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Solution of Carrier-Type Transport Models: General Solution for an Arbitrarily Complex Rapid Equilibrium Model

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Summary. A general framework for solving and analyzing rapid equilibrium carrier models is given. The basis of this work is the demonstration that the solution of an arbitrarily complex model of this type can be written in the form

$$J_S^{1\to 2} = \frac{C_o A_{12} F_{21}}{\alpha_1 F_{21} + \alpha_2 F_{12}}$$

where $J_S^{1\to 2}$ is the unidirectional flux of the substrate S from side 1 to side 2 of the membrane, C_o is the total number of carriers and A_{12}, F_{12}, F_{21}, α_1 and α_2 are sums of terms which can be written down simply and directly from knowledge of the basic properties of the model. The above relation not only leads to a simple and convenient method for solving transport models of this type, but also provides a powerful algebraic tool for analyzing the properties of individual models or groups of models. In this regard several examples of the potential utility of this formalism are given. The effects of "dead-end" inhibitors on rapid equilibrium carrier models are analyzed. Also the properties of carriers with one substrate binding site are studied in some detail. A parameterization of $J_S^{1\to 2}$ entirely in terms of experimentally measurable kinetic parameters as well as a set of generalized rejection criteria are derived for these models. Since the existence of a single substrate binding site is the only assumption made in these latter derivations, the results obtained necessarily apply to all rapid equilibrium models of this type, irrespective of complexity.

Key Words transport kinetics · carrier model · transport theory · coupled transport · active transport

Introduction

The behavior of many biomembrane transport systems is dependent on the presence or absence of a number of interacting chemical species not all of which are necessarily transported. Primary active systems such as Na/K-ATPase are classic examples of this kind of complexity, but more recently secondary active transporters involving multiple co- and/or countertransported activators as well as catalytic modifier sites have been described (*see*, for example, references 1 and 5). The kinetic behavior of these complex transporters is often difficult if not impossible to predict in an intuitive fashion even when considerable information regarding the details of the transport process (stoichiometry, order of binding, rate-limiting steps, etc.) is available. Accordingly, it is essential to have rigorous mathematical solutions to possible models of these systems to aid in the planning and interpretation of kinetic experiments designed to probe the details of the transport mechanism. Owing to the variety of transporters found in nature and to the number of different kinetic schemes potentially applicable to a given transport system, it is unlikely, however, that every model of possible biological interest will receive a detailed theoretical treatment in the literature. Indeed, rather than treating each of these models individually, a somewhat more general approach to this problem would also seem to be more productive and desirable.

The present paper deals with the derivation and application of such a general approach for solving and analyzing rapid equilibrium carrier models. The basis of this work is the demonstration that the flux equation of any model of this type can be written in the same simple mathematical form. The proof of this relation leads directly to a straightforward method for writing down the solution to an arbitrarily complex model. Of greater potential significance, however, is the fact that one can take advantage of the form of this general flux equation to determine many of the properties of an individual model or of families of related models in a relatively transparent way and without resorting to complete mathematical solutions. Moreover, this can often be done without specifying all of the details of the proposed transport mechanism, thus retaining considerable generality. Several examples of the potential utility of this formalism are given.

General Solution to an Arbitrarily Complex Rapid Equilibrium Carrier Model

Assumptions

The rapid equilibrium carrier model as treated here incorporates the following assumptions concerning the transport process and the experimental conditions under which fluxes are measured.

1. Carrier Assumption

The binding sites on the carrier for transported ligands are only accessible from one side of the membrane at a time and the transmembrane translocation of binding sites is a first-order process. It is also assumed that the binding sites for transported ligands are translocated in an all-or-none manner, i.e., conformational changes of the carrier which result in the translocation of some but not all binding sites for transported ligands are not allowed. Note that this does not exclude the translocation of partially loaded carrier species.

2. Rapid Equilibrium Assumption

The rate-limiting step in the transport process is the translocation of binding sites from one membrane face to the other; thus the transporters are in equilibrium with the ligands at the membrane faces.

3. Steady-State Assumption

The transporter has reached a steady state at the time of measurement, i.e., there is no net movement of transporter binding sites from one face of the membrane to the other.

4. Conservation of Carriers

The total number of carriers is constant and equal to C_o. Note that this assumption does not exclude most of the obvious forms of carrier recruitment (e.g., activation of dormant carriers by the binding or dissociation of modifier ligands, membrane potentials, etc.).

Since no assumptions regarding the energetics of the transport event are made, the treatment given here is equally applicable to active and passive transport systems.

Nomenclature

Let the various carrier species on side n of the membrane be denoted by C_{in}. Here $i = 0, 1, 2, \ldots, N_n$, where $N_1 + 1$ and $N_2 + 1$ are the total number of carrier species on sides 1 and 2, respectively, and C_{01} and C_{02} denote the unloaded forms of the carrier. Also let k_{12}^{ij} and k_{21}^{ij} be the first-order rate constants for the translocation events (Assumption 1) defined by

$$C_{i1} \underset{k_{21}^{ij}}{\overset{k_{12}^{ij}}{\rightleftharpoons}} C_{j2}. \qquad (1)$$

side 1 side 2

Many of the rate constants defined by Eq. (1) will, in fact, be equal to zero, since only translocations between carrier species with the same complement of bound ligands are possible.

In what follows it will be assumed that the carrier species assigned to side 1 of the membrane (i.e., the C_{i1}) are those with binding site(s) for the substrate of interest exposed on side 1. In some cases the assignment of a given carrier species to one or to the other side of the membrane may be rather arbitrary (e.g., antiporters with substrate binding sites exposed simultaneously on both sides of the membrane). However, this does not pose a serious problem. Once the assignment, however arbitrary, of one carrier species to side 1 or 2 of the membrane has been made, the assignment of all other carrier species will be uniquely determined according to whether or not a translocation event is required to reach it. The application of the results of this paper requires only that these assignments be treated consistently.

General Solution

The general solution to an arbitrarily complex rapid equilibrium model follows directly from the assumptions and nomenclature introduced above. (The solution of a specific example, which follows the same sequence as the more general solution presented below, is given in the Appendix. This Appendix is included both to illustrate the utility of the method derived here for solving rapid equilibrium carrier models and to complement the more abstract treatment below with a more concrete example.) The rapid equilibrium assumption (Assumption 2) requires that all of the carrier species C_{in} are in chemical equilibrium with the ligands at the membrane faces. Thus each is related to C_{On} by an equation of the form

$$C_{in} = \gamma_{in} C_{On} \qquad (2)$$

where γ_{i1} is simply the product of the concentrations of the various ligands bound to C_{in} divided by their respective dissociation constants (*see* Appendix for specific examples). The total number of car-

riers on side n of the membrane can then be written as

$$\sum_i C_{in} = C_{On} \sum_i \gamma_{in} = C_{On}\alpha_n$$

where

$$\alpha_n = \sum_i \gamma_{in} \tag{3}$$

and the sum Σ_i is over all $i = 0, 1, 2, \ldots, N_n$. The total number of carriers C_o thus has the form

$$C_o = \alpha_1 C_{01} + \alpha_2 C_{02}. \tag{4}$$

From the steady-state assumption (Assumption 3) one has that the total number of transporters moving from side 1 to side 2 of the membrane is equal to that moving from side 2 to side 1, or in mathematical terms that

$$\sum_{ij} k_{12}^{ij} C_{i1} = \sum_{ij} k_{21}^{ij} C_{j2} \tag{5}$$

where the sum Σ_{ij} is over all $i = 0, 1, \ldots, N_1, j = 0, 1, \ldots, N_2$ for which $k_{12}^{ij}, k_{21}^{ij} \neq 0$, i.e., over all mobile species. Substituting Eq. (2) into Eq. (5) yields

$$F_{12}C_{01} = F_{21}C_{02} \tag{6}$$

where

$$F_{12} = \sum_{ij} k_{12}^{ij}\gamma_{i1} \quad \text{and} \quad F_{21} = \sum_{ij} k_{21}^{ij}\gamma_{j2}. \tag{7}$$

Equations (4) and (6) can now be solved to give the following expressions for C_{01} and C_{02}.

$$C_{01} = \frac{F_{21}C_o}{\alpha_1 F_{21} + \alpha_2 F_{12}}$$

$$\text{and} \quad C_{02} = \frac{F_{12}C_o}{\alpha_1 F_{21} + \alpha_2 F_{12}}. \tag{8}$$

The unidirectional flux of substrate S from side 1 to side 2 of the membrane is given by

$$J_S^{1\to 2} = \sum_{ij}^{*} n_{i1} k_{12}^{ij} C_{i1} \tag{9}$$

where the sum Σ_{ij}^{*} is over all mobile species with bound substrate, and n_{i1} is the number of translocated substrate molecules on C_{i1}. Substituting again for C_{i1} from Eq. (2) one finds that Eq. (9) can be rewritten as

$$J_S^{1\to 2} = A_{12}C_{01} \tag{10}$$

where

$$A_{12} = \sum_{ij}^{*} n_{i1} k_{12}^{ij} \gamma_{i1}. \tag{11}$$

Combining Eqs. (8) and (10) yields

$$J_S^{1\to 2} = \frac{C_o A_{12} F_{21}}{\alpha_1 F_{21} + \alpha_2 F_{12}}. \tag{12}$$

The corresponding expression for $J_S^{2\to 1}$ is given simply by reversing the roles of the subscripts 1 and 2 in Eqs. (11) and (12).

Equation (12) gives the general solution to an arbitrarily complex rapid equilibrium carrier model. In order to write down the solution to a particular model one need only substitute the expressions for α_1, α_2, F_{12}, F_{21} and A_{12} obtained using their definitions (Eqs. 3, 7, and 11; *see* Appendix for specific example). Several authors have previously noted that the solutions of certain less general rapid equilibrium carrier models can be cast in the form of Eq. (12) [3, 4, 8–10].

Dead-End Inhibitors

As a simple example of the way in which Eq. (12) can be used to determine certain properties of transport models without resorting to complete mathematical solutions, consider the case of a rapid equilibrium carrier model with a single binding site for a "dead-end" inhibitor, I, i.e., an inhibitor whose binding renders the carrier immobile. Assume also that I is present only on side 1 of the membrane at concentration I_1. For the purposes of this analysis it is not necessary to specify the mode of inhibition by I (competitive, noncompetitive, etc.). Since A_{12}, F_{12} and F_{21} are sums over mobile species only (*cf.* Eqs. 7 and 11), they are necessarily independent of I_1. Also, since there is a single binding site for I, α_1 and α_2 must be linear functions of I_1. Again it is not necessary to specify whether I binds to all carrier species or only to a subset of carrier species. It follows from Eq. (12) that $J_S^{1\to 2}$ has the form $B/(C + DI_1)$ where B, C and D are expressions which are independent of I_1. Thus for inhibitors of the rather general class specified above, a Dixon plot of $1/J_S^{1\to 2}$ *vs.* I_1 will necessarily be linear. Likewise a Hill plot of $\log[J_{SI}^{1\to 2}/(J_S^{1\to 2} - J_{SI}^{1\to 2})]$ *vs.* $\log I_1$, where $J_{SI}^{1\to 2}$ and $J_S^{1\to 2}$ are fluxes in the presence and absence of inhib-

Table 1. Expressions for zero-trans (*zt*), infinite-trans (*it*) and equilibrium exchange (*ee*) kinetic constants for the rapid equilibrium carrier model with a single substrate binding site (*see text* for details)

$$V^{zt}_{S_1} = \frac{C_o F''_{12} F'_{21}}{\alpha''_1 F'_{21} + \alpha'_2 F''_{12}}$$

$$K^{zt}_{S_1} = \frac{\alpha'_1 F'_{21} + \alpha'_2 F'_{12}}{\alpha''_1 F'_{21} + \alpha'_2 F''_{12}}$$

$$V^{it}_{S_1} = \frac{C_o F''_{12} F''_{21}}{\alpha''_1 F''_{21} + \alpha''_2 F''_{12}}$$

$$K^{it}_{S_1} = \frac{\alpha'_1 F''_{21} + \alpha''_2 F'_{12}}{\alpha''_1 F''_{21} + \alpha''_2 F''_{12}}$$

$$V^{ee}_{S_1} = \frac{C_o F''_{12} F''_{21}}{\alpha''_1 F''_{21} + \alpha''_2 F''_{12}}$$

$$K^{ee}_{S_1} = \frac{F''_{21}(\alpha'_1 F'_{21} + \alpha'_2 F'_{12})}{F'_{21}(\alpha''_1 F''_{21} + \alpha''_2 F''_{12})}$$

$$R = \frac{F'_{12} F''_{21}}{F'_{21} F''_{12}}$$

itor, respectively, will also be linear with slope one. The generalization of this analysis to multiple inhibitor binding sites and other types of inhibitor behavior is obvious.

It is worth stressing that results similar to those derived in the preceding paragraph are well-known in enzymatic systems [7]. What is, however, novel in the above treatment is first, the application to transport systems, and second, the ease and generality with which these results were obtained. Note also the clear relationship between the assumptions made and the predictions derived. A more general example which further illustrates the potential utility of Eq. (12) follows.

Models with a Single Substrate Binding Site

Equation (12) reduces to an interesting and useful form when the carrier possesses a single substrate binding site. This result is derived below and used to determine a number of properties of this family of transport models.

General Solution for Models with a Single Substrate Binding Site

When the transporter has a single binding site for the transported substrate, S, it can be seen directly from Eqs. (3) and (7) that α_n and F_{nm} can be written in the form

$$\alpha_n = \alpha'_n + S_n \alpha''_n \tag{13}$$

and

$$F_{nm} = F'_{nm} + S_n F''_{nm} \tag{14}$$

where S_n is the concentration of S on side n of the membrane and α'_n, α''_n, F'_{nm}, F''_{nm} are expressions which are independent of both S_1 and S_2. It can also be shown from Eqs. (7), (11) and (14) that

$$A_{nm} = S_n F''_{nm}. \tag{15}$$

Substituting Eqs. (13), (14) and (15) into Eq. (12) and rearranging yields

$$J_S^{1\to 2} = \frac{C_o F''_{12} F_{21} S_1}{\alpha_1 F_{21} + \alpha_2 F_{12}} = \frac{C_o F''_{12}(F'_{21} + S_2 F''_{21}) S_1}{[(\alpha'_1 F'_{21} + \alpha'_2 F'_{12}) + S_1(\alpha''_1 F'_{21} + \alpha'_2 F''_{12}) + S_2(\alpha'_1 F''_{21} + \alpha''_2 F'_{12}) + S_1 S_2(\alpha''_1 F''_{21} + \alpha''_2 F''_{12})]} \tag{16}$$

Zero-trans, Infinite-trans and Generalized Equilibrium Exchange Experimental Conditions

Defining *zero-trans conditions* by $S_2 = 0$ and *infinite-trans conditions* by $S_2 \to \infty$ it can be seen from Eq. (16) that $J_S^{1\to 2}$ has Michaelis-Menten form in each case. Expressions for the resulting zero-trans and infinite-trans Michaelis constants and maximum velocities are given in Table 1.

Carrying out some straightforward algebraic manipulation with the aid of Table 1 it can be shown that Eq. (16) can be rewritten in the form

$$J_S^{1\to 2} = \frac{V^{zt}_{S_1} K^{it}_{S_2} S_1 + V^{it}_{S_1} S_1 S_2}{K^{zt}_{S_1} K^{it}_{S_2} + K^{it}_{S_2} S_1 + K^{it}_{S_1} S_2 + S_1 S_2} \tag{17}$$

Thus under a given set of experimental circumstances (*viz.*, all α'_n, α''_n, F'_{nm} and F''_{nm} fixed) the eight kinetic constants derived from zero-trans and infinite-trans procedures are sufficient to completely determine the behavior of the model, i.e., to predict the flux rate in either direction under any substrate conditions. As discussed in more detail below, only five of these eight kinetic constants are actually independent of one another.

It is useful for what follows to define a set of *generalized equilibrium exchange conditions* which are applicable to both active and passive transport systems as well as to situations where ligands which are co- and/or countertransported with the substrate are not at electrochemical equilibrium across

the membrane. Generalized equilibrium exchange conditions are defined here by the requirement that there is no net substrate flux across the membrane, i.e., that $J_S^{1\to 2} = J_S^{2\to 1}$. By applying this condition to Eq. (16) it can be shown that the constraint

$$\frac{S_1}{S_2} = \frac{F'_{12}F''_{21}}{F'_{21}F''_{12}} \tag{18}$$

is both a necessary and sufficient condition for generalized equilibrium exchange conditions in the rapid equilibrium carrier model with a single substrate binding site. In the remainder of the paper the quantity on the right-hand-side of Eq. (18) is referred to as R (*cf.* Table 1). It is worth stressing that R is an experimentally measurable constant; under a given set of experimental conditions (i.e., fixed α'_n, α''_n, F'_{nm}, F''_{nm}) one need only determine substrate concentrations S_1 and S_2, at which $J_S^{1\to 2} = J_S^{2\to 1}$, then R is given by $R = S_1/S_2$. Furthermore, it follows from the above discussion that once R has been determined for a given set of experimental conditions then $J_S^{1\to 2}$ necessarily equals $J_S^{2\to 1}$ for all S_1 and S_2 for which $S_1/S_2 = R$. This latter observation is an important prediction of the rapid equilibrium carrier model with a single substrate binding site.

The form of $J_S^{1\to 2}$ under generalized equilibrium exchange conditions can be obtained by substituting Eq. (18) directly into Eq. (16) (the algebra can be simplified considerably by using the relation $F_{12}F'_{21} = F'_{12}F_{21}$ which can be proven from Eqs. (14) and (18) for generalized equilibrium exchange conditions). The result has Michaelis-Menten form with kinetic constants $V_{S_i}^{ee}$ and $K_{S_i}^{ee}$ given in Table 1.

Rejection Criteria

As illustrated by Eq. (17), for the subset of rapid equilibrium carrier models with one substrate binding site, the eight kinetic constants derived from zero-trans and infinite-trans experimental procedures are sufficient to completely characterize the model for a given set of experimental conditions. In fact, only five of these constants are independent of one another since the relationships referred to as Eqs. (RC1), (RC2) and (RC3) in Table 2 can be proven directly from Table 1. It is also clear from Eq. (17) that the additional kinetic constants derived from generalized equilibrium exchange conditions (or for that matter from any experimental conditions) must necessarily be expressible in terms of zero-trans and infinite-trans parameters. Thus these conditions give rise to four additional relationships referred to as Eqs. RC4–RC7 in Table 2. Finally,

Table 2. Rejection criteria for the rapid equilibrium carrier model with a single substrate binding site (*see text*)

$V_{S_1}^{it} = V_{S_2}^{it}$	(RC1)
$K_{S_1}^{zt} K_{S_2}^{it} = K_{S_2}^{zt} K_{S_1}^{it}$	(RC2)
$\frac{V_{S_1}^{it} K_{S_1}^{zt}}{K_{S_1}^{it} V_{S_1}^{zt}} = 1 + \frac{V_{S_2}^{zt}}{V_{S_1}^{zt}} - \frac{V_{S_2}^{it}}{V_{S_1}^{it}}$	(RC3)
$\frac{V_{S_1}^{zt}}{K_{S_1}^{zt}} = \frac{V_{S_2}^{zt}}{K_{S_2}^{zt}} \frac{K_{S_2}^{ee}}{K_{S_1}^{ee}}$	(RC4)
$V_{S_1}^{ee} = V_{S_2}^{ee}$	(RC5)
$V_{S_1}^{ee} = V_{S_2}^{it}$	(RC6)
$\frac{V_{S_1}^{ee}}{K_{S_1}^{ee}} = \frac{V_{S_1}^{zt}}{K_{S_1}^{zt}}$	(RC7)
$R = \frac{K_{S_1}^{ee}}{K_{S_2}^{ee}} = \frac{V_{S_2}^{zt} K_{S_1}^{zt}}{K_{S_2}^{zt} V_{S_1}^{zt}}$	(RC8)

the experimentally measurable constant R can also be expressed in terms of various kinetic constants (Eq. RC8).

The eight relationships given in Table 2 represent a set of mathematical constraints on the commonly determined experimental parameters listed in Table 1. These constraints arise directly from the form of Eq. (16) and thus provide a set of rejection criteria for the rapid equilibrium carrier model with a single substrate binding site.

Concluding Remarks

This paper presents a general framework for solving and analyzing rapid equilibrium carrier models. It is demonstrated that the flux equation of any model of this type can be written in a simple mathematical form (Eq. 12) and that this relation leads directly to a straightforward method for writing down the solution to an arbitrarily complex model. The use of graph theoretic techniques [7] was unnecessary in these derivations owing to the mathematical simplifications arising from the rapid equilibrium assumption.

It is also emphasized that the general form of the flux equation for rapid equilibrium carrier models established here (Eq. 12) can be used to determine many properties of individual models or groups of models without resorting to complete mathematical analyses and without specifying all of the details of the proposed transport mechanism. This ability potentially allows one to screen models for certain types of behavior and/or to introduce modifications indicated by experimental observations in a relatively straightforward way. The utility of this procedure was demonstrated by analyzing

the kinetic effects of dead-end inhibitors and by characterizing the kinetic behavior of rapid equilibrium models with a single substrate binding site. In each case assumptions regarding only the interaction of the ligand of interest with the carrier were made, thus the results derived were otherwise completely general. In the latter case it was possible to derive a general rate equation (Eq. 17) and a set of rejection criteria (Table 2) which are similar to those found for several much simpler models [2, 6, 9].

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Appendix

The solution to a specific example is given here in order to demonstrate the use of Eqs. (3), (7), (11) and (12). The kinetic diagram for the model under consideration is shown in the Figure. This model incorporates a number of features for illustrative purposes and is not intended to represent any particular experimental system. The free carriers on sides 1 and 2 of the membrane are denoted by C_1 and C_2, respectively; neither C_1 nor C_2 is mobile. M is a nontransported modifier whose binding site is on side 1 of the membrane. M can bind to both C_1 and C_2 and this binding renders them mobile. The transported species are X, Y and Z with X and Y cotransported in exchange for Z. R is a dead-end inhibitor which can only bind to CM_1 from side 1. The various binding constants are defined below. These equations are equivalent to Eq. (2) in the body of the paper.

$$CX_n = \frac{X_n}{K_{X_n}} C_n \qquad CXY_n = \frac{Y_n}{K_{XY_n}} \frac{X_n}{K_{X_n}} C_n$$

$$C_{XYZ_1} = \frac{Z_2}{K_{XYZ_1}} \frac{Y_1}{K_{XY_1}} \frac{X_1}{K_{X_1}} C_1$$

$$C_{XYZ_2} = \frac{Z_1}{K_{XYZ_2}} \frac{Y_2}{K_{XY_2}} \frac{X_2}{K_{X_2}} C_2$$

$$CM_1 = \frac{M_1}{K_{M_1}} C_1 \qquad CM_2 = \frac{M_1}{K_{M_2}} C_2$$

and

$$CMR_1 = \frac{R_1}{K_{MR_1}} \frac{M_1}{K_{M_1}} C_1.$$

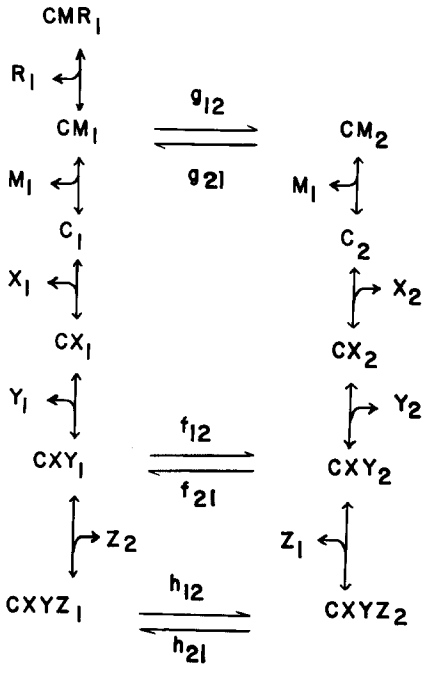

Fig. Schematic representation of the rapid equilibrium carrier model solved in the Appendix. *See text* for details

Thus (*cf.* Eq. 3)

$$\alpha_1 = 1 + \frac{M_1}{K_{M_1}} + \frac{R_1 M_1}{K_{MR_1} K_{M_1}} + \frac{X_1}{K_{X_1}} + \frac{Y_1 X_1}{K_{XY_1} K_{X_1}} + \frac{Z_2 Y_1 X_1}{K_{XYZ_1} K_{XY_1} K_{X_1}}$$

and

$$\alpha_2 = 1 + \frac{M_1}{K_{M_2}} + \frac{X_2}{K_{X_2}} + \frac{Y_2 X_2}{K_{XY_2} K_{X_2}} + \frac{Z_1 Y_2 X_2}{K_{XYZ_2} K_{XY_2} K_{Y_2}}.$$

F_{12} and F_{21} are given by similar sums over mobile species (*cf.* Eq. 7). Thus

$$F_{12} = g_{12} \frac{M_1}{K_{M_1}} + f_{12} \frac{Y_1 X_1}{K_{XY_1} K_{X_1}} + h_{12} \frac{Z_2 Y_1 X_1}{K_{ZYX_1} K_{XY_1} K_{X_1}}$$

and

$$F_{21} = g_{21} \frac{M_1}{K_{M_2}} + f_{21} \frac{Y_2 X_2}{K_{XY_2} K_{X_2}} + h_{21} \frac{Z_1 Y_2 X_2}{K_{ZYX_2} K_{XY_2} K_{X_2}}.$$

The complete solution to the model (Eq. 12) now requires only evaluation of A_{12} (Eq. 11). In order to find A_{12} one must first specify the substrate. If, for example, X is the substrate, then

$$A_{12} = f_{12} \frac{Y_1 X_1}{K_{XY_1} K_{X_1}} + h_{12} \frac{Z_2 Y_1 X_1}{K_{XYZ_1} K_{XY_1} K_{X_1}}.$$

Alternatively, if X is the substrate and X and Y are the same chemical species then

$$A_{12} = 2f_{12} \frac{X_1^2}{K_{XX_1} K_{X_1}} + 2h_{12} \frac{Z_2 X_1^2}{K_{XXZ_1} K_{XX_1} K_{X_1}}.$$