}K_{12} + K_{32}K_{12}$, $\epsilon_3 = K_{12}K_{23} + K_{13}K_{23} + K_{21}K_{13}$.

and

$$J = (\Pi_{ccw}/\epsilon_3)(C_3)(1 - \Pi_{cw}/\Pi_{ccw}),$$

or, rewriting:

$$J\epsilon_1 = C_1(\Pi_{ccw} - \Pi_{cw}),$$

$$J\epsilon_2 = C_2(\Pi_{ccw} - \Pi_{cw}),$$

$$J\epsilon_3 = C_3(\Pi_{ccw} - \Pi_{cw}).$$

In these saturable enzyme systems, the total amount of enzyme is usually both small and constant:

$$C_T = C_1 + C_2 + C_3.$$

If the three network descriptions are added, the sum is

$$J\epsilon = C_T(\Pi_{ccw} - \Pi_{cw}),$$

or

$$J = (C_T/\epsilon)(\Pi_{ccw} - \Pi_{cw}).$$

This is the "complete" solution to the problem, expressing the flow in terms of a conductance C_T/ϵ, and a force $(\Pi_{ccw} - \Pi_{cw})$, where

$$\epsilon = \epsilon_1 + \epsilon_2 + \epsilon_3.$$

A number of important observations should now be made. First of all, without going into detail, a correspondence between many of the expressions used here and the Hill diagram method can be made. The expressions ϵ, ϵ_1, ϵ_2, and ϵ_3 have exactly the same meaning as in the Hill treatment. (Hill uses the trees of the linear graph to formulate these in terms of the rate constants.) In the present context, they arise in the conductances. Here, the three trees were used as well, each as one network. The other correspondences are in the terms Π, Π_{cw}, and Π_{ccw}, which also appear in Hill's treatment. It should also be noted that the use of all three networks to arrive at a solution independent of the concentrations C_1, C_2, and C_3 is not necessary. Any one of the three networks would have been sufficient. The method of solution will be explained in detail in the next example. (This becomes *very* important when the kinetic schemes become more elaborate, since the linear graphs' forests may then contain hundreds, or even thousands, of trees. The network solution will always utilize only one tree.)

THE CHOICE OF REFERENCE STATE

Besides being a simple demonstration of the technique Peusner introduced for finding connected network representations of the kinetic description of enzyme, carrier and other systems, the above example illustrates the need to define a reference state [1,2]. These nonlinear systems have a thermodynamic description which is almost always more complicated than linear systems.

When kinetic models of the type being considered here are analyzed (saturable enzyme and/or carrier systems, for example), this description is nonlinear and, in general, has the form [1,2]:

$$J_K = F_K (C_1^A, C_1^B, C_2^A, C_2^B, \ldots, C_i^A, C_i^B),$$

where A and B now refer to the two sides of the system and $1, 2, \ldots, k$ to different chemical species. If these flows are to be expressed in terms of the X_i mentioned above, the resultant functional dependence is of the form:

$$J_K = G_K (X_1, X_2, \ldots, X_i; a_1, a_2, \ldots, a_i),$$

where the a_i's are some function of C_i^A and C_i^B and determine the *reference state* for the system's description. Thus, any relationship between the flows and the forces is reference state dependent. This suggests an experimental strategy which first fixes a reference state and then examines the flow-force relations for each reference state.

The three networks which were created to describe the Michaelis-Menten system have a very important relationship to the choice of reference state. We must examine them more carefully with respect to the pseudo-first-order rate constants. In this model, they are

$$K_{12} = \lambda_{12} \cdot S$$

$$K_{13} = \lambda_{13} \cdot P.$$

An examination of table 1 shows that there is one and only one network which has conductances independent of K_{13}, namely the network formed by starting at node 1. This is due to the fact that the last kinetic step to be formulated as a network branch was the only one containing this rate constant. Without too much trouble, it can be demonstrated (the argument follows trivially from symmetry) that if the same node were used as a starting point and the construction were to be carried out in a clockwise direction around the loop, the conductances would be independent of K_{12}. Once more, the rate constant K_{12} only occurs in the last branch constructed.

Two very convenient choices of reference state are to clamp either the substrate concentration $S = S^0$ or the product concentration $P = P^0$, and to cause the

flow to vary by varying the unclamped member of the pair. Each of these choices of reference state has associated with it a network whose solution is

$$J = LX,$$

where L is *constant* in this reference state. Thus, the network has produced a coordinate system which is linear in a given reference state.

Other reference state choices are possible given the networks we have created. For example, in table 1, the networks formed by starting at nodes 2 or 3 have constant conductances as long as $S/P = R^0$. The ratio of substrate to product concentrations must be held constant in these cases.

We have demonstrated the relationship between Peusner's networks and the choice of reference state for a simple, nonlinear system having only one degree of freedom (one force-flow relationship in a given reference state). It is possible to extend this development to single degree of freedom systems with more states. These would include completely coupled versions of more complicated systems, since completely coupled systems have only one cycle. It is easily shown that Kirchhoff's criteria for independence of loop flows is applicable in making this assessment of the number of degrees of freedom.

AN EXAMPLE WITH TWO DEGREES OF FREEDOM: LINEAR DESCRIPTION AND ONSAGER COEFFICIENTS FOR A GIVEN REFERENCE STATE

The system to be used as an example here is taken from Rothschild et al. [11]. This is shown in fig. 9(a), with one of its networks in fig. 9(b).

Table 2 lists the values of the networks potentials, conductances and source values. The network can be constructed as above, following this bath through the branches: $V_1 \rightarrow V_3 \rightarrow V_2 \rightarrow V_4 \rightarrow V_3^{\star}$ and $V_1 \rightarrow V_2^{\star}$. The starred steps indicate the need to "close the gap" with sources X_1 and X_2.

By using Kirchhoff's voltage law, the "potential drops" around two independent loops a and b can be related:

$$X_1 = (R_1 + R_2 + R_3) J_1 - R_3 J_2,$$

$$X_2 = - R_3 J_1 + (R_3 + R_4 + R_5) J_2,$$

where the R_i's are the resistances corresponding to the conductances L_i:

$$R_i = 1/L_i, \qquad i = 1, \ldots, 5.$$

These two equations can be solved simultaneously to express the flows in terms of the forces:

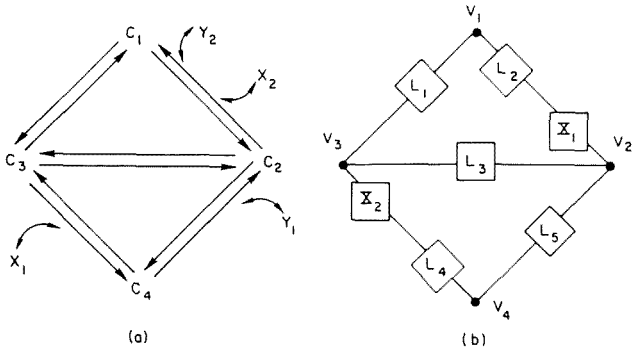

Fig. 9. (a) The kinetic scheme for a simple active transport system. Transitions $2 \longleftrightarrow 3$ involve "crossing" the membrane. Step $1 \longleftrightarrow 2$ involves a chemical reaction $S \longleftrightarrow P$, and steps $3 \rightarrow 4$ and $2 \rightarrow 4$ involve binding a transported ligand each on opposite sides of the membrane. (b) The network representing this system in the reference state $P = P^0$, $A_R = A_R^0$ (product concentration held constant and ligand concentration on the right-hand side held constant). In this reference state, the network has constant (but reference state dependent) conductances.

Table 2

Conductances, potentials, and source values for the network in fig. 9(b)

A. Node potentials

$$V_1 = C_1$$
$$V_2 = C_2/a$$
$$V_3 = C_3/b$$
$$V_4 = C_4/c$$

where the C_i's are the concentrations of the enzyme in each state, and a, b, and c are the scaling factors for the potentials:

$$b = K_{13}/K_{31}$$
$$a = bK_{32}/K_{23}$$
$$c = aK_{24}/K_{42}$$

B. Conductances

$$L_1 = K_{13}$$
$$L_2 = K_{12}$$
$$L_3 = K_{13}K_{32}/K_{31}$$
$$L_4 = K_{13}K_{32}K_{24}K_{43}/K_{31}K_{23}K_{42}$$
$$L_5 = K_{13}K_{32}K_{24}/K_{31}K_{23}$$

C. Sources

$$X_1 = C_2\,(1 - \Pi^a_{cw}/\Pi^a_{ccw})\,(K_{21}/K_{12})$$
$$X_2 = C_3\,(1 - \Pi^b_{ccw}/\Pi^b_{cw})\,(K_{31}/K_{13})$$

$$J_1 = [(R_3 + R_4 + R_5)/\Delta]\, X_1 + (R_3/\Delta) X_2,$$

$$J_2 = (R_3/\Delta) X_1 + [(R + R_2 + R_3)/\Delta]\, X_2,$$

where

$$\Delta = (R_1 + R_2)(R_4 + R_5) + R_3(R_1 + R_2 + R_4 + R_5).$$

Since X_1 and X_2 depend on the state populations C_2 and C_3, a more compact solution would eliminate these values. However, in this reference state, the above relations between the observable flows J_1 and J_2 and the network's *formal* forces X_1 and X_2 are both *linear* and *reciprocal* arbitrarily far from equilibrium!

SOLVING FOR THE STATE POPULATIONS

No matter which method one chooses, the solution of the problem in its most compact form should be independent of the state populations. If we wish to express this network's solution in terms of the network's *formal* forces, we have already accomplished this. However, if we wish to express the flows as functions of thermodynamic forces and/or concentration ratios, we must solve for the state populations. We will introduce a method which accomplishes this in a manner not dependent on the use of Cramer's rule of other linear algebraic manipulations. First, we formulate "Ohm's law" for each of the five conductances:

$$V_1 - V_3 = R_1 J_1$$

$$V_2 m - V_1 = R_2 J_1$$

$$V_2 - V_3 = R_3 (J_2 - J_1)$$

$$V_3 n - V_4 = R_4 J_2$$

$$V_4 - V_2 = R_5 J_2,$$

where $m = \Pi^a_{ccw}/\Pi^a_{cw}$ and $n = \Pi^b_{ccw}/\Pi^b_{cw}$, and a and b refer to the upper and lower cycles, respectively. Due to the principle of detailed balance, at equilibrium $n = 1$ and $m = 1$ for this reference state. All the flows go to zero as well, so that all the potentials become equal. The equilibrium state populations are not equal, however, due to the scaling factors. Also, the quantities m and n were introduced because X_1 and X_2 depend on V_2 and V_3, respectively, as follows:

$$X_1 = a_1 V_2$$

$$X_2 = a_2 V_3,$$

so that

$$V_2 - X_1 = (1 - a_1)\, V_2 = mV_2$$

$$V_2 - X_2 = (1 - a_2)\, V_3 = nV_3 .$$

In the set of five equations generated by Ohm's law, we have four state populations plus two flows as unknowns. We also have the enzyme conservation statement:

$$V_1 + aV_2 + bV_3 + cV_4 = C_T ,$$

which provides a sixth equation. The state populations are found first by eliminating the flows and then using the "voltage divider" relations derivable from the remaining set of equations to express the potential in a convenient way.

Combining the first two relations by eliminating J_1:

$$V_1 = m\gamma_1 V_2 + \gamma_2 V_3 ,$$

where the "voltage divider" ratios γ_1 and γ_2 are

$$\gamma_1 = R_1/(R_1 + R_2),$$

and

$$\gamma_2 = R_2/(R_1 + R_2).$$

The same procedure relates V_4 to V_2 and V_3:

$$V_4 = \gamma_3 V_2 + n\gamma_4 V_3 ,$$

where

$$\gamma_3 = R_4/(R_4 + R_5)$$

and

$$\gamma_4 = R_5/(R_4 + R_5).$$

Combining the first, third and fifth Ohm's law relations to eliminate J_1 and J_2:

$$(V_2 - V_3)/R_3 = (V_4 - V_2)/R_5 - (V_1 - V_3)/R_1 ,$$

or rearranging

$$R_5R_3V_1 + (R_1R_5 + R_3R_1)V_2 - (R_1R_5 + R_5R_3)V_3 - R_3R_1V_4 = 0,$$

the voltage divider relations can be used to eliminate V_1 and V_4:

$$V_2 = V_3 [R_5(R_1 + R_3) + R_3(R_1\gamma_4 n - R_5\gamma_2)] /$$

$$[R_1(R_3 + R_5) + R_3(R_5\gamma_1 m - R_1\gamma_3)]$$

$$= V_3 [(R_4 + R_5)(R_1 + R_2 + R_3) + R_3(R_1 + R_2)n] /$$

$$[(R_1 + R_2)(R_3 + R_4 + R_5) + R_3(R_4 + R_5)m].$$

At equilibrium, $m = n = 1$ and $V_2 = V_3$.

For convenience, let the numerator of the above expression be represented by N and the denominator by M. Then,

$$V_2 = (N/M)V_3.$$

Substituting this and the two voltage divider relations into the enzyme conservation expression results in an expression for V_3:

$$V_3 = MC_T / [(m\gamma_1 + a + c\gamma_3)N + (\gamma_2 + b + c\gamma_4 n)M].$$

Using this, explicit expressions for the other state populations are also obtained:

$$V_2 = NC_T/D,$$

$$V_1 = (\gamma_1 N + \gamma_2 M)C_T/D,$$

$$V_4 = (\gamma_3 N + \gamma_4 M)C_T/D,$$

where D is the denominator in the expression for V_3.

It is now possible to eliminate the state populations from the expressions for the network forces (source values):

$$X_1 = aNC_T(1 - \Pi^a_{cw}/\Pi^a_{ccw})(K_{21}/K_{12})/D$$

$$= NC_T\ (\Pi^a_{ccw}/\Pi^a_{cw} - 1)/D$$

$$X_2 = bMC_T(1 - \Pi^b_{ccw}/\Pi^b_{cw})/(K_{31}/K_{13})/D$$

$$= MC_T\ (1 - \Pi^b_{ccw}/\Pi^b_{cw})/D.$$

THE THERMODYNAMIC NETWORK AND THE ONSAGER COEFFICIENTS

By combining R_4 and R_5 into one conductance and doing the same for R_1 and R_2, the network becomes identical in form to the "canonical" representation of all two degree of freedom Onsager systems [3,4]. Near equilibrium, the network forces X_1 and X_2 smoothly become equivalent to the thermodynamic driving forces for this system, since m and n approach unity as

$$-(1 - \Pi^a_{cw}/\Pi^a_{ccw}) \rightarrow A,$$

$$(1 - \Pi^b_{ccw}/\Pi^b_{cw}) \rightarrow \Delta\mu,$$

where A and $\Delta\mu$ are the affinity of the chemical reaction and the chemical potential difference of the transported ligand, respectively. In this near-equilibrium domain (Onsager), the flow-force relations become the phenomenological equations of non-equilibrium thermodynamics:

$$J_1 = L_{11} A + L_{12} \Delta\mu,$$

$$J_2 = L_{21} A + L_{22} \Delta\mu.$$

Thus, the Onsager coefficients are directly obtained from the network as

$$L_{12} = L_{21} = R_3 C_T / 4Q,$$

$$L_{22} = (R_1 + R_2 + R_3) C_T / 4Q,$$

$$L_{11} = (R_3 + R_4 + R_5) C_T / 4Q,$$

where $Q = \Delta (1 + a + b + c)$.

3. Summary and conclusions

With two examples, we have demonstrated a very general method for finding a linear, reciprocal, reference state dependent coordinate system for thermokinetic descriptions. Moreover, we have demonstrated that these model-dependent networks all merge smoothly into the more general model-independent thermodynamic networks near equilibrium. We know that these thermodynamic networks provide a canonical representation of the Onsager systems in an orthogonal coordinate system, complete with metric structure [3,4,24–28].

It is now important to ask what the new thermokinetic coordinates furnish as a means of analyzing nonlinear systems. Since all the nonlinearity is "pushed" into the network sources, the remaining network is a linear, reciprocal object with the

stability properties, minimum dissipation (network), and other properties usually associated with Onsager systems near equilibrium. What remains to be carried out is a careful examination of the interplay between this well-behaved part of the network and its sources. We anticipate that further study of this question will lead to some interesting new ways of describing the properties of thermokinetic systems.

References

[1] F.A. Sauer, in: Appendix to *Handbook of Physiology*, Sect. 8, ed. J. Orloff and R.W. Berliner (Williams and Wilkins, Baltimore, MD, 1973) Ch. 12, p. 399.
[2] F.A. Sauer, in: *Intestinal Permeation*, ed. M. Kramer and F. Lauterbach (Exerpta Medica, Amsterdam, 1977) p. 320.
[3] L. Peusner, The principle of network thermodynamics and biophysics applications, Ph.D. Thesis, Harvard University, Cambridge, MA (1970).
[4] L. Peusner, *Studies in Network Thermodynamics* (Elsevier, Amsterdam, 1986).
[5] L. Peusner, D.C. Mikulecky, S.R. Caplan and B. Bunow, J. Chem. Phys. 83(1985)5559.
[6] D.C. Mikulecky, F.A. Sauer and L. Peusner, *Biophys. Memb. Transp. VIII*, ed. J. Kucera and S. Przestalski (Agricultural Academy of Wroclaw, Wroclaw, Poland, 1986) p. 217.
[7] L. Peusner, J. Theor. Biol. 122(1986)125.
[8] L. Peusner, J. Memb. Sci. (in press).
[9] L. Onsager, Phys. Rev. 37(1931)405.
[10] L. Onsager, Phys. Rev. 38(1931)2665.
[11] A. Katchalsky and P.F. Curran, *Nonequilibrium Thermodynamics in Biophysics* (Harvard University Press, Cambridge, MA, 1965).
[12] S.R. Caplan and A. Essig, *Bioenergetics and Linear Nonequilibrium Thermodynamics: The Steady State* (Harvard University Press, Cambridge, MA, 1983).
[13] K.J. Rothschild, S.A. Ellias, A. Essig and H.E. Stanley, Biophys. J. 30(1980)209.
[14] A. Essig and S.R. Caplan, Proc. Natl. Acad. Sci. USA 78(1981)1647.
[15] T.L. Hill, *Free Energy Transduction in Biology* (Academic Press, New York, 1977).
[16] T.L. Hill, Nature 84(1982)5878.
[17] G.R. Kirchhoff, English translation in: *Graph Theory 1736–1936*, ed. N.L. Briggs, F.K. Lloyd and R.J. Wilson (Oxford University Press, London, 1976) p. 131.
[18] G.F. Oster, A.S. Perelson and A. Katchalsky, Quart. Rev. Biophys. 6(1973).
[19] D. Waltz and S.R. Caplan, Biochim. Biophys. Acta 859(1986)151.
[20] D.C. Mikulecky, Amer. J. Physiol. 245(1983)R1.
[21] J.L. Wyatt, Jr., D.C. Mikulecky and J.A. DeSimone, Chem. Eng. Sci. 345(1980)2115.
[22] J.C. White and D.C. Mikulecky, Pharmac. Ther. 15(1982)251.
[23] K.M. Thakker, J.M. Wood and D.C. Mikulecky, Comput. Prog. Biomed. 15(1982)61.
[24] L. Peusner, J. Theor. Biol. 115(1985)319.
[25] L. Peusner, J. Chem. Phys. 83(1985)1276.
[26] L. Peusner, J. Chem. Soc. Faraday Trans. 2(1985)1151.
[27] L. Peusner, J. Chem. Phys. 77(1982)5500.
[28] L. Peusner, in: *Chemical Applications of Topology and Graph Theory*, ed. R.B. King (Elsevier, Amsterdam, 1983) p. 379.