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COMPLETE MEASUREMENT SETS IN MULTICOMPARTMENT SYSTEMS ANALYSIS--A REFERENCE COMPARTMENT CRITERION

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COMPLETE MEASUREMENT SETS IN MULTICOMPARTMENT SYSTEMS ANALYSIS- A REFERENCE COMPARTMENT CRITERION

by

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Abstract

COMPLETE MEASUREMENT SETS IN MULTICOMPARTMENT SYSTEMS ANALYSIS - A REFERENCE COMPARTMENT CRITERION

by

Phoebe G. Spetsieris

Adviser: Professor Hiram E. Hart

The identifiability of linear, contiguous, steady-state, n-compartment systems for which none of the n^2 transfer rates are known a priori, is considered. The concept of "minimal completeness" is introduced to characterize any n impulse-response measurement elements of the transition matrix which are capable of providing a unique solution or at most a discrete set of physically compatible solutions. It is shown initially for strongly connected systems, that all minimally complete sets require either measurement in all compartments or injection in all compartments. It is further shown that the existence of a reference compartment linking any n transition matrix elements that satisfy the previous requirement, is in general a necessary and sufficient condition for determining minimal completeness. An "eigenvector approach" for obtaining a solution is developed which can be applied to both strongly connected systems

and to certain non-strongly connected systems for which n distinct eigenvalues are obtainable. The extent of the applicability of the "reference compartment criterion" to non-strongly connected sytems and the generalization of the eigenvector approach to measurement sets involving simultaneous multiple inputs and weighted response measurements are also examined. The relation of some of these concepts to aspects of linear system control theory and structural identifiability is indicated. The constraints imposed on the topology of complete measurement sets by the structural topology of the system and the general implications of such relationships between measurement topologies and system topologies are considered.

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SECTION I

INTRODUCTION

The study of physiological transport phenomena and biochemical processes is evidenced by an immense volume of related scientific literature. However, the concepts of defining the appropriate system parameters and the required measurements necessary to mathematically describe and specify such processes did not achieve a significant degree of sophistication until relatively recently. In particular, the development of the methods of isotopic multicompartment tracer analysis had tremendous impact in the study of linear steady-state systems (Sheppard, 1962). The work of Sheppard and Householder (1951) first established that the general steady-state multicompartment system could be fully identified by measurements involving the use of multiple isotopes. It was later shown by Hart (1955) that only a single tracer was necessary to completely determine the transfer rates of such systems. A variety of increasingly more complex applications were developed for both systems that could be described by a set of linear first order differential equations having constant coefficients (Hearon, 1963) and for those that require an integral differential equation representation (Branson, 1946; Stephenson, 1960; Hart, 1967). In particular, with respect to the former systems, experimental designs have been applied either to solve general reversibly connected n compartment systems for which all transfer rate parameters are nonvanishing, or to solve a variety of constrained systems exhibiting one or more vanishing transfer rate parameters (Rescigno, 1966). In addition, procedures have been developed for specifying the multiple solution sets that are physically compatible with a set of incomplete data (Berman and Shoenfeld, 1956) and for determinning the sets of compatible null constraints that can be imposed to reduce a manifold of possible solutions (Rubinow and Winzer, 1971; Rubinow, 1973). Thus, an ideal objective of compartmental analysis would appear to be the unique determination of all n^2 transfer rate parameters but this, of course, can only be accomplished if suitable experimental data can be obtained. This is equivalent to the requirement in the linear system theory terminology of Bellman and Astrom (1970) that the compartmental system be "structurally identifiable". However, since the data obtained from different experiments may be independently sufficient for identifiability, it is fundamental to ask what, if any, common criteria can be found to characterize such "complete" sets of measurements. The data obtained by the impulse response measurement of the concentration of label in each compartment for an extended time period following tracer injection in a single, arbitrarily chosen compartment is often adequate and it is such a set of n measurement functions that is commonly assumed to be obtainable. However, if special flow topologies exist, so that, e.g., unidirectional transfer between some or all compartments occurs, it may be necessary to obtain a special set of "injection-measurement functions" for a complete solution, where it may no longer be possible to limit injection to a single compartment or where it may not be necessary to measure all of the compartments if the reduced connectivity of the system is known a priori. Thus, the topology of the measurement sets that can identify the system must be related to the underlying system structural topology.* Based on the system theory concepts defined by Bellman and Åstrom, Cobelli and Romanin-Jacur (1975,1976) established additional criteria for observability, controllability and structural identifiability. They provide a computer flow-chart for testing these criteria for a specified set of measurements and a given system model. A generalized standard type of experiment may be assesed, where input occurs at a single compartment or is simultaneously distributed to a multiple set of compartments and each output measurement may be influenced by one or more compartments. Note that the measurements considered by Cobelli and Romanin-Jacur are restricted to a single impulse in time and, moreover, that some prior knowledge of the system

* see appendix E for the prior use of topological concepts in network and multicompartment systems analysis.

structural topology is assumed. In general, the number of measurements required to identify the system is less than n, since some of the null transfer rates are known a priori. However, if none of the n^2 transfer rates are previously known, then, as expected, at least n measurements must be obtained. Bertrand, Walter and Le Cardinal (1975,1976,1978) noted that these measurements need not be restricted to a standard type of experiment. Thus, assuming that only the order of the system is known, they showed that the identifiability of the system may be established by experiments involving topologically independent impulse response measurements for which inputs are separated in time. Specifically, they noted that all the transfer rate parameters could be determined by repeatedly measuring label as a function of time in a single arbitrarily chosen compartment, each time following injection in a different compartment. This provides an alternate minimally complete set* of n injection-measurement functions which, in a way, reverses the standard procedure. The existence of two distinct classes of minimal experimental measurement sets for determining the transfer rate parameters, suggested that other sets of n injection-measurement functions might also provide complete transfer rate information. Such alternate complete measurement sets have been identified and a general principle involving the concept of a

* A class of complete sets requiring more than n measurements is also defined by Walter et. al. (1976).

reference compartment has been presented as a criterion for characterizing minimal completeness (Spetsieris and Hart, 1976) for systems for which none of the transfer rates are known a priori. This reference compartment completeness requirement will be more thoroughly described here and the basic criteria for its applicability will be developed. It will be shown that a discrete number of multiple solutions may be obtained for some of the parameters. In such cases, even if no additional knowledge of the system is available, one may sometimes distinguish the true values by considering the physical constraints imposed on the system. Both those sets of measurements leading to unique solutions for all of the parameters and those which may lead to a finite set of physically compatible solutions for some of the parameters are defined here under the term "complete sets of measurements", whereas, on the other hand, "incomplete sets of measurements" do not provide a solution for all parameters (i.e., only an infinite class of solutions can be defined). Further, it should be noted that although the identifiability of a system is subject to the limitations imposed by experimental factors, this practical consideration is irrelevant to the theoretical concept of completeness that we are concerned with here. Thus, it would be useless to attempt to determine a solution from a set of otherwise "perfect" measurements if they do not innately contain sufficient information for such a solution. It is exclusively this innate quality that we attempt to define here. Therefore, we may consider a set of measurements to be sufficient for "completeness" even though the magnitude of the experimental errors or the mathematical complexity of obtaining a precise solution may be essentially prohibitive.

A review of the mathematical considerations leading to the concept of completeness is presented in section II and an algebraic approach for studying the general problem is outlined. An alternate "eigenvector approach" for obtaining a solution is presented and the conditions for which both methods are applicable are discussed. In section III the digraph-theoretic concepts of injection-measurement sets are revealed. It is shown in sections IV and V, respectively, that the existence of a reference compartment is in general a necessary and sufficient condition for solution for strongly connected systems. The general applicability of the criterion and the eigenvector approach for obtaining a solution are discussed in section VI. It is suggested that the overall applicability of the reference compartment criterion may be more generally extendable for systems exhibiting n distinct eigenvalues in the measurement functions. The extent of the applicability of the criterion is specifically examined for non-strongly connected systems for which the eigenvector relations are partially applicable assuming that none of the transfer rates (including the null values) are known initially.

Although the discussion is here primarily confined to tracer analysis, the relation of the measurement topology to the system structural topology and criteria for complete measurement sets developed in this context are of more general interest. Immediate analogs can be found in other areas, such as electrical and mechanical linear control system models, that conform to a similar mathematical representation.

SECTION II

MATHEMATICAL FORMULATION OF THE PROBLEM

a. General Considerations

Consider a steady-state system of n contiguous compartments that can be described by a set of first order linear differential equations having constant coefficients. In vector-matrix representation,*

$$\dot{\underline{x}}(t) = \mathcal{A}\underline{x}(t) \tag{1}$$

where, $\mathcal{A}$ is the nxn constant transfer rate matrix and x(t) is the state vector. If the components $x_i(t)$, i=1,2,...,n of $\underline{x}(t)$ are defined as the concentrations of label in each compartment i at time t, then the parameters a_{ij}, $i \neq j$ represent the fractional transfer rate of material from compartment j to compartment i per unit volume of compartment i, as defined below, and a_{ii} is the negative of the total fractional rate of exit from compartment i to the exterior and to all other compartments. The total amount of label in each compartment at time t is given by the vector

$$\underline{q} = V\,\underline{x}$$

where V is an nxn diagonal matrix with volume elements

$$V_{ij} = V_i\,\delta_{ij} \;.$$

The vector $\underline{q}$ satisfies an equation of the same form as (1):

$$\dot{\underline{q}} = K\,\underline{q} \;,$$

*Equivalently, the system may be expressed by the equations $\dot{\underline{x}} = \mathcal{A}\underline{x} + b\underline{u}$, $\underline{y} = c\underline{x}$, where $\underline{u}$ is the input vector, $\underline{y}$ is the output vector and b and c are constant matrices (Bellman and Astrom, 1970).

where the elements k_{ij}, $i \neq j$ of K represent the fraction of material (including tracer) in compartment j that is transferred to compartment i per unit time. The matrices $\mathcal{a}$ and K are related by the similarity transformation:

$$a = V^{-1} K V \quad (a_{ij} = k_{ij} V_j / V_i)$$

(see e.g., Rubinow and Winzer, 1971). Since the determined quantity is usually the concentration* of label in each compartment x_i, rather than the total q_i, only knowledge of the compartment volumes of the injected compartments is necessary for the complete determination of the transfer rate parameters a_{ij}, as will be further noted.

* One may consider x_i to be the specific activity in μc/mg in which case V_i will be given in mg..

b. Standard (Column) Solution

The standard methods for determining the transfer rate matrix $\mathfrak{a}$ in general involve injecting label in a single* compartment j and measuring the concentration of label in each of the n compartments as a function of time. Ideally, assuming that $\mathfrak{a}$ has n distinct eigenvalues, the set of experimental "injection-measurement" curves can be fitted by sums of n exponential terms:

$$x_{ij} = \sum_{k=1}^{n} A_{ik}^{j} e^{\lambda_k t}, \quad i=1,2,\ldots,n \qquad (2)$$

The n eigenvalue parameters λ_k, $k=1,2,\ldots,n$, can be extracted from any single curve for which all n coefficients A_{ik}^{j} are not zero or from any combination of curves that explicitly exhibit the n eigenvalues as a whole. The coefficients A_{ik}^{j}, $i,k=1,2,\ldots,n$, associated with injection into the jth compartment, form an nxn matrix $A^j \equiv (A_{ik}^{j})$, $i,k=1,2,\ldots,n$:

$$A^j = \begin{pmatrix} A_{11}^{j} & A_{12}^{j} & \cdots & A_{1n}^{j} \\ A_{21}^{j} & A_{22}^{j} & \cdots & A_{2n}^{j} \\ \vdots & & & \\ A_{n1}^{j} & A_{n2}^{j} & \cdots & A_{nn}^{j} \end{pmatrix}$$

The n column vectors of A^j are defined by

$$\underline{A}_k^{j} = \begin{pmatrix} A_{1k}^{j} \\ A_{2k}^{j} \\ \vdots \\ A_{nk}^{j} \end{pmatrix} \qquad k=1,2,\ldots,n \qquad (3)$$

* A more generalized standard type of experiment is described in the Introduction and in Appendix D.

As is well known (Hart, 1955) and as shown in Appendix A, each column vector $\underline{A}_k^j$ must satisfy the equation:

$$\mathcal{a}\, \underline{A}_k^j = \lambda_k \underline{A}_k^j \qquad (4)$$

Thus, a vector $\underline{A}_k^j$ represents an eigenvector of $\mathcal{a}$ corresponding to the eigenvalue λ_k, unless it is a null vector. Since the eigenvectors of a matrix with distinct eigenvalues are linearly independent (Gantmacher, 1960), A^j must be non-singular, assuming that none of the vectors $\underline{A}_k^j$ are null vectors. One can then determine $\mathcal{a}$ from the inverse diagonalization relationship:

$$\mathcal{a} = (A^j)\, \lambda\, (A^j)^{-1} \qquad (5)$$

where λ is the nxn matrix having elements $\lambda_{kp} = \lambda_k \delta_{kp}$.

$$\lambda = \begin{pmatrix} \lambda_1 & & & 0 \\ & \lambda_2 & & \\ & & \ddots & \\ 0 & & & \lambda_n \end{pmatrix} \qquad (6)$$

Thus, equation 5 expresses the fact that complete transfer rate information can be derived from a standard set* of n injection-measurement functions under the assumption that the eigenvalues λ_k are distinct and that A^j is non-singular. It is obviously** implied that a flow path must exist from the injected compartment to all of the compartments, even though some of the direct transfer rates may be zero.

*Hart, 1955; Berman and Schoenfeld, 1956

**Since the inclusion of a null measurement function directly implies that A^j is singular or simply from the fact that n distinct eigenvalues are obtainable.

The latter requirement corresponds to the necessary and sufficient condition for controllability described by Cobelli and Romanin-Jacur (1976). The observability condition is also satisfied since all compartments are measured, assuming, of course, that every compartment is accessible to measurement (Bellman and Astrom, 1970). We will refer to this standard type of solution as a "column" solution because of its relation to a column of the transition matrix discussed below (Bertrand et.al., 1975).

c. Transition Matrix Representation

For arbitrary initial conditions, assuming that α has n distinct eigenvalues, the state vector $\underline{x}(t)$ can be written as

$$\underline{x}(t) = A\,\underline{e}(t) \tag{7}$$

where,

$$\underline{e}(t) = \begin{pmatrix} e^{\lambda_1 t} \\ e^{\lambda_2 t} \\ \vdots \\ e^{\lambda_n t} \end{pmatrix} \tag{8}$$

is an n component vector and A is an nxn constant matrix. The matrix A will, of course, depend on the specific initial conditions and will reduce to the previously defined matrix A^j when initial injection occurs in the jth compartment.

It will be useful for the following discussion to express $\underline{x}(t)$ more explicitly in terms of initial conditions. This is immediately accomplished by multiplication of the initial state vector $\underline{x}(0)$ by the dimensionless, time dependent state transition matrix $T(t)$, commonly referred to in systems engineering (see, e.g. Perkins and Cruz, 1969). Thus,

$$\underline{x}(t) = T(t)\,\underline{x}(0) \quad . \tag{9}$$

Clearly, $T(t)$ is initially equal to the nxn identity matrix

$$T(0) = I \quad . \tag{10}$$

Each element $T_{ij}(t)$ of $T(t)$, where $T_{ij}(0) = \delta_{ij}$, represents, in effect, an impulse-response relationship or in the present terms, an injection-measurement function between two compartments j and i. It is evident from equation 9 that the dimensionless element $T_{ij}(t)$ is numerically equal to the time response of a compartment i as given by the value of $x_{ij}(t)$, equation 2 , for initial conditions of unit dose (concentration) in compartment j and zero initial dose in all other compartments.* The transition matrix for n=2 is given by 13 For an arbitrary initial dose in j ($x_{jj}(0)\neq 0$), the values $T_{ij}(t)$ are, of course, proportional to the measured values $x_{ij}(t)$:

$$T_{ij}(t) = \frac{x_{ij}(t)}{x_{jj}(0)} \qquad (11)$$

Thus, by carrying out a standard set of measurements, we are determining a column of the transition matrix. It follows from the considerations leading to equation 5 that the n injection-measurement functions represented by the elements of any column j of T(t), are a complete set of measurements with respect to transfer rate information assuming, of course, that A^j is not singular. In addition, the volume of the injected compartment j is given by

$$V_j = \frac{D_j}{x_{jj}(0)} \qquad (12)$$

where D_j is the dose administered in j. Complete volume information cannot be obtained from 12 unless further measurements of the initial concentrations $x_{jj}(0)$ are made

* Hearon (1963) also defines such a matrix.

following injection in each of the n compartments*.

Transition Matrix for the Two Compartment System

$$T = \begin{pmatrix} \frac{(\lambda_1 - a_{22})}{(\lambda_1-\lambda_2)} e^{\lambda_1 t} - \frac{(\lambda_2 - a_{22})}{(\lambda_1-\lambda_2)} e^{\lambda_2 t} & \frac{a_{12}}{(\lambda_1-\lambda_2)} e^{\lambda_1 t} - \frac{a_{12}}{(\lambda_1-\lambda_2)} e^{\lambda_2 t} \\ \frac{a_{21}}{(\lambda_1-\lambda_2)} e^{\lambda_1 t} - \frac{a_{21}}{(\lambda_1-\lambda_2)} e^{\lambda_2 t} & \frac{(\lambda_1 - a_{11})}{(\lambda_1-\lambda_2)} e^{\lambda_1 t} - \frac{(\lambda_2 - a_{11})}{(\lambda_1-\lambda_2)} e^{\lambda_2 t} \end{pmatrix} \quad (13)$$

* Alternatively, the volumes can be calculated if the exterior entry rates r_{io} (assuming they are not zero) or the exterior exit rates r_{oi} are measured for each compartment and assuming that the values a_{ij} have been previously determined. In the first case, the volumes are obtained from the expressions:

$$r_{io} = -V_i \sum_{j=1}^{n} a_{ij} , \quad r_{io} \neq 0$$

In the second case, the following system of equations must be solved:

$$r_{oi} = - \sum_{j=1}^{n} a_{ji} V_j$$

d. Row Solution

It has been observed by Bertrand, Walter and Le Cardinal (1975,1976,1978) that the transfer rate parameters can also be determined by injecting successively in each of the n compartments and measuring only one of the compartments repeatedly. In this case, the determined set of n injection-measurement functions correspond to the n elements of a single row of the transition matrix T. The method of analysis developed by Bertrand et. al. incorporates a least-square minimization of experimental errors and is equally applicable for determining α from the knowledge of a single row or column of T. As indicated by Walter et. al.(1976) and as shown in Appendix B, equation 14 below, in analogy to eq. 5, enables the determination of the transfer rate matrix (transpose) directly from the knowledge of a single row i of T. It should here be noted that there must exist a flow path into the measurement compartment i from each of the other compartments*, otherwise, $|A_i| = 0$. The latter assumption is usually sufficient to insure that the matrix A_i, defined below, is non-singular. Thus,

$$\alpha^{\tau} = (A_i)\,\lambda\,(A_i)^{-1} \qquad (14)$$

where, $A_i = (A^j_{ik})$, $j,k=1,2,\ldots,n$ is an nxn matrix whose elements are the coefficients A^j_{ik} of the elements T_{ij} of row i

* Note that this assumption is equivalent to the necessary and sufficient condition for observability in a structural sense described by Cobelli and Romanin-Jacur (1976) for experiments involving simultaneous input in the n compartments. Controlability would also be satisfied for such experiments since all compartments would be injected.

of T:

$$A_i = \begin{pmatrix} A^1_{i1} & A^1_{i2} & \cdots & A^1_{in} \\ A^2_{i1} & A^2_{i2} & \cdots & A^2_{in} \\ \vdots & & & \\ A^n_{i1} & A^n_{i2} & \cdots & A^n_{in} \end{pmatrix} , \qquad (15)$$

where $\sum_{k=1}^{n} A^j_{ik} \, e^{\lambda_k t} = T_{ij}(t) = x_{ij}(t)/x_{jj}(0)$

The column vectors of A_i,

$$\underline{A}i_k = \begin{pmatrix} A^1_{ik} \\ A^2_{ik} \\ \vdots \\ A^n_{ik} \end{pmatrix} , \qquad (16)$$

must satisfy the equation

$$\alpha^{\tau} \underline{A}i_k = \lambda_k \underline{A}i_k \qquad (17)$$

and are, therefore, eigenvectors of α^{τ} unless they are null vectors. Further if $\underline{x}'$ is a column vector whose components are the elements of any row i of T(t),

$$\underline{x}' = A_i \underline{e} ,$$

then the following relationship, analogous to 1 must be true:

$$\dot{\underline{x}}' = \alpha^{\tau} \underline{x}' \qquad (18)$$

This equation is of interest since it relates values obtained in the same compartment under different initial conditions.

The following must be noted in comparing the solutions obtained by equations 5 and 14 . For equation 5 the elements A^j_{ik} of A^j may represent either the coefficients of the measured quantities x_{ij}(eq. 2)or the coefficients of the transition matrix elements T_{ij}, since the value $x_{jj}(0)$ relating T_{ij} and x_{ij} is constant for all measurements and will cancel in the two factors (A^j) and $(A^j)^{-1}$. The quantity $x_{jj}(0)$ is, however, directly obtained as part of the procedure, anyway. On the other hand, for equation 14, the elements A^j_{ik} of A_i must be coefficients of the transition matrix elements T_{ij} corresponding to row i of T. Thus, the n values $x_{jj}(0)$, j=1,2,...,n, relating the elements T_{ij} with the measured quantities x_{ij}, are necessary for solution by equation 14 . However, only the value $x_{ii}(0)$ would normally be obtained since only compartment i is measured. Thus, either the volumes of the remaining compartments must be known a priori, in which case the constants $x_{jj}(0)$ can be determined from equation 12 , or the measurement of the values $x_{jj}(0)$ must be incorporated as part of the procedure for all n values of j. The latter approach would deviate somewhat from the primary restriction that only one compartment i be measured, though slightly since only the initial value is needed. Further, this requirement may not be so demanding in view of the fact that each compartment is injected as part of the procedure at widely separated times and it may even seem natural to measure the initial concentration at the time of injection assuming that there is instantaneous mixing.

The disadvantage is that the initial value is not always accurate and a backward extrapolation of the first few measurements may be needed. However, in any case it should be clear that both the column and row solutions imply knowledge of only n elements, corresponding to a single column or row of T, respectively.

e. <u>Hybrid Solutions</u>

Having established the existence of two distinct methods for determining α employing either any column or any row of the transition matrix, a logical question is whether other sets of n elements of T can provide complete transfer rate information as well. It will be shown in the theory to be developed in subsequent sections that the existence of solutions derivable from minimal sets of n elements* of the matrix T, involves a relationship between the topology of the system and the topology of the measurement set. Thus, a set of n elements of T, randomly selected, may or may not provide the information necessary for a solution. Both the "direct approach" for studying the problem developed below and the "eigenvector approach" developed later on in this section are generally applicable for strongly connected systems and to some extent for non-reversibly connected systems. The direct method of analysis is revealing for systems with a small number of compartments. However, for a larger number of compartments, the eigenvector approach is the preferred method of analysis since it can be presented in a more concise algebraic form even though the actual number of equations may be greater.

* Hybrid measurement sets involving more than n elements can, of course, also be defined (Walter et.al. (1976)).

f. Direct Approach

The amount of information that can be extracted from any single injection-measurement function has been examined in detail by Rubinow and Winzer (1971) and by Rubinow (1973). Thus, the equations derived below have previously been studied in relation to a single measurement or a set of standard measurements. Here, we will attempt to determine the information that is obtained for n random injection-measurements.

We will assume initially that a single injection of tracer occurs in the jth compartment. Therefore, $\underline{x} = A^j \underline{e}$. Differentiating equation 1 repeatedly one obtains:

$$\frac{d^p \underline{x}}{dt^p} = \alpha^p \underline{x} = A^j \lambda^p \underline{e} \qquad (19)$$

The ith component of the vector 19 evaluated at t=0 determines the ijth element of the pth power of α :

$$\sum_{k=1}^{n} (\alpha^p)_{ik} x_{kj}(t) = \sum_{k=1}^{n} A^j_{ik} \lambda^p_k e^{\lambda_k t}$$

and therefore,

$$(\alpha^p)_{ij} = \frac{1}{x_{jj}(0)} \sum_{k=1}^{n} A^j_{ik} \lambda^p_k \quad . \qquad (20)$$

The expression on the right hand side of equation 20 can be evaluated after measurement of the element $x_{ij}(t)$ for any power p assuming that $x_{jj}(0)$ is known or measured.

The matrix element $(\alpha^{p})_{ij}$ for any value of p can, therefore, be evaluated by measuring the element $T_{ij}(t)$ of T.
It follows from the Cayley-Hamilton theorem that only the first n-1 values of p result in independent expressions for the matrix element $(\alpha^{p})_{ij}$. Therefore, only n-1 independent equations can be obtained from equations 20 since higher powers of α result in redundant equations. The first of these (p=1) gives the value of α_{ij} directly*. If n elements of T are measured n^2-n such nonlinear algebraic equations relating the elements of α are obtained. An additional n equations are provided by the n invariants (trace, determinant, etc.) of the matrix α which can be expressed in terms of the n eigenvalues λ_k. Thus, a total of n^2 equations relating the n^2 elements of α are made available by any set of n known elements of T assuming n distinct eigenvalues are obtained. Whether this total set of n^2 equations is independent or not depends on the choice of n elements of T comprising the set. While the n elements of a row or column of T in general define a single unique solution, single, multiple or incomplete solutions may arise from other sets of n elements of T. If multiple solutions occur for some of the α_{ij} parameters one may consider that the true physical solution must also satisfy the following physical constraints imposed on the transfer rate parameters (Hearon, 1963):

* Equivalently determined form the initial slope $\dot{T}_{ij}$

$$a_{ij} \geq 0 \qquad i,j=1,2,\ldots,n \quad i \neq j \tag{21}$$

$$a_{ii} \leq 0 \qquad i=1,2,\ldots,n \tag{22}$$

$$\sum_{j=1}^{n} a_{ij} \leq 0 \qquad i=1,2,\ldots,n \tag{23}$$

$$\sum_{i=1}^{n} a_{ij} \frac{V_i}{V_j} \leq 0 \qquad j=1,2,\ldots,n \tag{24}$$

The first two conditions are true in accordance with the physical definition of the transfer rate parameters. The third condition expresses the fact that under steady-state conditions the fractional entry rate from all compartments into compartment i, $\sum_{j=1}^{n} a_{ij}$, $j \neq i$, plus the rate of entry from the exterior must equal the rate of exit from i, $-a_{ii}$. Thus, $\sum_{j=1}^{n} a_{ij}$ is the negative of the fractional exterior entry rate into i. The last condition expresses the fact that $\sum_{i=1}^{n} a_{ij} V_i/V_j$ is equal to the negative of the fractional excretion rate from compartment j to the exterior. Since each of the above conditions represents a multiple number of constraints it may be expected that the information totally provided by these constraints would often be decisive in determining a unique physically realizable solution when more than one mathematical solution is obtained.

These conditions are further examined in this respect for the two and three compartment system and their applicability is illustrated in the examples.

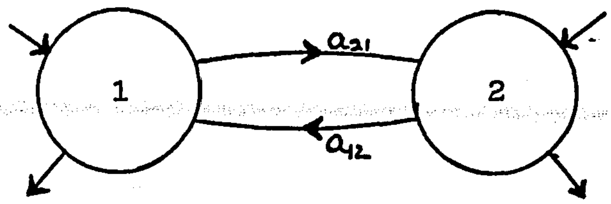


fig. 1. General Two Compartment Model

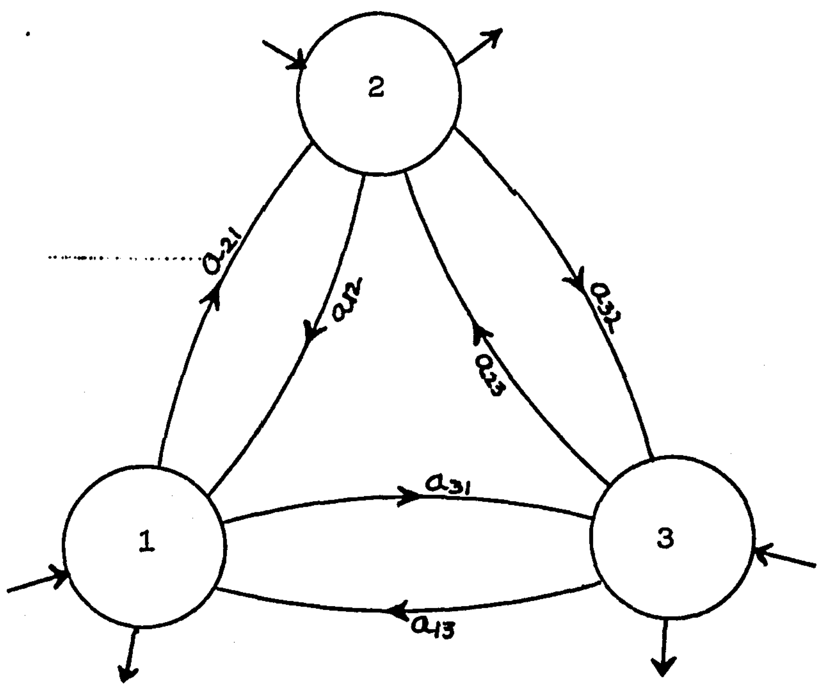


fig. 2. General Three Compartment Model

1. Two Compartment System (fig.1a)

Let us first examine the two compartment system*. Consideration of the invariants leads to two equations in the four unknowns, a_{11}, a_{12}, a_{21}, a_{22}:

$$a_{11} + a_{22} = \lambda_1 + \lambda_2 \quad \text{(trace)} \quad (25)$$

$$a_{11} a_{22} - a_{12} a_{21} = \lambda_1 \lambda_2 \quad \text{(det)} \quad (26)$$

Substituting the results of the measurement of an element T_{ij} in equation 20 , the corresponding element a_{ij} of a is determined. It is evident that if the two elements of any row or column of T are known, so that the corresponding elements of a are also known, the remaining two unknown elements of a can be uniquely determined from equations 25 and 26 assuming, of course, that the eigenvalues can also be determined. For example, if T_{11} and T_{12} are known then a_{21} and a_{22} can be determined; if T_{11} and T_{21} are known then a_{12} and a_{22} can be determined. If only the set of diagonal elements, T_{11} and T_{22} can be determined, a complete solution for the matrix a is not obtainable since only the product of a_{12} and a_{21} can be specified in addition to a_{11} and a_{22}. Moreover, if the off-diagonal elements T_{12} and T_{21} are measured, the elements a_{12} and a_{21} are uniquely specified and two possible

* Walter et. al.(1976) also examined this case but did not take into account the additional information provided by the physical constraints 21-24 .

symmetric solutions for the pair a_{11} and a_{22} result from equations 25 and 26:

$$a_{ii} = \frac{(\lambda_1 + \lambda_2) \pm \sqrt{(\lambda_1 - \lambda_2)^2 - 4a_{12}a_{21}}}{2} \quad i=1,2$$

Thus, assuming that the discriminant is not zero, an ambiguity arises as to the assignment of the two values obtained for a_{11} and a_{22}. The first two sets of constraints, 21 and 22 are satisfied for both solutions. The third set, 23 , will only be satisfied for both solutions if the magnitude of each of the off-diagonal elements is less than or equal to the magnitude of each diagonal element. This condition is not necessarily satisfied unless both compartments have equal volumes* . Even if one assumes that the third set of constraints is satisfied, one can determine that the fourth set of constraints, 24 , can only be satisfied for both solutions if the volume ratio V_2/V_1 is within a certain limited range of the determined parameters:

$$-\frac{a_{12}}{a_{ss}} \leq \frac{V_2}{V_1} \leq -\frac{a_{ss}}{a_{21}}$$

where a_{ss} is the smaller in magnitude of the two determined diagonal elements.

* since then the third condition for one solution is equivalent to the fourth condition for the other solution

Example 1

a) Consider a two compartment system, represented by fig. 1, for which the measurement of the off-diagonal elements T_{12} and T_{21} of T gives the following values:

$$T_{12} = \frac{1}{4} e^{-1t} - \frac{1}{4} e^{-5t}$$

$$T_{21} = \frac{3}{4} e^{-1t} - \frac{3}{4} e^{-5t}$$

Employing equation 20 we obtain:

$$a_{12} = 1 , \quad a_{21} = 3$$

From equations 25 and 26 the following two symmetric solutions are obtained for the diagonal elements:

$$a_{11} = -2 , \quad a_{22} = -4$$

$$\text{and,} \quad a_{11} = -4, \quad a_{22} = -2 \quad .$$

Thus the two possible mathematical solutions are represented by the following two transfer rate matrices:

$$a_1 = \begin{pmatrix} -2 & 1 \\ 3 & -4 \end{pmatrix} \quad ; \quad a_2 = \begin{pmatrix} -4 & 1 \\ 3 & -2 \end{pmatrix}$$

However, only the first solution given by a_1 can represent a physical model since for the second solution given by a_2, the elements of the second row do not satisfy constraint 23 which requires that the sum of the elements in each row be less or equal to zero.

b) Let us assume now that the values obtained for T_{12} and T_{21} are:

$$T_{12} = \frac{2}{7} e^{-4t} - \frac{2}{7} e^{-11t}$$

$$T_{21} = \frac{3}{7} e^{-4t} - \frac{3}{7} e^{-11t}$$

Employing equations 20, 25 & 26, the following two solutions result for a:

$$a_1 = \begin{pmatrix} -5 & 2 \\ 3 & -10 \end{pmatrix} ; \qquad a_2 = \begin{pmatrix} -10 & 2 \\ 3 & -5 \end{pmatrix}$$

In this case both solutions satisfy the first three sets of constraints, 21 , 22 , 23 . However, in accordance with the fourth set of constraints, 24 , the volume ratio for solution a_1 must be in the region:

$$\frac{1}{5} \leq \frac{V_2}{V_1} \leq \frac{5}{3}$$

For solution a_2 the volume ratio must be in the region,

$$\frac{2}{5} \leq \frac{V_2}{V_1} \leq \frac{10}{3}$$

Thus the fourth set of constraints can only be satisfied for both solutions if the volume ratio is within the region,

$$\frac{2}{5} \leq \frac{V_2}{V_1} \leq \frac{5}{3}$$

2. Three Compartment System (fig. 2)

For a three compartment system each element T_{ij} permits a_{ij} and $\sum_{k=1}^{n} a_{ik} a_{kj}$ to be determined from equations 20 . In addition, the three invariants, trace, determinant, and the sum of the cofactors of the diagonal elements of a, can be expressed in terms of the eigenvalues λ_k. Of the eighty-four (i.e. $C_{9,3}$) physically different ways of selecting three elements of the transition matrix, the number of essentially distinct types of experiments is reduced to seventeen when the effects of simple relabeling of compartments are considered. From algebraic analysis it may be seen that seven cases can lead to complete solutions for a (fig.4) while there are ten cases leading to a partial solution only (fig. 5). This classification into complete and incomplete measurement sets can be concisely described for the general n compartment system by introducing a few simple concepts. These basic concepts, which are more formally described in section III, were suggested by the two and three compartment results described below.

Upon examination of figures 3 and 4 , it can be seen that the sets of complete injection-measurement functions fall into two classes corresponding, in a sense, to either a complete column or a complete row of the transition matrix employed by the two basic solutions. The column sets, e.g., $\{T_{11}, T_{21}, T_{31}\}$, reflect experiments in which one compartment is injected and all of the n compartments are measured.

TWO COMPARTMENT SYSTEM

(n=2)

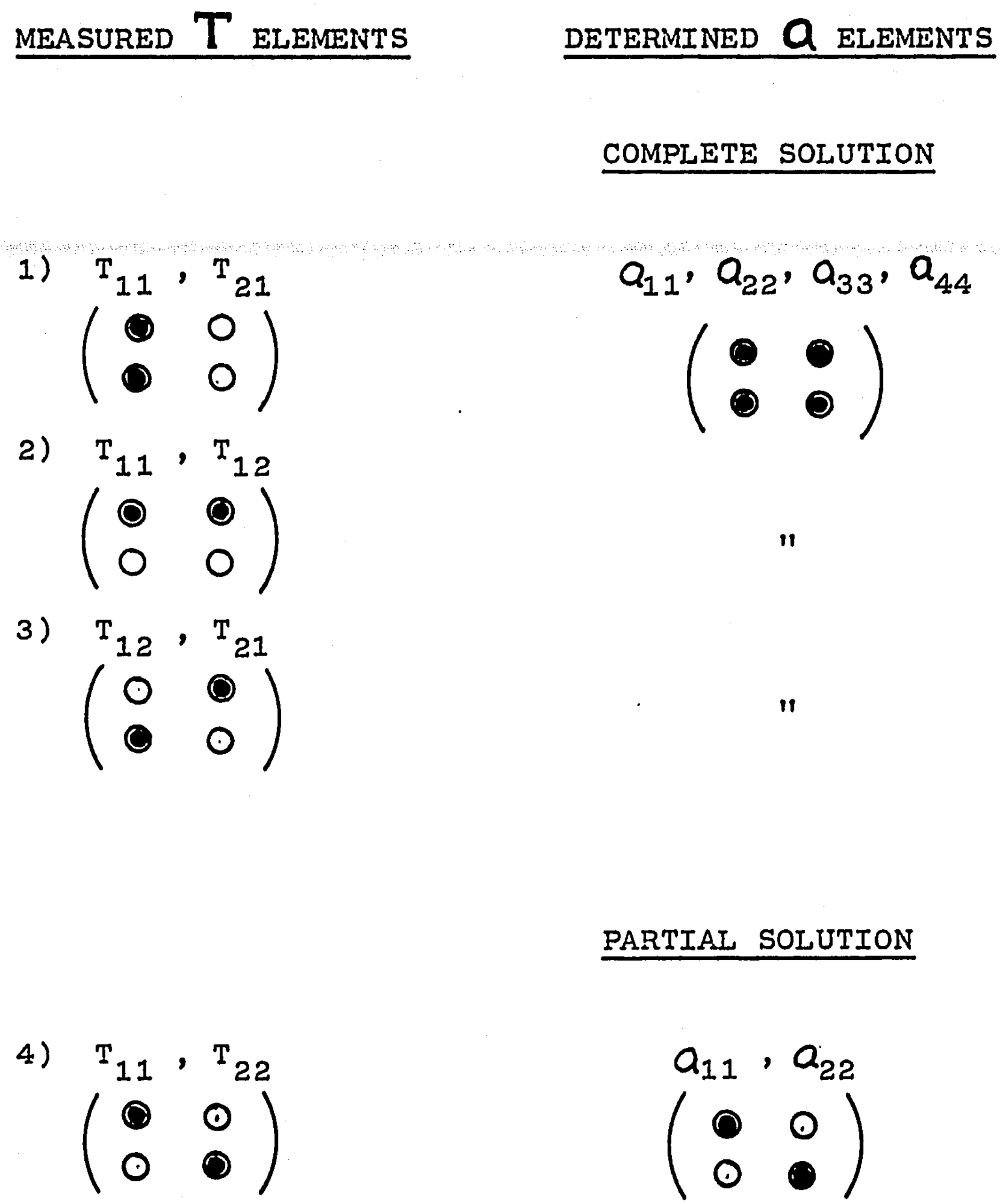


fig. 3

Complete and Partial Solution Sets for n=2

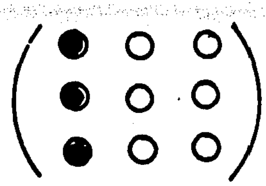
THREE COMPARTMENT SYSTEM

(n=3)

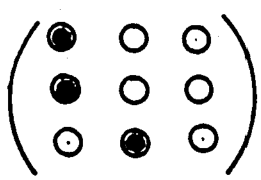
MEASURED T ELEMENTS ALLOWING COMPLETE DETERMINATION OF α

Column Type Experiments

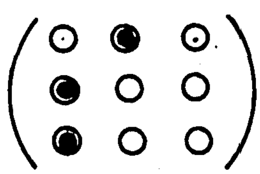
1) T_{11}, T_{21}, T_{31}



3) T_{11}, T_{21}, T_{32}

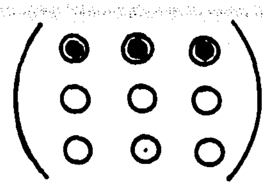


5) T_{12}, T_{21}, T_{31}

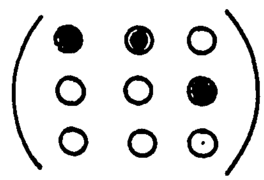


Row Type Experiments

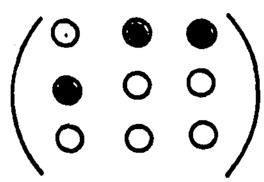
2) T_{11}, T_{12}, T_{13}



4) T_{11}, T_{12}, T_{23}



6) T_{21}, T_{12}, T_{13}



7) T_{13}, T_{32}, T_{21}

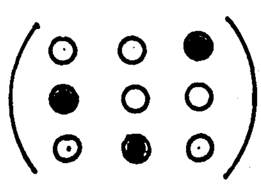


fig. 4

Complete Solution Sets for n=3

PARTIAL SOLUTION (n=3)

MEASURED T ELEMENTS | DETERMINED a ELEMENTS

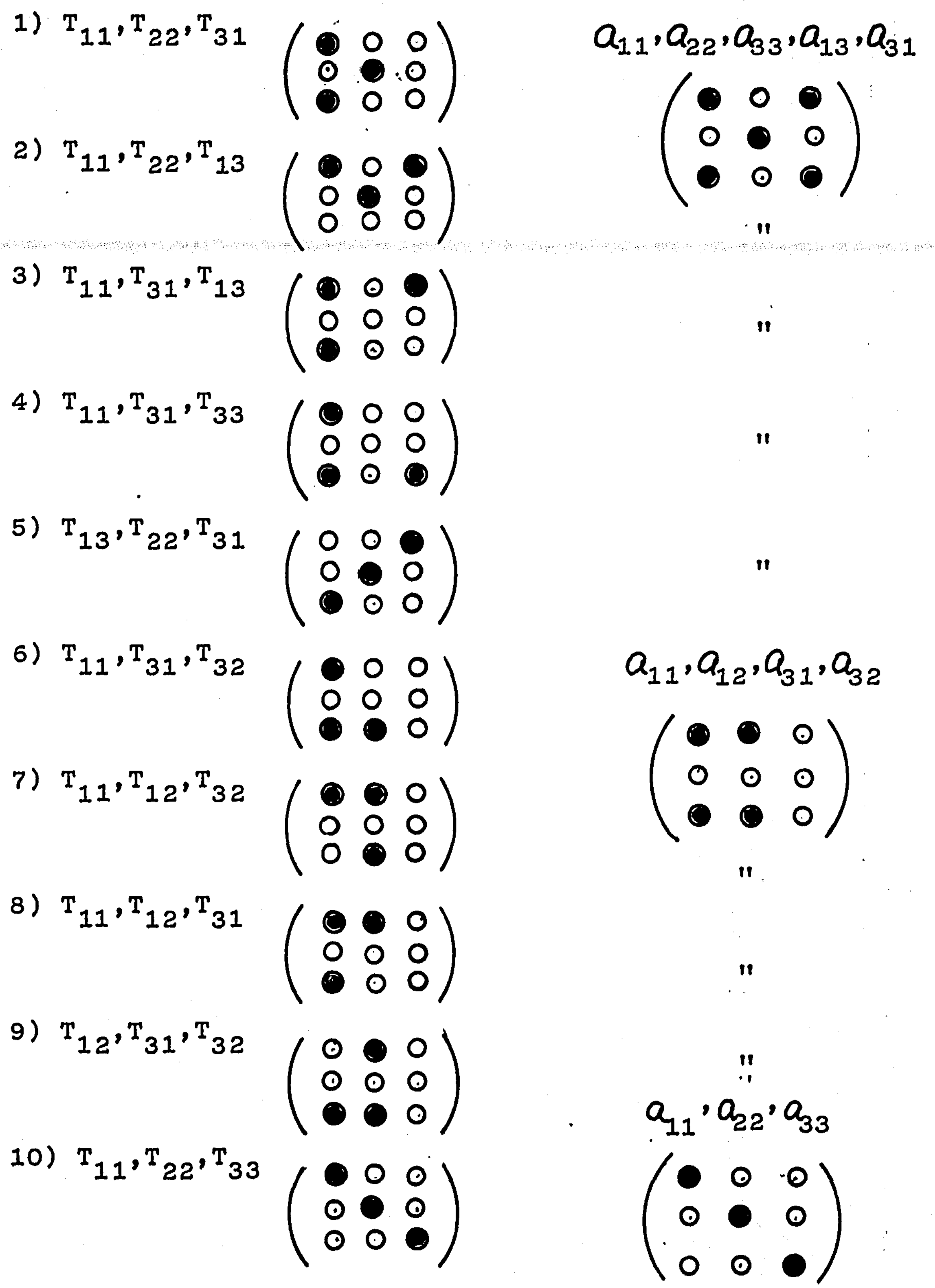


fig. 5
Partial Solution Sets for n=3

The injected compartment can be viewed as a "reference injection compartment" with respect to which all measurements are made. Generalizing this concept, a set of transition matrix elements such as $\{T_{11}, T_{21}, T_{32}\}$, may also be viewed as exhibiting a reference injection compartment. Clearly, T_{11} and T_{21} refer to the common reference injection compartment 1 directly. However, T_{32} may also be considered to refer to the injection compartment 1, albeit indirectly through compartment 2. The corresponding generalization applies for the common reference measurement compartment exhibited by row sets representing experiments where all n compartments are injected separately and the measured compartment is the reference compartment or refers to it. If a set exhibits a common measurement reference compartment, then the transposed set of elements having reversed subscripts exhibits a common injection reference compartment. Thus, the sets corresponding to a complete solution may be thaught of as having a common reference compartment, whereas, for example, the sets $\{T_{11}, T_{21}, T_{23}\}$ or $\{T_{11}, T_{21}, T_{33}\}$ which correspond to incomplete solutions, would not. For many sets of transition matrix elements more than one common reference compartment is operative (e.g. for n=3: cases 5, 6 and 7 of fig.4). In case 7, all three compartments can be viewed as either a common injection or common measurement reference compartment. Viewing compartment 1 as a common injection reference compartment, T_{21} indicates that compartment 2 is being measured directly with respect to compartment 1; T_{32} indicates that compartment 3 is being measured with respect to compartment 1 indirectly through 2; T_{13} indicates that 1 is being measured doubly indirectly with respect to 1, first through 3 and then through 2. Notice, however, that even though a common reference compartment existed in case 3 of figure 5 for those compartments participating in the injection-measurement process, a complete solution cannot be obtained. Such cases suggested an additional requirement for a complete solution, namely, that all n compartments must participate either as injection or measurement sites in the data acquisition process.

A direct extension of the above approach to a general 4-compartment system results in extremely unwieldy and tedious calculations*. It was decided, therefore, to numerically evaluate the Jacobian determinant (see section V) by computer calculation for several arbitrarily selected strongly-connected systems. Based on the observations made for the 2, 3 and 4 compartment systems, the following hypothesis was postulated for n-compartment systems for which none of the n^2 transfer rates are known a priori, assuming that n distinct eigenvalues from the data and that any needed volume information is obtainable:

* For this case there are 1820 ($C_{16,4}$) different experimental designs which can be grouped into 95 distinct classes of measurement topologies. Of these, only 17 can lead to complete solutions (see fig. 8).

<u>For a complete solution, all n compartments must be measured or all must be injected with respect to a common reference compartment.</u> *

The principal objective of the analysis presented in the following sections is to examine more formally to what extent this requirement constitutes a necessary and sufficient condition for a complete solution.

* The above reference compartment condition can be represented analytically as the requirement that either a complete column or row of elements of the following path matrix P are not zero:

$$P = \mathfrak{m}^1 + \mathfrak{m}^2 + \ldots + \mathfrak{m}^n$$

where $\mathfrak{m}$ is an nxn matrix whose elements $\mathfrak{m}_{ij}$ are equal to 1 whenever the corresponding transition matrix elements T_{ij} have been measured and are zero otherwise. A non-zero element P_{ij} indicates that compartment i has been measured w.r.t. compartment j either directly or indirectly. The above relationship between the matrix P and the measurement matrix $\mathfrak{m}$ is analogous to the relationship between the structural reachability and adjacency matrices R and A (Rescigno, 1964). Note, however, that a diagonal element of $\mathfrak{m}$ can be non-vanishing.

g. Eigenvector Approach

It is evident that the algebraic complexity of equations 20 and the invariant relationships become increasingly prohibitive with increasing values of n. An alternate, more convenient approach, directly involving the unknown coefficients A_{ik}^{j} rather than the unknowns α_{ij} as parameters in the equations, which allows for the determination of α via equations 5 or 14, is developed below.*

Consider two non-zero vectors $\underline{A}_k^j$ and $\underline{A}_k^{j'}$, as defined by equation 3, corresponding to unit initial concentrations in two different compartments j and j'. Since $\underline{A}_k^j$ and $\underline{A}_k^{j'}$ are eigenvectors of α having the same eigenvalue λ_k, they must be linearly related, assuming, of course, that α has n distinct eigenvalues. Let the constant non-zero factor relating the two vectors be $R_k^{j'j}$. Then,

$$\underline{A}_k^j = R_k^{j'j} \underline{A}_k^{j'} , \quad R_k^{j'j} \neq 0 \tag{27a}$$

and, therefore, the corresponding vector components A_{ik}^j and $A_{ik}^{j'}$ are also related by the same constant factor:

$$A_{ik}^j = R_k^{j'j} A_{ik}^{j'} , \quad i=1,2,\ldots,n \tag{27b}$$

Similarly, any two non-trivial vectors $\underline{A}_{ik}$ and $\underline{A}_{i'k}$, as defined by equation 16, must be linearly related by a

* This method of analysis may be shown to be related to the "General Identifiability Procedure" proposed by Walter, Le Cardinal and Bertrand (1976).

constant $R_{i'ik}$ since both are eigenvectors of α^T having the same eigenvalue λ_k. Thus,

$$\underline{Ai}_k = R_{i'ik}\,\underline{Ai'}_k \;, \quad R_{i'ik} \neq 0 \qquad (28a)$$

and,

$$A^j_{ik} = R_{i'ik}\,A^j_{i'k} \;, \quad j=1,2,\ldots,n \qquad (28b)$$

If equations 27 are true then since the factors $R^{j'j}_k$ are independent of i, the coefficients A^j_{ik} that satisfy these equations must also satisfy the following non-trivial product relationships. Equivalently, these relationships are also valid for the coefficients A^j_{ik} that satisfy equations 28 since the constants $R_{i'ik}$ are independent of j.

$$A^j_{ik}\,A^{j'}_{i'k} = A^{j'}_{ik}\,A^j_{i'k} \;, \quad \begin{matrix} j\neq j' \\ i\neq i' \end{matrix} \qquad (29a)$$

In the case that the coefficients A^j_{ik} are all different from zero, equations 29a can always be written as ratio relationships as in 29a' or 29a".

We will generally refer to equations 27 and 28 as the eigenvector relations where we will say that equations 27 belong to the column set of eigenvector relations and equations 28 belong to the row set of eigenvector relations since they refer to columns and rows of T respectively.

Since we have a linear system, these equations would also apply for non-unit initial concentrations in the injected compartments. In this case, each value A_{ik}^{j} would equal the value corresponding to unit initial concentration in j times the magnitude of the initial concentration in j, as previously indicated by equation 11.

In solving a system using the eigenvector approach, either eqs. 27 or 28 and the n^2 initial requirements of the transition matrix (eq. 10) are employed to determine the unknown coefficients A_{ik}^{j}. The transfer rate matrix a can then be immediately obtained from (5) or (14). In the case that a discrete set of multiple solutions is obtained, the physical constraints 21-24 may be considered, as previously described for the direct approach.

In a more general sense, we may extend the applicability of such a set of equations to include experiments for which weighted measurement functions may be obtained, with or without simultaneous initial input into a multiple set of injection sites. This generalization is treated in Appendix D.

1. Strongly Connected Systems

For a strongly connected system, a flow path must exist from each compartment to all other compartments. Thus, no element T_{ij} of the transition matrix can be zero. Usually*, each element T_{ij} will exhibit n distinct components so that none of the n^3 coefficients A^j_{ik} will be zero.

$$T_{ij} = \sum_{k=1}^{n} A^j_{ik} e^{\lambda_k t} , \qquad i,j=1,2,\ldots,n$$

The condition, $A^j_{ik} \neq 0$ for all i,j,k implies that the eigenvector relations 27 and 28 will be valid for any set of vectors $\underline{A}^j_k$ or $\underline{A}i_k$, respectively. One can then express equations 29 as coefficient ratio relationships:

$$\frac{A^j_{ik}}{A^{j'}_{ik}} = \frac{A^j_{i'k}}{A^{j'}_{i'k}} = R^{j'j}_k , \qquad \begin{matrix} i,j,k=1,2,\ldots,n \\ j\neq j' \\ i\neq i' \end{matrix} \qquad (29a')$$

or, alternatively, rewriting the same equations:

$$\frac{A^j_{ik}}{A^j_{i'k}} = \frac{A^{j'}_{ik}}{A^{j'}_{i'k}} = R_{i'ik} , \qquad \begin{matrix} i,j,k=1,2,\ldots,n \\ j\neq j' \\ i\neq i' \end{matrix} \qquad (29a'')$$

For a fixed compartment $j'=j_o$, the factors $R^{j'j}_k$ are independent of i and, similarly, for a fixed $i'=i_o$, the factors $R_{i'ik}$ are independent of j.

* As shown in section VI (see also Hart(1955,1967)), exceptional cases exist where some of the coefficients A^j_{ik} of a strongly connected system are zero because of a particular combination of the system parameter values and not because of the connectivity of the system model. Cobelli and Romanin-Jacur (1975,1976) indicate whenever they are excluding such special cases from consideration by using the qualifying terminalogy "in a structural sense only".

Further, by equations C-7 and C-13 it can be seen that either the n^2-n factors $R_k^{j'j}$, j,k=1,2,...,n; $j \neq j'$ or the n^2-n factors $R_{i'ik}$, i,k=1,2,...,n, $i \neq i'$, in conjunction with the n eigenvalues λ_k can serve to uniquely define the system*. Therefore, either set of factors must represent a set of n^2-n independent parameters for the system.

Varying i,j and k over all possible values for which $i \neq i_o$ and $j \neq j_o$, equations 29a represent $n(n-1)^2$ independent** relations involving the n^3 coefficients A_{ik}^j. An additional n^2 relations are provided by equation 10, noting that the values A_{ik}^j here represent coefficients of the transition matrix elements:

$$\sum_{k=1}^{n} A_{ik}^{j} = \delta_{ij} \quad , \qquad i,j=1,2,\ldots,n \qquad (29b)$$

Totally, the system of equations 29 consists of n^3-n^2+n independent relations involving the n^3 coefficients A_{ik}^j. Thus, as expected, at most n^2-n coefficients can be independent. Note that in the absence of any additional

* The relationship between the parameters $R_k^{j'j}$ and the parameters $R_{i'ik}$ for strongly connected systems is derived in the appendix C.

**It is evident that these equations in the n^3 coefficients A_{ik}^j are formally independent if all coefficients are viewed initially as unknown parameters since no equation can result from any set of others.

information about the system, such as a priori knowledge of the transfer rates or of the exterior entry and exit rates (which would eliminate some of the inequalities in eqs. 21-24), there can be no additional independent relations involving the coefficients A^j_{ik} , since this would imply that the system can be determined by specifying less than n^2 independent parameters (including the n eigenvalue parameters).

The set of n^2-n independent coefficient parameters are not a priori necessarily limited to any specific rows or columns. (Note, an equivalent argument to the above holds if selection of the coefficients A^j_{ik} is initially restricted to any subset of m rows or columns of T. In this case the number of A^j_{ik} coefficients is reduced to n^2m and the number of independent equations involving them would be reduced to $n^2m-n^2 + n$.) In order for a solution to be formally possible, the values of such a set of n^2-n independent A^j_{ik} parameters must be either known or obtained by measurement.

Although n-1 elements of the transition matrix contain n^2-n parameters A^j_{ik}, it follows from equations 29b that not all of the A^j_{ik} parameters are independent and, therefore, a complete solution cannot be obtained. Thus, n is the minimal number of transition matrix elements that must be measured and even these n elements may be insufficient for a complete solution if the A^j_{ik} parameters are further related by equations 29a . Since n of the equations 29b will always involve the measured coefficients exclusively, the remaining equations 29a and 29b will formally constitute a system of at most n^3-n^2 equations in the remaining n^3-n^2 undetermined unknowns. However, if the experimentally determined coefficients are further related, the remaining equations will either not be sufficient or independent for the remaining unknowns. An obvious case where the equations are not sufficient is if some of the equations 29a involve measured coefficients exclusively.* As such, they will, of course, not be useful for determining any of the remaining unknowns. Thus, for example, if n=4 the measurement of all four corners of a "rectangle of elements" $(T_{11},T_{12},T_{21},T_{22})$ or in general $(T_{i_1j_1},T_{i_1j_2},T_{i_2j_1},T_{i_2j_2})$ is immediately precluded as a complete measurement set, and if $n>4$ it cannot be part of a minimally complete measurement set.

In the following sections we will further examine the implications w.r.t. determining a complete solution when a random set of elements of T are known. It will be noted that it is not always necessary to include all of the equations 29 to determine a solution for the transfer rate matrix $\mathcal{a}$. Only the subsystem of n^2m-n^2+n involving, exclusively, the $m\leq n$ columns or rows of T containing measured elements need be considered. As will be shown in section IV, the problem is further reduced essentially to that of solving a subset of $nm-n$, generally, non-linear equations.

* See also Walter et. al. (1976).

2. Systems With Limited Flow Connectivity

For systems with limited flow connectivity, some of the transfer rates are, of course, zero. However, since it is assumed here that the values of all n^2 transfer rates are not known prior to the analysis, only general assumptions with respect to the system connectivity can be made.

Column Type Experiments

For column type experiments, the n measured elements are selected from a set of columns of T corresponding to the set of injected compartments. If a flow path, either direct or indirect, does not exist from an injection compartment j to a measurement compartment i, then the corresponding element T_{ij} of the transition matrix and the corresponding coefficients A^j_{ik} k=1,2,...,n will, of course, all be zero. Thus, the matrix A^j will be singular, having a row i of zero elements and, therefore, eq. 5 cannot be applied. It is also implied that one of the columns of A^j will be zero, otherwise, the column vectors $\underline{A}^j_k$ would all be eigenvectors of α and as such A^j would not be singular. These observations may be expected if one considers that under such an initial stimulus, the system cannot exhibit completely the transport behavior with respect to at least one of its compartments. Thus, the measurement functions will only provide evidence for the existence of at most n-1 compartments. However, if one can limit the choice of injection compartments to those with either direct or indirect flow to all compartments*, then eq. 5, as a rule, is still valid for the selected values of j and, therefore, a column type experiment can still be performed. The vectors $\underline{A}_k^j$ for these values of j may be eigenvectors of $\mathcal{A}$ even though some of the coefficients A_{ik}^j are zero. The presence of zero coefficients A_{ik}^j, indicates in a structural sense** that the measured compartments i do not have influx from all compartments and is, therefore, expected for non-strongly connected systems. Although, of course, the parameters $R_k^{jj'}$ relating two eigenvectors must be different from zero, some of the ratios $A_{ik}^j/A_{ik}^{j'}$ will be undefined (zero over zero). Therefore, one may wish to avoid equations 29a altogether, utilizing equations 28 directly as in the procedure followed in section IV for strongly connected systems with a limited number of injection compartments. Here, one considers the factors $R_k^{jj'}$ relating the injected compartments as the unknown parameters of immediate interest. (Note, in this case, in contrast to the factors $R_k^{jj'}$, some of the factors $R_{ii'k}$ can in fact be zero or undefined, since the column vectors of A_i and $A_{i'}$ are not necessarily eigenvectors of $\mathcal{A}^T$ as in the case of the row

* This implies that the system must be completely controllable in a structural sense (Cobelli and Romanin-Jacur, 1976) for each separate input.

**see footnote page 40.

type experiments discussed below.)

Row Type Experiments

For row type experiments, the measured elements are selected from a set of rows i of T corresponding to the set of measured compartments. Each selected measurement compartment i must have influx from all compartments*, for the matrix A_i in eq. 14 to be non-singular. The column vectors of A_i are then eigenvectors of $\mathcal{a}^T$. For hybrid cases, if the selected measurement compartments i are thus limited, so that $|A_i| \neq 0$ for each i, the corresponding column vectors $\underline{A}i_k$ and $\underline{A}i_{k'}$ of any two matrices A_i and $A_{i'}$ will be related by non-zero parameters $R_{ii'k}$. Equations 28a can, therefore, be utilized in seeking a solution, where the determination of the non-zero parameters $R_{ii'k}$ is now of immediate interest. (Note, in this case, in contrast to the factors $R_{ii'k}$, some of the factors $R_k^{jj'}$ may be zero or undefined since the vectors $\underline{A}_k^j$ and $\underline{A}_k^{j'}$ are not necessarily eigenvectors of $\mathcal{a}$ here.)

In general, utilizing the eigenvector approach for obtaining a solution, the transfer rate parameters are determined once an alternate set of independent parameters is obtained. Knowledge of the latter set of parameters

* This requirement corresponds to the condition that the system be completely observable in a structural sense for each i .

(which may be the coefficients A^{j}_{ik}, or related to them, in conjuction with the n eigenvalue parameters) implies that the former set can be uniquely specified and vice-versa. Therefore, for systems for which both sets of parameters can be defined, any necessary and sufficient conditions derived for the determinability of one set of parameters must also be necessary and sufficient for the determinability of the other set as well. Furthermore, assuming the n eigenvalue parameters can be easily obtained, for the remaining parameters we may limit our consideration to the eigenvector relations and to the initial conditions, since, as previously noted, there can be no other independent relation involving these parameters unless prior knowledge of the system is available. These principles will be basically assumed in the following sections.

SECTION III

SET THEORETIC REPRESENTATION - DIGRAPH ANALOG

Thus far, measurement concepts have been referred to in terms whose meaning was more-or-less apparent. Here, it will be useful to define more precisely and further extend some of these concepts.

Expressed in terms of set theory, the correspondence between injection-measurement relationships among a set of compartments, and linear graph related concepts becomes evident (For a review of graph theory see Bertziss(1971)). Thus, a set of compartments C can be represented by the nodes of a linear graph, here, represented by circles. If M is a set of injection-measurement functions, as described in I and II, or simply "measurement functions", involving the elements of C, then each element $T_{ij} \in M$ can correspond to an arc(j,i) directed from j to i (fig. 6). Thus, the "measurement-graph" corresponding to the pair (C,M) is a digraph (directed graph)*. Of course, it is well known (Rescigno, 1966) that the underlying transport flow can also be represented by a digraph network. For example, figures 1 & 2 may be considered transport flow digraphs of the general two and three compartment model, where external (compartment 0) input and output are also depicted.

The pictorial visualization offered by the digraph analog is helpful in understanding the measurement theory

* To our knowledge digraphs, at least for compartment systems, have not been previously used to represent the topology of measurement sets.

concepts presented in the next sections. In the following definitions, the digraph related terminology, referring always to the measurement topology, is presented in parentheses.

DEF. We will say that the element $T_{ij}(t)$ of the transition matrix is measured or is a measurement function if and only if its value is obtained as a function of time. Then j is an injected compartment and i is a measured compartment (initial node-terminal node). If i=j, then i is diagonally measured (arc(j,i) is a loop); otherwise, it is off-diagonally measured.(fig. 6).

DEF. Compartment i is measured w.r.t. compartment j and, equivalently, compartment j is injected w.r.t. compartment i (cf. there exists a path from j to i) if and only if there exists a set of $q \geq 1$ measurement functions M(q) that can be placed in a sequence of the form

$$T_{i_p j_p}\ , \quad p=1,2,\ldots,q \qquad \text{where, } i_1 = i\ ,\ j_q = j\ ,\ i_{p+1} = j_p\ ,\ p < q$$

If q=1, then i is measured directly w.r.t. j (fig. 6); otherwise, it is indirectly measured w.r.t. j (fig. 7). Equivalently, j is directly or indirectly injected w.r.t. i.

Clearly, the latter property is transitive. Thus, if i is measured w.r.t. j and j is measured w.r.t. k, then i is measured w.r.t. k .

DEF. In the previous definition if $i=j$, then i is cyclically measured and injected and the set $M(q)$ is a cycle (the path from j to i is a cycle),(fig. 7b).

Clearly, every compartment i_p, $p=1,2,\ldots,q$ in the cycle is measured w.r.t. all compartments in the cycle.and hence is also cyclically measured. Equivalently, every compartment in the cycle is cyclically injected.

DEF. A set M is cyclic if it contains at least one measurement cycle (fig. 8), otherwise, it is acyclic.

Consider the set of compartments C associated with a set of measurement functions M. It is convenient to consider C as the union of two sets I and J, where I is the set of all measured compartments and J is the set of all injected compartments. Generally $I \neq J$, unless M is a cycle or a union of cycles*.

DEF. A set M that contains the same number of elements T_{ij} as the number of measured compartments ($|M| = |I|$) has a common injection reference compartment $j \in J$ if and only if every compartment $i \in I$ is measured w.r.t. j. A set M for which $|M| = |J|$ has a common measurement reference compartment $i \in I$ if and only if i is measured w.r.t. every $j \in J$. Equivalently, every $j \in J$ is injected w.r.t. i . (see also footnote on page 36)

* Of course a union of cycles may involve more measurements than the number of compartments.

DEF. A set M is complete for a multicompartment system if it contains, in principle, enough information to determine all transfer rate parameters of that system*; otherwise, it is incomplete. It is minimally complete if and only if it is complete and all proper subsets are incomplete.

* Not considering experimental errors and including cases where a discrete set of physically compatible solutions can be obtained. Note, that while in general we will be considering systems for which no prior knowledge of the values of the transfer rates is assumed, the above definition could be applied to the case where a priori knowledge is in fact available.

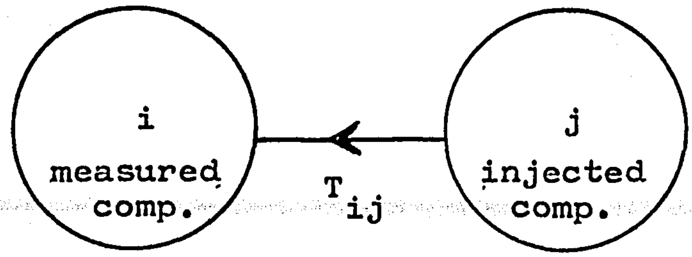


Figure 6a . Digraph representation of the off-diagonal measurement function T_{ij} .

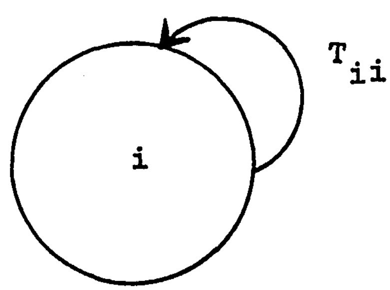


Figure 6b . Digraph representation of a diagonally measured compartment .

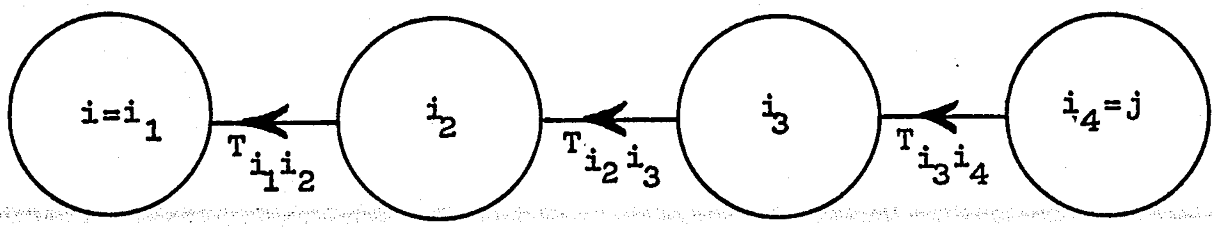


Figure 7a . Indirect measurement of i w.r.t. j

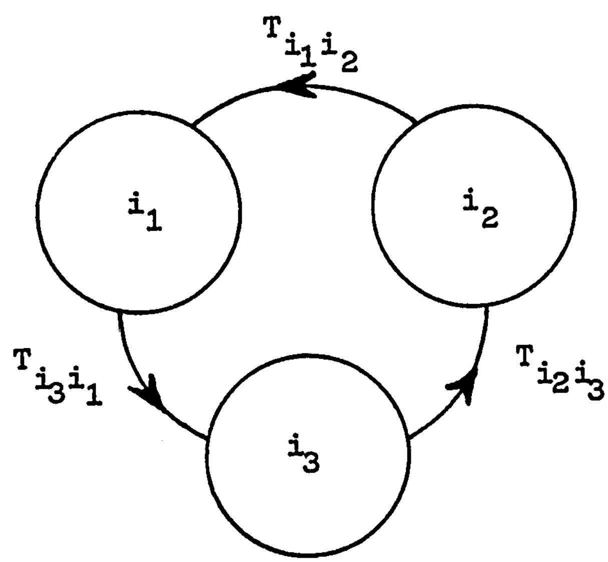


Figure 7b . Example of a measurement cycle

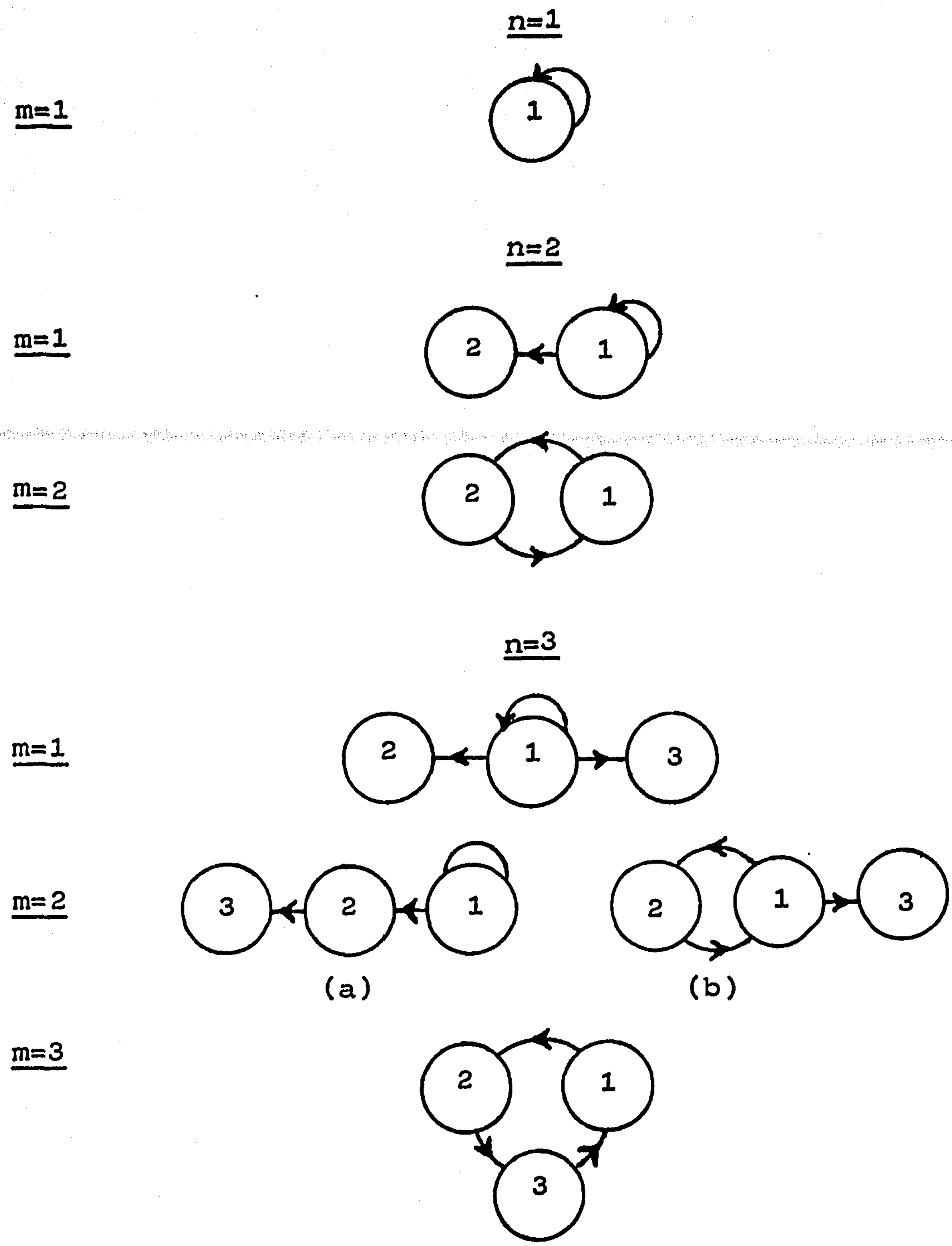
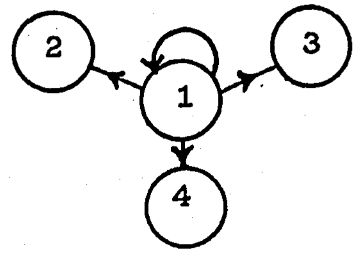


Figure 8 . Distinct types of experiments for n measurement functions exhibiting a common injection reference compartment (comp. 1), assuming all compartments are measured. The number of injected compartments is given by m.

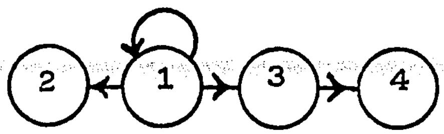
If the arrows are reversed, then compartment 1 becomes a common measurement reference compartment, where all n compartments are injected.

n=4

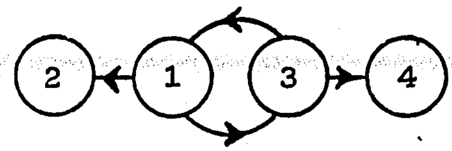
m=1



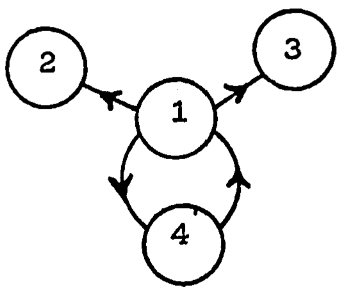
m=2



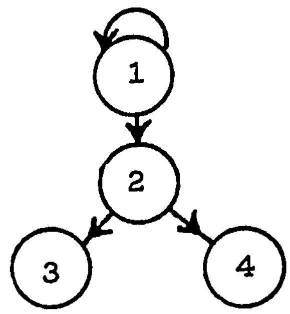
(a)



(b)

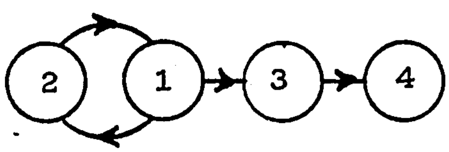


(c)

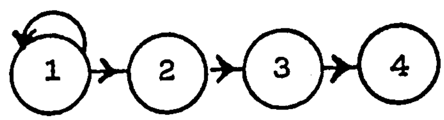


(d)

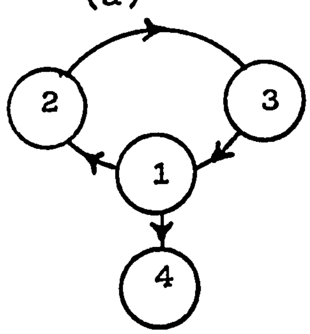
m=3



(a)

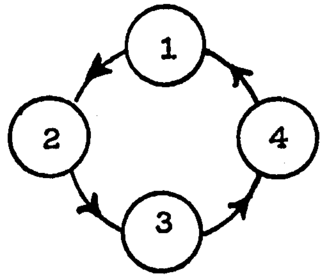


(b)



(c)

m=4



SECTION IV

NECESSARY CONDITION FOR COMPLETENESS

THEOREM T1: The existence of a common reference compartment for a set M of n measurement functions, randomly selected from the n^2 elements of the transition matrix T, is a necessary condition for completeness for strongly connected n-compartment systems for which all* $A^j_{ik} \neq 0$, assuming that none of the transfer rates are known a priori.

It will be useful for the subsequent proof of T1 to establish the following lemmas. Note, that lemma L1 describes an additional necessary condition for completeness.

LEMMA L1: A set of measurement functions M containing any number of elements of T is incomplete for the n-compartment system described in theorem T1, if both the set of measured compartments I and the set of injected compartments J contain less than n compartments.

This implies that at least one element (for $|M| = n$, exactly one) must be measured in each row or in each column of T, i.e., all compartments must be measured or all must be injected.

Proof of L1: Since a total of n^2 independent parameters are needed to specify the system and since n independent eigenvalue parameters are assumed to be obtainable from the set of measured elements M, an additional n^2-n independent

* Thus, the theorem refers to the general class of strongly connected systems in a structural sense only (see footnote on page 40).

parameters must be determined from M. Assume that the measured elements can be selected from any n-a rows and any n-b columns of T. The total number of elements of T that can belong to both one of these rows and one of these columns is (n-a)(n-b). Hence, the total number of coefficient parameters A^j_{ik} corresponding to these elements is n(n-a)(n-b). However, the number of independent equations 29a and 29b linking these parameters alone is, upon reflection, n(n-a-1)(n-b-1) + (n-a)(n-b). Since the number of parameters is greater than the total number of equations by n^2-n-ab, the maximum number of independent parameters present here cannot be greater than n^2-n-ab (i.e., n^2-n-ab are the available degrees of freedom in the absence of any measurement of A^j_{ik} at all if the measurements are to be selected from n-a rows and n-b columns of T). Thus, n^2-n independent parameters cannot be determined from these elements even if all such elements are measured, unless either a or b or both are equal to zero. Hence, a necessary requirement for complete determination of T is that all compartments be measured ($|I| = n$) or all must be injected ($|J| = n$).

COROLLARY: A complete set M that contains n elements is is minimally complete.

This is evident from L1. Since a set M is incomplete if both $|I|$ and $|J| \angle n$, at least n measurement functions are required for a complete set.

LEMMA L2: Consider a finite set of q measurement functions M for which $|I| = |M| = q$ and $J \subseteq I$. If there exists an acyclic subset $M^- \subset M$ that contains one less element than M, then M must have at least one common injection reference compartment a.

Proof*of L2: Let $M^- \equiv M - \{T_{ab}\}$ be an acyclic subset of M. Then $I^- = I - \{a\}$. Let $T_{i_1 i_2}$ be an element of M^-. Then since M^- is acyclic, $i_2 \neq i_1$. Further, since $J \subseteq I$, i_2 is either equal to a or belongs to I^-. If i_2 equals a, then i_1 must be measured w.r.t. a; if $i_2 \neq a$ ($i_2 \in I^-$), then there exists an element $T_{i_2 i_3} \in M^-$. In this case, consider the sequence

$$T_{i_1 i_2}, T_{i_2 i_3} .$$

Since M^- is acyclic, $i_3 \neq i_1$ (thus, both i_2 and $i_3 \neq i_1$). If i_3 equals a, then both i_1 and i_2 are measured w.r.t. a; otherwise, if $i_3 \neq a$, then there exists an element $T_{i_3 i_4} \in M^-$. Thus, one can define a finite sequence of elements of M^-,

$$T_{i_1 i_2}, T_{i_2 i_3}, \ldots, T_{i_p i_{p+1}}, \quad 1 \leq p \leq q-1$$

where, $i_{p+1} \neq i_1, i_2, \ldots, i_p$ and $i_1, i_2, \ldots, i_p \neq a$. Since M^- is finite the maximum number of elements of M^- that can belong to such a sequence is $p_m \leq q-1$.

* Alternatively, in graph theoretic terms, one can show that the node base of the digraph corresponding to (C,M) contains exactly one node that lies on a cycle.

If $p_m < q-1$, then it is implied that $i_{p_m+1} = a$. If $p_m = q-1$, then again $i_{p_m+1} = a$, since $i_{p_m+1} \neq i_1, i_2, \ldots, i_{q-1}$ and, therefore, $i_{p_m+1} \notin I^-$. Since $i_{p_m+1} = a$, in either case i_1 is measured w.r.t. a, and since the choice of the element $T_{i_1 i_2} \in M^-$ was arbitrary, every $i \in I^-$ must be measured w.r.t. a. Furthermore, since in the set M, a is measured w.r.t. b and since b can either equal a or belong to I^-, a must be cyclicaly measured either directly or indirectly with respect to itself. Thus, a is a common injection reference compartment for M as well as for M^-. If $b \neq a$, then the cycle in which a is measured involves more than one compartment each of which, including b, is also a common injection reference compartment for M since any compartment measured w.r.t. a is necessarily measured w.r.t. any other compartment in the cycle. (For example, notice in fig. 8 that any compartment that lies on the same cycle as compartment 1 is also a common injection reference compartment.)

LEMMA L3: Consider a finite set of q measurement functions for which $|J| = |M| = q$ and $I \subseteq J$. If there exists an acyclic subset $M^- \subset M$ that contains one less element than M, then M must have at least one common measurement reference compartment.

The proof of L3 is analogous to that of L2.

<u>Proof of T1:</u> Consider a strongly connected n-compartment system for which all coefficients $A_{ik}^{j} \neq 0$. By lemma L1 we have already shown that a random set of n measurement functions M(n) is incomplete if both the set of measured compartments I and the set of injected compartments J contain less than n elements. To further examine the requirements for completeness, it will first be assumed that I contains n elements and that J contains $m \leq n$ elements and seek conditions which permit equations 29a and 29b to be solved.

For the case $|I| = n$, $|J| = m \leq n$ exactly one element T_{ij_i} is measured in each row i, where $j_i \in J$ specifies the column containing the measured element in row i**. If we let $j' = j_i$ in equations 27b , it follows that all of the unknowns A_{ik}^{j}, $j \neq j_i$ can be expressed as products of the as yet unknown parameters $R_k^{j_i j}$ and the known values determined from measurement $A_{ik}^{j_i}$ (enclosed for clarity in brackets below). Thus,

$$A_{ik}^{j} = R_k^{j_i j} \left[A_{ik}^{j_i} \right] \qquad (31)$$

* As previously noted (see Section II), the condition $A_{ik}^{j} \neq 0$ for all i,k,j implies that the eigenvector relations are valid for any set of vectors $\underline{A}_k^j$ or $\underline{A}i_k$.

** Physically, of course, j_i is the compartment injected prior to measurement of compartment i.

Substituting equations 31 into equations 29b, we obtain a system of n^2-n non-trivial eqs. in the parameters* $R_k^{j_i j}$:

$$\sum_{k=1}^{n} \left[A_{ik}^{j_i}\right] R_k^{j_i j} = \delta_{ij}, \qquad i,j=1,2,\ldots,n \quad j\neq j_i \tag{32}$$

Although these n^2-n equations are linear in the $R_k^{j_i j}$, the number of distinct $R_k^{j_i j}$ parameters is $m(n^2-n)$ since there are m injected compartments j_i. Therefore, this system by itself is inadequate for determining the unknowns except in the trivial case where m=1. An additional set of $(m-1)(n^2-n)$ non-trivial equations are provided from equations 27 or 29a' by relating the parameters $R_k^{j_i j}$ for all j_i to the parameters $R_k^{j_o j}$ for a fixed $j_o \in J$:

$$R_k^{j_i j} = R_k^{j_o j} / R_k^{j_o j_i} \qquad j_i \in J \quad j,k=1,2,\ldots,n \quad j \neq j_i \tag{33}$$

Substituting in 32 one obtains the nonlinear system

$$\sum_{k=1}^{n} \left[A_{ik}^{j_i}\right] \frac{R_k^{j_o j}}{R_k^{j_o j_i}} = \delta_{ij}, \qquad i,j=1,2,\ldots,n \quad j\neq j_i \tag{34}$$

for the n^2-n unknowns $R_k^{j_o j}$, $j,k=1,2,\ldots,n; j\neq j_o$. If one restricts j to the m columns containing measured elements, $j \in J$, the equations 34 of interest are reduced to the

* Here, the parameters $R_k^{j_i j}$ are, of course, non-zero constants since they relate non-trivial vectors.

subset of n(m-1) equations in the n(m-1) unknowns $R_k^{j_o j}$, where $j, j_o \in J$ & $j \neq j_o$. If this subsystem can be solved, the unknowns A_{ik}^{j}, corresponding to any single $j \in J$, can be immediately obtained from equations 31 and 33 , which essentially would complete the solution since α is then given by equation 5 . Thus, the remaining equations of 34, for $j \notin J$, are not essential although they simply represent a linear system for the remaining unknown parameters once the $R_k^{j_o j_i}$ are determined.

In seeking necessary conditions for solution of the system of equations 34 , where we will continue to consider only those equations for which $j \in J$, it is convenient to first divide each equation by the parameter ratio $R_{k_o}^{j_o j} / R_{k_o}^{j_o j_i}$, where k_o can be arbitrarily selected since the parameter ratios are by assumption all not equal to zero. The resulting equivalent set of equations is

$$\left[A_{ik_o}^{j_i}\right] + \sum_{\substack{k=1 \\ k \neq k_o}}^{n} \left[A_{ik}^{j_i}\right] \frac{X_k^j}{X_k^{j_i}} = \frac{X_{k_o}^{j_i}}{X_{k_o}^{j}} \delta_{ij} \qquad \begin{array}{l} i=1,2,\ldots,n \\ j \in J \\ j \neq j_i \end{array} \qquad (35)$$

where,*

$$X_k^j \equiv \begin{cases} R_{k_o}^{j_o j}, & k=k_o \\ R_k^{j_o j}/R_{k_o}^{j_o j}, & k \neq k_o \end{cases} \qquad \begin{array}{l} j \in J \\ k=1,2,\ldots,n \end{array}$$

* Note, the dependence of the X_k^j parameters on the indices k_o, j_o is not expressed since these indices are assumed to be fixed.

It is evident that the n·m parameters X_k^j define an alternate set of n(m-1) non-vanishing independent unknowns for values of $j \neq j_o$ and are equal to one for $j = j_o$. Of these, only the parameters for which $k \neq k_o$ appear on the left hand side of equations 35. The remaining m parameters $X_{k_o}^j$ appear on the right hand side of the equations in ratios, $X_{k_o}^{ji}/X_{k_o}^j$. However, since these ratios are modified by kronecker deltas, the total number of parameters in fact present on the right side depends on the values of the deltas, varying from zero if all deltas are zero, to at most m. Furthermore, even if all m ratios appear on the right hand side of equations 35, the maximum number of independent such ratios is m-1 since they involve at most m-1 independent parameters $X_{k_o}^j, j \neq j_o$ (note again that $X_{k_o}^{j_o}$ is just the constant one). If less than m-1 of the deltas are non-vanishing, less than m-1 modifying ratios appear in equations 35 and all of the unknowns cannot be evaluated (even if equations 35 could be solved for the ratios that do appear). Therefore, if a solution is to be feasible, there must exist a set of m-1 non-vanishing deltas ($\delta_{ij}=1$), modified by independent ratios $X_{k_o}^{ji}/X_{k_o}^i$ on the right hand side of equations 35.

The implications of the above observation in limiting the choice of measured elements can now be considered. Let $M(m) \subseteq M(n)$ be the set of m measured elements in the subset of m rows $I(m) \subseteq I$ for which $I(m)=J$. Therefore, each measured compartment in I(m) is included in the class of injected compartments. Notice that the condition $\delta_{ij}=1$, $j \neq j_i$ implies that the measured element in row i, T_{ij_i}, must belong to M(m) since $i=j \in J$ and must be off-diagonal since $i \neq j_i$. Furthermore, since there must be at least m-1 non-vanishing deltas involving, of course, different values of i, at least m-1 of the measured elements M(m) must be off-diagonal. Physically, this implies that not more than one of the injected compartments can be measured w.r.t. itself. Notice, the remaining measured elements $T_{ij_i} \notin M(m)$ are necessarily off-diagonal since $i \notin J$. Thus, at most one of the n measured elements $T_{ij_i} \in M(n)$ can be diagonal. Moreover, the choice of measured elements is further limited as a result of the requirement that m-1 of the ratios $X_{k_o}^{j_i}/X_{k_o}^{i}$, each associated with a measured off-diagonal element $T_{ij_i} \in M(m)$ must be independent. It is evident that a set of such ratios cannot be independent if the associated set of m-1 measurement functions is cyclic*.

* If the associated set of m-1 measurement functions M(m-1) is cyclic, then it must contain at least one measurement cycle $M(s) \subseteq M(m-1)$ having $s \leq m-1$ elements, where $s>1$ since $i \neq j_i$ for every $T_{ij_i} \in M(m-1)$. Let us number the elements of M(s) with the index p, $M(s) \equiv \{T_{i^p j^p},\ p=1,2,\ldots,s\}$ and consider the product of the ratios

$$X_{k_o}^{j_i}/X_{k_o}^{i} \equiv X_{j^p}/X_{i^p}$$

that are associated with the elements of M(s):

$$\prod_{p=1}^{s} (X_{j^p}/X_{i^p}), \quad i^p \neq j^p$$

Since M(s) is a cycle, we can define $i^1 = j^s$ and $i^{p+1} = j^p$ for $p=1,2,\ldots,s-1$. Since each factor in the product numerator cancels with one in the product denominator, the product of the parameters must be identically equal to one. Therefore, the parameters (X_{j^p}/X_{i^p}) $p=1,2,\ldots,s$ must be dependent.

Therefore, there must exist an acyclic subset $M^{-} \subset M(m)$ containing m-1 elements. But then by Lemma L2, M(m) must have at least one common injection reference compartment. Let j_o be such a reference compartment. Then every compartment $i \in I(m)$ is measured w.r.t. j_o. In addition consider the compartments $i \notin I(m)$. Since they are necessarily measured w.r.t. some $j_i \in J$ and since $J=I(m)$, they must also be measured w.r.t. j_o, either directly, if $j_i = j_o$, or indirectly, if $j_i \neq j_o$. Thus, all n compartments $i \in I$ must be measured w.r.t. a common injection reference compartment. Hence, the existence of a common injection reference compartment for M(n) is a necessary condition for completeness when the n measured elements are selected from m columns of T.

If one now assumes that the set of injected compartments J contains all n compartments and the set of measured compartments I contains $m \leq n$ compartments ($|J|=n$, $|I| = m \leq n$), then in this case exactly one element $T_{i_j j}$ is measured in each column j, where i_j is defined* as the row containing the measured element in column j. The algebraic formalism for determining the requirements for solution will parallel that of the previous case because of the symmetrical way i and j enter into equations 29a and 29b. For example, the equations corresponding to equations 32 are:

* Physically i_j is, of course, the compartment measured after injection in compartment j .

$$\sum_{k=1}^{n} R_{i_j i k} \left[A^{j}_{i_j k} \right] = \delta_{ij} \qquad \begin{matrix} i,j=1,2,\ldots,n \\ i \neq i_j \end{matrix}$$

Because of the complete algebraic analogy between the two cases, it will be sufficient to adapt the results obtained in the previous case, where all compartments were measured, to the present case where all compartments are injected, by interchanging the identities of the measurement and injection compartments. Thus one may conclude that the existence of a common measurement reference compartment is a necessary condition for completeness when the n measured elements are selected from m rows of T. For a random set of n measured elements, either a common measurement or a common injection reference compartment must exist for a complete solution.

SECTION V

SUFFICIENT CONDITION FOR COMPLETENESS

Consider a general strongly connected n-compartment system for which all coefficients A^{j}_{ik} are not zero*. It has been shown that the existence of a reference compartment for a random set of n injection-measurement functions is a necessary condition for the determination of the transfer rate matrix α, assuming that no element of α is previously known. The extent to which the reference compartment criterion can be considered sufficient for determining a solution will now be examined for the case where the n injection-measurement functions are selected from m columns of the transition matrix T, i.e., corresponding to the set J of m injected compartments. As was noted for the necessary condition, the algebraic formalism will be identical when n measurement functions are selected from m rows of T.

In defining a complete set of measurements, we have pointed out that we are not considering the external effects of experimental errors or the mathematical complexity of obtaining a solution but, rather, the informational potential inherently contained as a result of the topology of these measurements. This consideration is essential in making a distinction between sufficiency for obtaining a numerically precise solution and sufficiency for completeness. Furthermore, it should again be noted that a finite

* see footnote pg. 56

set of solutions may be obtained in some cases and that the true physically compatible solution must at least satisfy the inequalities 21-24. Moreover, in determining the transfer rate matrix, it is assumed that all n eigenvalue parameters are obtainable from the measurement data. This, sometimes, may not be possible for a random set of measurements unless the eigenvector relations are valid. That the validity of the eigenvector relations for any set of vectors $\underline{A}_k^j$ corresponding to the injected compartments $j \in J$, insures that all n eigenvalues can, at least theoretically, be determined from n measurements if all n compartments are measured, is evident from the following: Assume that an eigenvalue λ_k is not obtainable. This implies that the $k\underline{th}$ term $A_{ik}^{ji} e^{\lambda_k t}$ in all n measurements T_{ij_i}, $i=1,2,\ldots,n$, $j_i \in J$, is zero, i.e. $A_{ik}^{ji} = 0$ for every $i=1,2,\ldots,n$. If we assume that the eigenvector relations 27 are valid for all $j \in J$, then A_{ik}^{j} would be zero for all values of j since $A_{ik}^{j} = R_k^{j_i j} A_{ik}^{ji}$. However, then the vectors $\underline{A}_k^j$, $j \in J$ could not, in fact, be eigenvectors of α for that value of k, since their elements would all have to be zero. Hence, if the eigenvector relations are valid, then all n eigenvalues must be obtainable from n such measurements.

Assuming that all $A_{ik}^{j} \neq 0$, the eigenvector relations will, of course, be valid for any set of columns of T. Thus, the sufficiency requirement for completeness will essentially be satisfied if the system of equations 34 can be solved.

There remains to establish conditions for solutions for this system of equations subject to the considerations mentioned above. As in the previous section, one need only be directly concerned with the subset of $n(m-1) \equiv N$ equations for which $j \in J$. Since these equations are not linear in the unknowns, one must resort to somewhat indirect means of examining under what conditions a solution would be feasible. Thus, it will be convenient to view the parameters $R_k^{j_o j}$ of equations 34 as if they were a particular set of values for a corresponding set of variables $U_k^{j_o j}$ where, to be consistent, for $j=j_o$, $U_k^{j_o j}$ is not a variable but a constant equal to one. Then, in analogy with the left-hand side of equations 34, the functions f_{ij} of these variables are defined by the expressions

$$f_{ij} = \sum_{k=1}^{n} \left[A_{ik}^{j_i}\right] \frac{U_k^{j_o j}}{U_k^{j_o j_i}} \qquad \begin{array}{l} i=1,2,\ldots,n \\ j \in J \\ j \neq j_i \end{array} \qquad (36)$$

Further, in comparison with equations 34, the equations

$$f_{ij} = v_{ij} \qquad (37)$$

must, obviously, be satisfied by the set of values $v_{ij} = \delta_{ij}$ and $U_k^{j_o j} = R_k^{j_o j}$. If the functions f_{ij} are continuous and have continous derivatives in the neighborhood of this set of values and if the Jacobian determinant Δ (Goursat, 1904) formed from the partial derivatives of each of the functions f_{ij} with respect to each of the variables $U_k^{j_o j'}$, symbolized by

$$\Delta \equiv \left| \frac{\partial f_{ij}}{\partial U_k^{j_o j'}} \right| , \qquad (38)$$

does not vanish at the point having this set of values, then there must exist a unique set of N continuous functions $U_k^{j_o j}$ of the N variables $v_{ij'}$,

$$U_k^{j_o j} = U_k^{j_o j}(\{v_{ij'}\}) \qquad \begin{matrix} j, j' \in J \\ j \neq j_o \\ j' \neq j_i \end{matrix} ,$$

which for values $v_{ij'} = \delta ij'$, reduce to the values $U_k^{j_o j} = R_k^{j_o j}$ and which satisfy equations 37 in the neighborhood of this point. This would, of course, imply that the functions 37 are reversible for the desired solution $U_k^{j_o j} = R_k^{j_o j}$. Since the values $R_k^{j_o j}$ are all different from zero, it is evident that the functions 36 are continuously differentiable in the neighborhood of interest. Hence, there remains to show under what circumstances the jacobian does not vanish for the point having values $U_k^{j_o j} = R_k^{j_o j}$.

Consider the partial derivatives $\partial f_{ij} / \partial U_k^{j_o j'}$, $j' \neq j_o$ given by the expression below, where at most one of the two terms can be different from zero since $j \neq j_i$.

$$\frac{\partial f_{ij}}{\partial U_k^{j_o j'}} = \left[A_{ik}^{j_i}\right]\left(\frac{\delta_{j'j}}{U_k^{j_o j_i}} - \frac{U_k^{j_o j}\,\delta_{j' j_i}}{(U_k^{j_o j_i})^2}\right) \qquad \begin{matrix} j, j' \in J \\ j \neq j_i \\ j' \neq j_o \end{matrix}$$

For the systems under consideration, we have at the point of interest $U_k^{j_1 j_2} = R_k^{j_1 j_2}$ and $A_{ik}^{j_2} = A_{ik}^{j_1} R_k^{j_1 j_2}$ for any two values $j_1, j_2 \in J$. Thus,

$$\frac{\left[A_{ik}^{j_i}\right]}{U_k^{j_o j_i}} = \frac{\left[A_{ik}^{j_i}\right]}{R_k^{j_o j_i}}\,\frac{R_k^{j_i j}}{R_k^{j_i j}} = \frac{A_{ik}^{j}}{R_k^{j_o j}},$$

and

$$\left[A_{ik}^{j_i}\right]\frac{U_k^{j_o j}}{(U_k^{j_o j_i})^2} = \frac{\left[A_{ik}^{j_i}\right]}{R_k^{j_o j_i}}\,\frac{R_k^{j_o j}}{R_k^{j_o j_i}} = \frac{\left[A_{ik}^{j_i}\right] R_k^{j_i j}}{R_k^{j_o j_i}} = \frac{A_{ik}^{j}}{R_k^{j_o j_i}}$$

Substituting in the above equation and letting $U \rightarrow R$ represent evaluation at the point of interest, we obtain

$$\left.\frac{\partial f_{ij}}{\partial U_k^{j_o j'}}\right|_{U \rightarrow R} = \begin{cases} \dfrac{A_{ik}^{j}}{R_k^{j_o j}}, & j' = j \\ -\dfrac{A_{ik}^{j}}{R_k^{j_o j_i}}, & j' = j_i \\ 0, & j' \neq j, j_i \end{cases} \qquad \begin{matrix} j, j' \in J \\ j \neq j_i \\ j' \neq j_o \end{matrix} \qquad (39)$$

The elements $\partial f_{ij} / \partial U_k^{j_o j'}$ of Δ can be arranged so that the functions f_{ij} vary for each row and the variables $U_k^{j_o j'}$ vary for each column in some consecutive order. First let us number the m distinct elements of J with a superscript that varies from 1 to m . Choosing the index $j_o \equiv j^1$, the N variables $U_k^{j_o j'}$, $j' \neq j_o$ can then be placed in the following consecutive order:

$$U_k^{j_o j^2}, {}_{k=1,2,\ldots,n},\ U_k^{j_o j^3}, {}_{k=1,2,\ldots,n},\ \ldots,\ U_k^{j_o j^p}, {}_{k=1,2,\ldots,n},\ \ldots,\ U_k^{j_o j^m}, {}_{k=1,2,\ldots,n}$$

Consider now, the positioning of elements for each column. Since each column corresponds to a different variable $U_k^{j_o j^p}$, it can be identified by a constant pair of indices (p,k). Similarly, each row can be identified by an index pair (i,j). Setting $j' = j^p$ in equations 39 , the elements of Δ , evaluated for U→R, are:

$$\Delta_{(i,j),(p,k)} = \begin{cases} A_{ik}^{j}/R_k^{j_o j^p}, & j^p = j \\ -A_{ik}^{j}/R_k^{j_o j^p}, & j^p = j_i \\ 0, & j^p \neq j\ ;\ j_i \end{cases} \qquad \begin{matrix} j, j^p \in J \\ j \neq j_i \\ j^p \neq j_o \end{matrix} \qquad (39b)$$

Since p and k are constant for each column, the denominator factors $R_k^{j_o j^p}$ can be factored from Δ , leaving only the coefficients $\pm A_{ik}^{j}$ and the values zero. Thus, $\Delta_{U \to R} = D/d$, where D is a NxN determinantal factor, where N=n(m-1).

and $d = \prod_{p=2}^{m} \prod_{k=1}^{n} R_k^{j_o j^p}$. Since d is always a finite non-zero factor, we need only consider whether D is different from zero or not.

To represent D in a meaningful manner, we must also place the row index pairs (i,j) in some meaningful order. The n compartments i can be grouped according to the injected compartments with respect to which they are directly measured. Thus, we may label the compartments measured after injection in j^q by i_s^q $s=1,2,\ldots,n_q$, where n_q corresponds to the number of measured elements in column j^q of T. Thus, the set of n compartments I can be represented by $\left\{ i_s^q \middle| \begin{matrix} q=1,2,\ldots,m \\ s=1,2,\ldots,n_q \end{matrix} \right\}$ Taking consecutive values of the row index j, $j=j^v$, $v=1,2,\ldots,m$, i varies over all values for each j^v, excluding the values of i for which $j^v=j_i$. This indexing is, of course, consistent with the restriction on i and j in equations 39 that the index pairs (i,j_i) be excluded. Thus, using the above symbolism, for each j^v, i varies over all values i_s^q, $q=1,2,\ldots,m$, $q\neq v$, $s=1,2,\ldots,n_q$. Consider now the row index pair $(i,j)=(i_s^q,j^v)$. Clearly, for all $i=i_s^q$, $s=1,2,\ldots,n_q$, the index j_i is equal to j^q. Thus, from equations 39b it is evident that the non-zero entries $\pm A_{ik}^j$, $k=1,2,\ldots,n$ of row $(i,j)=(i_s^q,j^v)$ will appear in columns (p,k) for p=v (with the positive sign) and for p=q (with the negative sign). Varying s from 1 to n_q for each set of values v and q, these non-zero entries will be conveniently grouped together in submatrix elements symbolized by $\pm(A_{ik}^j)^{vq}$, $v\neq q$.

Each submatrix $(A^j_{ik})^{vq}$, $v \neq q$ has $n_q n$ elements

$$A^j_{ik} = A^{j^v}_{i^q_s k}, \quad \begin{matrix} s=1,2,\ldots,n_q \\ k=1,2,\ldots,n \end{matrix},$$

where s is the row variable, k is the column variable and v and q are fixed indices, $v \neq q$. Thus, in the general representations of D given by the determinants in equations 40-42 below, a submatrix $(A^j_{ik})^{vq}$ is positioned under the columns of D corresponding to the index pairs (p,k) for p=v and k=1,2,...,n and is repeated, preceded by a minus sign, for columns for which p=q and k=1,2,...,n. However, if either v or q is equal to 1, such a submatrix will only appear once, since p=1 is not a column index here. Of course, for $p \neq v,q$ the entries of D will be zero for elements having column index p and row indices v and q. For example, for m=2 and for m=3, the general representation of D is given by equations 40 and 41 for any value of n. Equation 42 gives the general representation of D for both any value of n and any value of m.

$$D_{m=2} = \begin{vmatrix} -(A_{ik}^{j})^{12} \\ (A_{ik}^{j})^{21} \end{vmatrix} \qquad (40)$$

$$D_{m=3} = \begin{vmatrix} -(A_{ik}^{j})^{12} & (0) \\ (0) & -(A_{ik}^{j})^{13} \\ (A_{ik}^{j})^{21} & (0) \\ (A_{ik}^{j})^{23} & -(A_{ik}^{j})^{23} \\ (0) & (A_{ik}^{j})^{31} \\ -(A_{ik}^{j})^{32} & (A_{ik}^{j})^{32} \end{vmatrix} \qquad (41)$$

$$D = \begin{vmatrix}
-(A^{j}_{ik})^{12} & (0) & \cdots & (0) & (0) \\
(0) & -(A^{j}_{ik})^{13} & \cdots & (0) & (0) \\
\cdot & \cdot & & \cdot & \cdot \\
(0) & (0) & \cdots & -(A^{j}_{ik})^{1(m-1)} & (0) \\
(0) & (0) & \cdots & (0) & -(A^{j}_{ik})^{1m} \\
(A^{j}_{ik})^{21} & (0) & \cdots & (0) & (0) \\
(A^{j}_{ik})^{23} & -(A^{j}_{ik})^{23} & \cdots & (0) & (0) \\
\cdot & \cdot & & \cdot & \cdot \\
(A^{j}_{ik})^{2(m-1)} & (0) & \cdots & -(A^{j}_{ik})^{2(m-1)} & (0) \\
(A^{j}_{ik})^{2m} & (0) & \cdots & (0) & -(A^{j}_{ik})^{2m} \\
(0) & (A^{j}_{ik})^{31} & \cdots & (0) & (0) \\
-(A^{j}_{ik})^{32} & (A^{j}_{ik})^{32} & \cdots & (0) & (0) \\
\cdot & \cdot & & \cdot & \cdot \\
(0) & (A^{j}_{ik})^{3(m-1)} & \cdots & -(A^{j}_{ik})^{3(m-1)} & (0) \\
(0) & (A^{j}_{ik})^{3m} & \cdots & (0) & -(A^{j}_{ik})^{3m} \\
\vdots & \vdots & & \vdots & \vdots \\
(0) & (0) & \cdots & (A^{j}_{ik})^{(m-1)1} & (0) \\
-(A^{j}_{ik})^{(m-1)2} & (0) & \cdots & (A^{j}_{ik})^{(m-1)2} & (0) \\
(0) & -(A^{j}_{ik})^{(m-1)3} & \cdots & (A^{j}_{ik})^{(m-1)3} & (0) \\
\cdot & \cdot & & \cdot & \cdot \\
(0) & (0) & \cdots & (A^{j}_{ik})^{(m-1)m} & -(A^{j}_{ik})^{(m-1)m} \\
(0) & (0) & \cdots & (0) & (A^{j}_{ik})^{m1} \\
-(A^{j}_{ik})^{m2} & (0) & \cdots & (0) & (A^{j}_{ik})^{m2} \\
(0) & -(A^{j}_{ik})^{m3} & \cdots & (0) & (A^{j}_{ik})^{m3} \\
\cdot & \cdot & & \cdot & \cdot \\
(0) & (0) & \cdots & -(A^{j}_{ik})^{m(m-1)} & (A^{j}_{ik})^{m(m-1)}
\end{vmatrix} \qquad (42)$$

For further illustration consider the following examples, where the set of measured elements of T is given by M :

1) n=2, m=2, $M = \{T_{11}, T_{22}\}$; $j^1 \equiv 1$, $j^2 \equiv 2$

$$D = \begin{vmatrix} -A^1_{21} & -A^1_{22} \\ A^2_{11} & A^2_{12} \end{vmatrix} \qquad (43)$$

2) n=3, m=2, $M = \{T_{13}, T_{23}, T_{32}\}$; $j^1 \equiv 2$, $j^2 \equiv 3$

$$D = \begin{vmatrix} -A^2_{11} & -A^2_{12} & -A^2_{13} \\ -A^2_{21} & -A^2_{22} & -A^2_{23} \\ A^3_{31} & A^3_{32} & A^3_{33} \end{vmatrix} \qquad (44)$$

3) n=3, m=3, $M = \{T_{11}, T_{23}, T_{32}\}$; $j^1 \equiv 1$, $j^2 \equiv 2$, $j^3 \equiv 3$

$$D = \begin{vmatrix} -A^1_{31} & -A^1_{32} & -A^1_{33} & 0 & 0 & 0 \\ 0 & 0 & 0 & -A^1_{21} & -A^1_{22} & -A^1_{23} \\ A^2_{11} & A^2_{12} & A^2_{13} & 0 & 0 & 0 \\ A^2_{21} & A^2_{22} & A^2_{23} & -A^2_{21} & -A^2_{22} & -A^2_{23} \\ 0 & 0 & 0 & A^3_{11} & A^3_{12} & A^3_{13} \\ -A^3_{31} & -A^3_{32} & -A^3_{33} & A^3_{31} & A^3_{32} & A^3_{33} \end{vmatrix} \qquad (45)$$

In general, note again that the elements of D are either zero or equal to one of the coefficients $\pm A^{j}_{ik}$, $j \in J$. Further, it is quite clear that all unknown coefficients A^{j}_{ik} for every $j \in J$ appear explicitly in this representation of D, whereas all known coefficients $A^{j_i}_{ik}$ are excluded since $j \neq j_i$ for the elements appearing in D. Specifically, notice that the coefficients A^{j}_{ik} appearing in any one column (p,k) of D are the k<u>th</u> component coefficients of the $(n-n_p)$ unmeasured elements that are in the j^p<u>th</u> column of T together with the k<u>th</u> component coefficients of the $n_p(m-1)$ unmeasured elements that are in the m-1 columns $j \in J$, $j \neq j^p$ and in the n_p rows containing the measured elements of column j^p. Topologically, one may easily picture these elements of T by placing an X in the position they occupy with respect to the T matrix. Thus, the following scheme develops, where only the m columns containing measured elements are shown and where, for purposes of visualization, the measured elements in column j^p are assumed to belong to a single shaded portion of the column (fig. 9). Finally, one should note that for every row (i,j) of D, the corresponding set of coefficients A^{j}_{ik}, k=1,2,...,n , which appear in that row, cannot appear in any other row, since every row corresponds to a unique pair (i,j).

Equating Δ with zero, of course, implies that the column vectors of D are linearly dependent. From the simplified representation of D given by 42 , it is evident

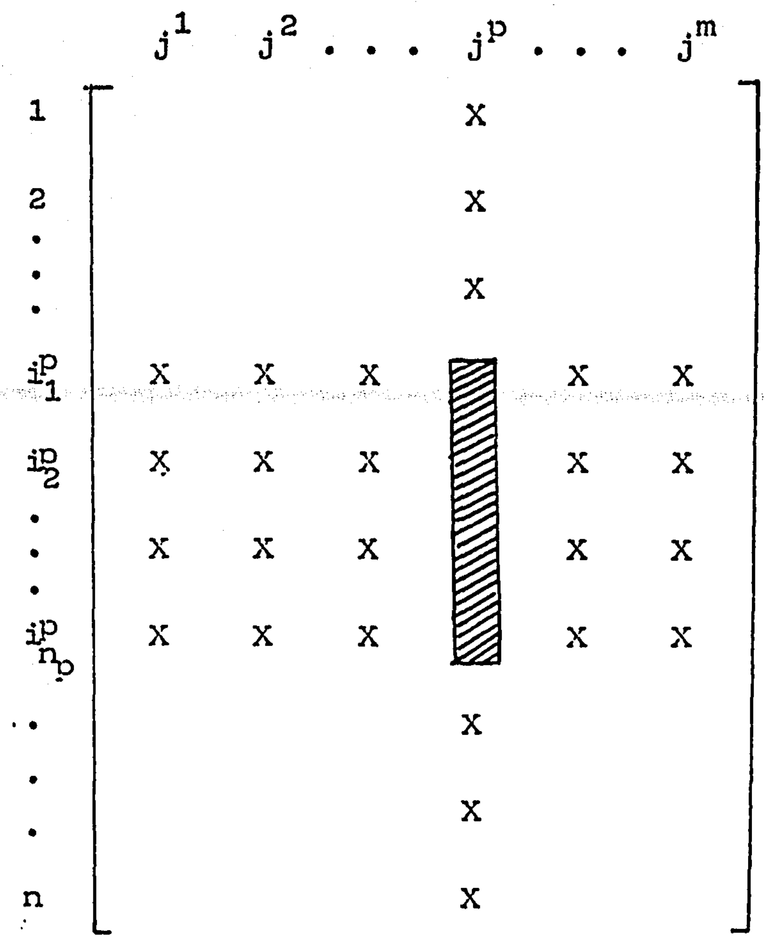


Fig. 9 Schematic representation of the topological positioning of the elements of T whose coefficients appear in the columns of D having indices (p,k), k=1,2,...,n (denoted by X).

that this, in turn, implies the linear relation of the coefficients A^{j}_{ik} . Since, the coefficients appearing in any one column all have the same value of k, it is evident that the addition of n columns for values of k from 1 to n may, in some cases, directly result in null values for the sum in each row, in view of equations 29b. Similarly, the total may be zero upon addition of multiple sets of n columns, as will be further demonstrated.

In the following, it is shown that the addition of a properly selected number of columns will always give zero if the chosen measured elements of T have no common injection reference compartment and, therefore, the Jacobian will be zero. This result is expected since if the Jacobian were not zero for such cases, a solution would be obtainable which would contradict the necessary condition for the existence of a common reference compartment, previously established. In addition, certain interesting characteristics with respect to the measurement topology are revealed.

Consider first, the following conditions which imply that the value of the determinant D is zero. In each case, the direct addition of r sets of columns of D gives a sum of zero for the corresponding elements in each row, where $r \leq m-1$ and where each set corresponds to a column index set (p,k), $k=1,2,\ldots,n$. As shown, the resultant conditions may be expressed by an equation $S_r = 0$.

(r=1): Adding a single set of n columns of D corresponding to column indices (p,k), p constant and k=1,2,...,n.

As previously noted, the elements of D corresponding to column indices (p,k) and row indices (i_s^q, j^v) will be zero if both v and q are not equal to p, and will be equal to $A^{j^v}_{i_s^q k}$ for v=p and to $-A^{j^v}_{i_s^q k}$ for q=p. Therefore, summation over all values of k for constant p will either give a value of zero or a value of $\pm\delta_{i_s^q j^v}$ for each row, in agreement with equations 29b. Thus, for constant p, we obtain the following three possible sums for each row, depending on the row indices (v,q,s):

0	for	$v \neq p,\ q \neq p$
$\delta_{i_s^q j^v}$	for	$v=p$ $q=1,2,\ldots,m\ ,\ q \neq p$ $s=1,2,\ldots,n_q$
$-\delta_{i_s^q j^v}$	for	$q=p$ $v=1,2,\ldots,m\ ,\ v \neq p$ $s=1,2,\ldots,n_p$

The addition of this set of n columns of D will result in a value of zero for the sum for each row, only if all of the above values are zero. This condition can be concisely expressed by setting the total sum of all of the above kronecker deltas (ignoring the negative signs) to zero:

$$S_1 = \sum_{\substack{q=1\\q\neq p}}^{m} \sum_{s=1}^{n_q} \delta i_s^q\, j^p + \sum_{\substack{v=1\\v\neq p}}^{m} \sum_{s=1}^{n_p} \delta i_s^p\, j^v = 0$$

Since in this expression, v and q serve as dummy variables, we may write more concisely,

$$S_1 = \sum_{\substack{q=1\\q\neq p}}^{m} \left(\sum_{s=1}^{n_q} \delta i_s^q\, j^p + \sum_{s=1}^{n_p} \delta i_s^p\, j^q \right)$$

Thus, if the condition $S_1=0$ is true for any value of p ($p=1,2,\ldots,m$)*, then Δ must be zero.

(r=2): Adding two sets of n columns of D corresponding to column indices (p_1,k) and (p_2,k), $k=1,2,\ldots,n$.

In this case, summation over p and k gives the following values for each row, depending on the row indices (v,q,s):

0	for	$v\neq p_1,p_2,\ q\neq p_1,p_2$
$\delta i_s^q\, j^v$	for	$v=p_1,p_2$ $q=1,2,\ldots,m\ ,\ q\neq p_1,p_2$ $s=1,2,\ldots,n_q$
$-\delta i_s^q\, j^v$	for	$q=p_1,\ p_2$ $v=1,2,\ldots,m\ ,\ v\neq p_1,p_2$ $s=1,2,\ldots,n_q$
0	for	$v=p_1,\ q=p_2$ and $v=p_2,\ q=p_1$

*Although p=1 is not a selected column index for D as here defined, this more general condition will be proven subsequently.

Notice, that all elements in the first category are zero, while the sums in the last category are also zero since they refer to rows containing coefficients with opposite signs in the added columns. Therefore, the first and last category need not be considered in the following condition which results by equating the remaining values with zero and changing the dummy index v to q, as before:

$$S_2 = \sum_{\substack{q=1 \\ q \neq p_1, p_2}}^{m} \left(\sum_{p=p_1,p_2} \left(\sum_{s=1}^{n_q} \delta_{i_s^q\, j^p} + \sum_{s=1}^{n_p} \delta_{i_s^p\, j^q} \right) \right) = 0$$

If this condition is true for any two column indices p_1, p_2, then $\Delta = 0$. Of course, if $p_1 = p_2$, this condition reduces to the previous condition $S_1 = 0$.

<u>General Case</u>

$(r \leq m-1)$: Adding $r \leq m-1$ sets of n columns having indices (p,k) for $p = p_1, p_2, \ldots, p_r$, $k = 1, 2, \ldots, n$.

Employing the same reasoning as in the previous cases, it is clear that the sum of the r column sets will be zero for the corresponding elements in each row, if the following condition in true:

$$S_r = \sum_{\substack{q=1 \\ q \neq p_1, p_2, \ldots, p_r}}^{m} \left(\sum_{p=p_1,p_2,\ldots,p_r} \left(\sum_{s=1}^{n_q} \delta_{i_s^q\, j^p} + \sum_{s=1}^{n_p} \delta_{i_s^p\, j^q} \right) \right) = 0$$

Although this condition has been derived for values of $p\neq 1$, it can be seen, that if the indices p and q are interchanged, then the condition $S_r=0$ for $p\neq 1$, will be identical to the condition $S_{(m-r)}=0$, if the value p=1 is permitted. In particular, consider the case where all columns of D are added. Since there are n(m-1) columns, this corresponds to a value of r=m-1 in S_r. Since if $p\neq 1$ and $q\neq p_1, p_2, \ldots, p_{m-1}$, the only possible value for q is q=1, the condition $S_r=0$ becomes

$$S_{m-1} = \sum_{p=2}^{m}\left(\sum_{s=1}^{n_q} \delta_{i_s^q j^p} + \sum_{s=1}^{n_p} \delta_{i_s^p j^q}\right) = 0$$

where, q=1 .

Interchanging the indices p and q, we can see that this condition is identical to condition $S_1=0$ if one allows the value p=1. Generally, the value p=1 may be permitted for the condition $S_r=0$ for any value of r. This result is expected since j_o was arbitrarily selected to have the value j^1.

Interpretation of Condition $S_r=0$ w.r.t. the Measurement Topology

First, notice that since i_s^q is a compartment measured directly w.r.t. j^q, a condition of the form $\sum_{s=1}^{n_q} \delta_{i_s^q j^p} = 0$ implies that j^p is not measured directly w.r.t. j^q, i.e., $T_{j^p j^q}$ is not a measured element of T ($T_{j^p j^q} \notin M$). Similarly, if $\sum_{s=1}^{n_p} \delta_{i_s^p j^q} = 0$, then $T_{j^q j^p} \notin M$. For a given value of r, the above observation implies that the following group of elements is not measured in each case:

Unmeasured Elements For Condition $S_1=0$:

$$T_{j^p j^q} \; , \quad T_{j^q j^p} \; \underset{q \neq p}{(q=1,2,\ldots,m)}$$

Denoting such unmeasured elements with an X, the following scheme represents the topological positioning of these elements w.r.t. the submatrix of T where only the columns corresponding to the injected compartments $j\epsilon J$ and the rows corresponding to the same columns $i\epsilon J$ are depicted.

	j^1	j^2	...	j^p	...	j^{m-1}	j^m
j^1				X			
j^2				X			
⋮				⋮			
j^p	X	X	..	O	...	X	X
⋮				⋮			
j^{m-1}				X			
j^m				X			

fig. 10a. Topological Representation For Condition $S_1=0$

Since an element must be measured in each row of T and since only the columns j_1 to j_m of T can contain measured elements, it is evident that $T_{j^p j^p}$ must be a measured element. Thus, j^p is the only possible common reference compartment if one exists, since j^p is only measured with respect to itself. However, no other of the injected compartments is measured directly w.r.t. j^p and as a consequence none can be indirectly so measured either.

Therefore, j^p cannot be a common injection reference compartment for all compartments; there can be none.

<u>Unmeasured Elements For Condition $S_2=0$:</u>

$$T_{j^p j^q}\ ,\ T_{j^q j^p} \quad (p=p_1, p_2\ ,\ \begin{array}{l} q=1,2,\ldots,m \\ q\neq p_1, p_2 \end{array})$$

	j^1	j^2	...	j^{p_1}	j^{p_2}	...	j^{m-1}	j^m
j^1				X	X			
j^2				X	X			
⋮				⋮	⋮			
j^{p_1}	X	X	...	O	O	...	X	X
j^{p_2}	X	X	...	O	O	...	X	X
⋮				⋮	⋮			
j^{m-1}				X	X			
j^m				X	X			

X: Unmeasured element

O: Uncertain whether measured

fig. 10b. Topological Representation For Condition $S_2=0$

Notice, j^{p_1} and j^{p_2} can only be measured w.r.t. j^{p_1} or j^{p_2}. Thus, the only possible reference compartments are j^{p_1} amd j^{p_2}. However, no injected compartment other than j^{p_1} and j^{p_2}, is either directly or indirectly measured w.r.t. j^{p_1} or j^{p_2}. Therefore, since $m\geq 2$, there can be no common injection reference compartment. (note, $m\geq 2$ since $r=2$ and $r\leq m-1$)

<u>Unmeasured Elements For Condition $S_r=0$:</u>
(General Case)

$$T_{j^p j^q}\ ,\ T_{j^q j^p} \quad (p=p_1,p_2,\ldots,p_r \ {}_{r\le m-1}, \quad q=1,2,\ldots,m \ ;\ q\ne p_1,p_2,\ldots,p_r)$$

	j^1	j^2	...	j^{p_1}	j^{p_2}	...	$j^{p_{r-1}}$	j^{p_r}	...	j^{m-1}	j^m
j^1				X	X	...	X	X			
j^2				X	X	...	X	X			
⋮				⋮	⋮		⋮	⋮			
j^{p_1}	X	X	...	O	O		O	O	...	X	X
j^{p_2}	X	X	...	O	O		O	O	...	X	X
⋮	⋮	⋮								⋮	⋮
$j^{p_{r-1}}$	X	X	...	O	O		O	O	...	X	X
j^{p_r}	X	X	...	O	O		O	O	...	X	X
⋮				⋮	⋮		⋮	⋮			
j^{m-1}				X	X	...	X	X			
j^m				X	X	...	X	X			

X: Unmeasured element

O: Uncertain whether measured

fig.10c. Topological Representation For Condition $S_r=0$

Employing similar reasoning as in the previous cases, it is evident that the only possible reference compartments for the set $\{j^{p_1}, j^{p_2}, \ldots, j^{p_r}\}$ must belong to the same set. However, since $m > r$ and since no other injected compartment is measured w.r.t. any of these compartments, such a reference compartment, if it exists for the set $\{j^{p_1}, j^{p_2}, \ldots, j^{p_r}\}$, will not be a common injection reference compartment for

for all of the compartments $j^1, j^2, \ldots, j^m$.

Thus, we see that if the direct summation of r column sets of D gives a value of zero for the corresponding elements of each row, represented by the general condition $S_r=0$, $r \leq m-1$, then a common injection reference compartment cannot exist for all compartments. The set theoretic representation of condition $S_r=0$ is given below.

<u>Set Theoretic Representation of Condition $S_r=0$.</u>

Let J^- represent a proper subset of the injected compartments

$$J^- \equiv \{j^{p_1}, j^{p_2}, \ldots, j^{p_r}\} \ , \quad r \leq m-1$$

It is clear from fig. 10c above that the general condition $S_r=0$ is equivalent to the requirement that a proper subset J^- of J exists such that for any element T_{ij} of T for which both i and j belong to J, if $i \in J^-$ and $j \notin J^-$ or if $j \in J^-$ and $i \notin J^-$, then T_{ij} is not a measured element ($T_{ij} \notin M$).

We have already seen that the measurement topology described by the general condition $S_r=0$ implies that a common injection reference compartment cannot exist for M. In the proof of the following theorem, it is shown that if a reference compartment does not exist, then such a measurement topology is alway implied and, therefore, Δ will be equal to zero.

THEOREM T2: Consider an n compartment system such that the eigenvector relations 27 are valid for any set of vectors $\underline{A}_k^j$ for which $j \in J$. If a set of n measurement functions M for which all n compartments are measured ($|I|=n$) and $m \leq n$ compartments are injected ($J \subseteq I$, $|J|=m$) does not have a common injection reference compartment than the Jacobian determinant Δ must be zero.

<u>Proof of T2</u>: Consider a set of n measurement functions M for which all compartments are measured, $|I|=n$ and $|J|=m \leq n$. Let $M_J \subset M$ be the subset of m measurement functions for which the set of measured compartments I_J is equal to J ($I_J = J$). Since each of the n compartments in I is measured w.r.t. a compartment in J, M will not have a common injection reference compartment if and only if M_J does not. Further, assuming that M does not have a common reference compartment, in accordance with Lemma L2, every subset of M_J having (m-1) elements must be cyclic, otherwise, M_J and, therefore, M also would have a common injection reference compartment. Since M_J is cyclic, let M_c be such a cycle in M_J. Then $I_c=J_c$ and, since every compartment in the cycle is measured w.r.t. any other compartment in that cycle, all compartments in the cycle can be considered reference compartments for the elements of the cycle. Let J^- be the subset of all compartments in J that are measured either directly or indirectly w.r.t. some com-

partment in J_c (note $J^- \supseteq J_c$). Thus, the associated set of measurements M^- has as a common injection reference compartment any element of J_c. However, since M_J itself cannot have a common reference compartment, M^- must be a proper subset of M_J. Hence, also $J^- \subset J$. Now, consider any element T_{ij} of T such that both i and j belong to J. It is evident that if T_{ij} is measured and if $j \in J^-$, then also $i \in J^-$ or if $i \in J^-$, then also $j \in J^-$. Therefore, T_{ij} is not a measured element for both $i \in J$ and $j \in J$, if either $j \in J^-$ and $i \notin J^-$ or if $i \in J^-$ and $j \notin J^-$. Thus, condition $S_r = 0$ is satisfied, which implies that the Jacobian determinant is zero at the desired solution point, for any case for which M does not have a common injection reference compartment.

The fact that the Jacobian vanishes at the desired solution point for the cases considered in the above theorem, substantiates the necessary condition for the existence of a reference compartment since a non-zero Jacobian would indicate that a solution is obtainable. However, it is not a proof for the necessary condition in itself since, as previously noted, the fact that the Jacobian vanishes for a "point" does not give a definite indication that the set of functions 36 cannot be inverted at that point. It should be clear that in evaluating the Jacobian at a given point for different sets of measurements, we are in effect evaluating different Jacobians corresponding to a varying set of functions. If the Jacobian were zero identically in some region for a fixed set of functions, the functions would be dependent in that region and no solution for the corresponding equations would be possible since the equations would also be dependent. However, there is no apparent reason for the Jacobian to vanish identically in the region of the solution point and, therefore, a different argument must be presented in explaining why a solution cannot be obtained for equations 34 for cases where there is no common reference compartment. This has already been accomplished in the proof of theorem T1. Moreover, we may want to distinguish the fact that even though in order to agree with theorem T1 one may have to consider equations 34 dependent for the cases exhibiting no common reference compartment, this does not necessarily imply that the corresponding functions, given by 36, are also dependent and so there is no conflict with the observation that the Jacobian does not vanish identically.

THEOREM T3: The existence of a common reference compartment for a set M of n measurement functions is in general a sufficient condition for completeness for a strongly connected n compartment system, assuming that one measured element is selected from each row or from each column of T.

<u>Proof of T3</u>: One need only consider the case where a common injection reference compartment exists, since, for a common measurement reference compartment the proof will be similar. The assumption of a strongly connected system implies that the column and row eigenvector relations are in general valid for all sets of vectors $\underline{A}_k^j$ and $\underline{A}_{i_k}$, respectively, as has been previously noted. For such systems a non-zero value of the Jacobian Δ at the true solution point would establish sufficiency for completeness, since the functions 36 would then be reversible at this point. Assuming, the contrary, that the Jacobian equals zero, implies that its column vectors are linearly dependent. It will now be shown that this contrary assumption in general leads to a contradiction.

In the first place, upon close examination of the representation of D, given by equation 42, such a case does not seem likely, considering the topological distribution of elements of T (as illustrated in fig. 9) whose coefficients belong to each column set (p,k), k=1,2,...,n of D. In particular, since a reference compartment exists, i.e., condition $S_r = 0$ is not satisfied, the direct summation of any column set or sets (p,k), k=1,2,...,n cannot be equal to zero. Therefore, the linear dependence of the columns of D here, would imply the existence of a set of distinct constants m_{pk}, where not all constants can be zero, such that if each column (p,k) of D is multiplied by the corresponding constant m_{pk}, the resulting column sum must be zero. Since, the elements A^j_{ik} of any single row (i,j), where to use the previously introduced notation, $i=i^q_s$ and $j=j^v$, appear (with opposite signs) only in the columns (p,k) for p=v and for p=q, where $p \neq 1$, the resulting linear relation for these elements would have the form

$$\sum_{k=1}^{n} \mu_k A^j_{ik} = 0 \quad , \qquad (46)$$

where

$$\mu_k = m_{vk} - m_{qk} \equiv \mu^{vq}_k \; , \; q \neq v \;\; \text{and} \;\; m_{1k} \equiv 0 \; . \qquad (47)$$

Clearly, all sets of elements A^j_{ik}, k=1,2,...,n , belonging to a common submatrix $(A^j_{ik})^{vq}$ would be related by the same set of constants μ^{vq}_k. Further, it is evident from 47 , that these sets of constants would have to be linearly related by the following equations:

$$\mu_k^{vq} = \mu_k^{v1} - \mu_k^{q1}, \; v,q \neq 1 \qquad \begin{array}{l} v,q=1,2,\ldots,m \\ q \neq v \\ k=1,2,\ldots,n \end{array} \qquad (48a)$$

$$\mu_k^{vq} = -\mu_k^{qv} \qquad (48b)$$

From a topological viewpoint, these equations express the following observations. For both v and q not equal to one, the submatrix $(A_{ik}^{j})^{vq}$ belongs to both of the column sets (v,k) and (q,k) of D, with opposite signs. Thus, each constant μ_k^{vq} $v,q \neq 1$ must be the difference of the constants μ_k^{v1} and μ_k^{q1} which relate elements that belong to a single column set, (v,k) and (q,k), respectively. The second set of equations (which for $v,q \neq 1$ also follows from the first) signifies the fact that the elements of any submatrix $(A_{ik}^{j})^{vq}$ and the elements of the submatrix $-(A_{ik}^{j})^{qv}$, having reversed indices v,q , must be related by the same constants μ_k^{vq}, since the elements of both submatrices belong to the same column sets of D.

It can now be seen that each constant of a set of constants μ_k that satisfy equation 46 can be expressed as a linear combination of the n-1 parameters $R_k^{jj'}$, $j' \neq i$ that satisfy the same equation. This is evident from the fact that in order for the homogeneous system of n equations which includes equations 46 and the n-1 equations,

$$\sum_{k=1}^{n} R_k^{jj'} A_{ik}^{j} = \delta_{ij'} = 0, \quad \begin{matrix} j'=1,2,\ldots,n \\ j' \neq i \end{matrix} ,$$

to have a non-trivial solution, the coefficient determinant for these equations must be zero. Thus, it follows

$$\mu_k = \sum_{\substack{j'=1 \\ j' \neq i}}^{n} C_{j'} R_k^{jj'} \tag{49}$$

However, since for the submatrix $(A_{ik}^{j})^{vq}$, i assumes n_q different values $i=i_s^q$, $s=1,2,\ldots,n_q$ and since as previously noted, all sets of coefficients A_{ik}^{j} $k=1,2,\ldots,n$, that belong to the submatrix $(A_{ik}^{j})^{vq}$ must be related by the same set of constants μ_k^{vq}, the index j' can only range over $n-n_q$ different values for each set of factors μ_k^{vq}. Thus,

$$\mu_k^{vq} = \sum_{\substack{j'=1 \\ j' \neq i_s^q,\ s=1,2,\ldots,n_q}}^{n} C_{j'}^{vq} R_k^{j^v j'} \tag{50}$$

Clearly, for different values of v, the expressions 50 cannot explicitly contain any common parameters $R_k^{j^v j'}$ except, possibly, the value $R_k^{j^v j^v} = 1$, which, in fact is not a true parameter. However, by equations 48 the magnitudes of such expressions must be linearly related or equal. As a whole, in view of 50, equations 48 can be viewed as a set of linear homogeneous equations in the unknown factors $C_{j'}^{vq}$ with the values $R_k^{j^v j'}$ regarded as known coefficients in these equations.

It may now be seen from the following, that since the condition $S_r=0$ is not satisfied, these equations cannot have a non-trivial solution, except, possibly, for certain constrained systems. In the first place, the latter can easily be shown to be the case if the values $C_{j'}^{vq}$ are, initially, assumed to all be constants which are independent of the system. Each of the equations 48 , for example, would then necessarily correspond to an unvarying linear relation of the sets of constants $R_k^{jj'}$ for two different values of j, $j=j^v$ and $j=j^q$, and a multiple set of values j'. However, since each value $R_k^{j^q j'}$ can be expressed as

$$R_k^{j^q j'} = R_k^{j^v j'} / R_k^{j^v j^q} ,$$

each equation would represent a relationship for the set of parameters $R_k^{j^v j'}$ $\underset{j' \neq j^v}{j'=1,2,\ldots,n}$, unless all of the values $C_{j'}$ corresponding to such an equation are zero, excepting those that modify the constants $R_k^{j\,j}=1$. Since, by appendix C , the parameters $R_k^{j^v j'}$ are independent for an unconstrained system, we must conclude that equations 48 cannot in general relate the parameters $R_k^{j^v j'}$, i.e., that the values of the constants μ_k^{vq} , given by 50, are generally either equal to zero or are equal to a constant $C_{j^v}^{vq}$. Thus, the constants μ_k^{vq} would not truly depend on k. Thus, also, the multiplicative constants $m_{pk} = \mu_k^{p1}$ could then at most depend on p, i.e, it would be implied that we can define:

$$m_{pk} \equiv m_p \ , \ k=1,2,\ldots,n$$

However, if condition $S_r=0$ is not satisfied, i.e., if the direct sum of any number of sets (p,k) of columns of D, does not give a value of zero for the sum of the elements in each row, then the multiplication of each column in each set (p,k), k=1,2,...,n by a constant m_p will not make the sum zero unless the values m_p are zero for all values of p. This is evident from the fact that only one set of coefficients A^j_{ik} k=1,2,...,n can appear in any given row (i,j) of D, either under one set of columns (p,k) or under two sets of columns (p,k) with opposite signs. Thus, the direct sum of elements in any row can only be different from zero if these elements appear under only one set of columns (p,k) and if the sum $\sum_{k=1}^{n} A^j_{ik}$ is not zero. Multiplication by a non-zero constant m_p cannot, of course, make the sum zero. Thus, in the case that the values $C^{vq}_{j'}$ are assumed to be constants, independent of the system parameters, equations 48 cannot all be non-trivially satisfied (i.e., $D \neq 0$) for the general unconstrained system, unless condition $S_r=0$ is satisfied, that is, unless a common reference compartment does not exist. However, since we have assumed the existence of a reference compartment, we must consider at least some of the values $C^{vq}_{j'}$ to be dependent on the system parameters and examine whether such an assumption can be satisfied for the general unconstrained system.

In attempting to solve the homogeneous system of equations 48 and 50 for the factors $C_{j'}^{vq}$ or, more precisely, their ratios, it will be convenient at this point to represent equations 48 more concisely by the single equivalent set of equations:

$$\mu_k^{vq} = \mu_k^{v1} - \mu_k^{q1}, \text{ where } \mu_k^{11} \equiv 0 \qquad \begin{array}{l} v,q=1,2,\ldots,m \\ q\neq v,\ q\neq 1 \\ k=1,2,\ldots,n \end{array} \tag{51}$$

The value q=1 is omitted since for this value, equation 51 is a trivial identity. Substituting equations 50 into equations 51 , we obtain a set of equations relating the unknowns $C_{j'}^{vq}$ $q\neq v$, $q\neq 1$ on the left hand side with the unknowns $C_{j'}^{v1}$ $v\neq 1$ on the right hand side, where $C_{j'}^{11} \equiv 0$:

$$\sum_{j'\neq i_s^q,\ s=1,2,\ldots,n_q} C_{j'}^{vq} R_k^{j^v j'} = \sum_{j'\neq i_s^1,\ s=1,2,\ldots,n_1} C_{j'}^{v1} R_k^{j^v j'} - \sum_{j'\neq i_s^1,\ s=1,2,\ldots,n_1} C_{j'}^{q1} R_k^{j^q j'} \qquad \begin{array}{l} v=1,2,\ldots,m \\ q=2,3,\ldots,m \\ q\neq v \\ k=1,2,\ldots,n \end{array} \tag{52}$$

For each value of k a subset of $(m-1)^2$ of equations 52 is formed. No equation in such a subset can be a linear combination of the remaining equations in the subset since each equation introduces a distinct set of unknowns $C_{j'}^{vq}$. Clearly, all unknowns will be represented in a single such subset since the unknowns are independent of the index k. The number of unknowns $C_{j'}^{vq}$ is given by the expression:

$$\sum_{v=1}^{m} \sum_{\substack{q=1 \\ q\neq v}}^{m} (n-n_q) = n(m-1)^2$$

The total number of equations in these unknowns is also equal to $n(m-1)^2$ since there are n subsets of equations 52, corresponding to the n values of k, and $(m-1)^2$ equations in each subset. Being that the system is homogeneous in the unknowns $C_{j'}^{vq}$, a non-trivial solution can only exist if the coefficient determinant formed from the values $R_k^{jj'}$ modifying the unknowns, is zero. This, formally implies that at least one equation must be a linear combination of the remaining equations. Such an equation may be considered redundant in the sense that it would not, of course, be necessary in determining a solution for the ratios of the unknowns from the remaining equations. In view of the symmetrical way in which the system 52 is constructed with respect to the index k, the question now arises as to whether any equation of 52, which may be concisely represented by $e_k^{vq}=0$, can be considered redundant selectively for a particular k or set of values k.

Certainly, for constants v and q, such an equation cannot be redundant for all n values of k since the unknowns $C_{j'}^{vq}$ that appear on the left hand side of equations 52 are not involved in any of the remaining equations. Therefore, one must assume that if all redundant equations are excluded, the same set of unknowns cannot be contained in every subset of equations 52 having constant index k. As a consequence, a solution obtained for the ratios of the unknowns $C_{j'}^{vq}$ from these equations, could not be a set of functions each of which involves the parameters $R_k^{jj'}$ symmetrically for all values of k, i.e., that reduce to identical expressions under any relabelling of the k indices. On the other hand, since the unknowns $C_{j'}^{vq}$ are independent of k and since we have previously excluded the case that the values $C_{j'}^{vq}$ can be constants that are independent of the system parameters, we must assume that some or all of the values $C_{j'}^{vq}$ are functions of the system parameters $R_k^{jj'}$ and that the value of these functions is unchanged under any relabelling of the k indices. However, in view of the previous observation that the values $C_{j'}^{vq}$ cannot all be functions that are algebraically symmetrical with respect to all values of k, this implies that each interchange of k indices will in general result in an altered set of functions whose values, though, must not change; but, any equation relating two non-trivial, non-identical functions of an independent set of system parameters must represent a constraint for the general system. In fact by equating the various expressions for the values of the ratios of the unknowns $C_{j'}^{vq}$ that are obtained under each relabelling of the k indices, a multiple set of constraints would be obtained.

Therefore, for the general case, a non-trivial solution for the $C_{j'}^{vq}$, which would imply that the value of the jacobian is zero, cannot exist except when the parameters $R_k^{jj'}$ satisfy all of the above constraints or those previously obtained by assuming the $C_{j'}^{vq}$ to be constants. These constraints are included in one general constraint for the parameters $R_k^{jj'}$ given by the expression equating the coefficient determinant of the homogeneous system 52 with zero.

For example, this constraint can obviously be satisfied when the values $R_k^{jj'}$ corresponding to two or more similar equations, $e_k^{vq} = 0$, differing only in k, are linearly related. Such a case is exhibited in the following examples. Of course, the expression D=0 itself represents the general constraint expressed in terms of the coefficients A_{ik}^{j}. Assuming this constraint is satisfied, the jacobian will equal zero at the correct solution point even though a reference compartment exists. However, as previously indicated, this does not necessarily imply that a solution for the transfer rate parameters cannot be obtained since the jacobian does not vanish identically. In example 1 , for n=2, it is seen that, in fact, a unique solution is obtained, for systems satisfying such a constraint. For $n > 2$, it would be difficult to determine whether a solution can always be obtained for any existing constrained systems. In any case, the number of systems satisfying such constraints must be small in the space of all strongly connected systems.

EXAMPLE 1:

Consider a strongly connected two compartment system for which the measured elements are the off-diagonal elements of T:

$$n=2, \quad m=2, \quad M = \{T_{12}, T_{21}\}$$

Let,

$$A_1 \equiv A^1_{11}, \quad A_2 \equiv A^1_{12}, \quad A_3 \equiv A^2_{21}, \quad A_4 \equiv A^2_{22}$$

$$R_1 \equiv R^{12}_1, \quad R_2 \equiv R^{12}_2, \quad R_3 \equiv R^{21}_1 = 1/R_1, \; R_4 \equiv R^{21}_2 = 1/R_2$$

Then,

$$D = -\begin{vmatrix} A_1 & A_2 \\ A_3 & A_4 \end{vmatrix}$$

The following equations follow from the conditions $\sum_{k=1}^{2} A^j_{ik} = \delta_{ij}$ and the relations 27b:

1) $A_1 + A_2 = 1$

2) $R_1A_1 + R_2A_2 = 0$

3) $A_3 + A_4 = 1$

4) $R_3A_3 + R_4A_4 = 0$

If we assume that D=0, then there must exist multiplicative constants m_1 and m_2 for columns one and two, not both zero, such that

5) $m_1A_1 + m_2A_2 = 0$

6) $m_1A_3 + m_2A_4 = 0$

From equations 1 and 2 we have,

$$A_1 = R_2/(R_2-R_1) \qquad R_1 \neq R_2$$

$$A_2 = -R_1/(R_2-R_1)$$

Substituting in 5, we obtain

$$m_1R_2 - m_2R_1 = 0 \quad \text{or*} \quad \begin{pmatrix} m_1 \\ m_2 \end{pmatrix} = C_2 \begin{pmatrix} R_1 \\ R_2 \end{pmatrix}$$

(The latter equation may have been written directly from 49)

Similarly, from equations 3, 4 and 6, we obtain

$$\begin{pmatrix} m_1 \\ m_2 \end{pmatrix} = C_1 \begin{pmatrix} R_3 \\ R_4 \end{pmatrix}$$

Therefore, the equations corresponding to the system 52 for this example are:

$$C_2\, R_1 = C_1\, R_3$$

and

$$C_2\, R_2 = C_1\, R_4$$

Hence, since $R_3=1/R_1$ and $R_4=1/R_2$, we obtain

$$C_1/C_2 = (R_1)^2 = (R_2)^2$$

* since R_1, $R_2 \neq 0$

Thus, either

$$R_1 = R_2 \quad \text{or} \quad R_1 = -R_2$$

The first case can never be true since if $R_1=R_2$ then

$$R_1A_1 + R_2A_2 = R_1(A_1 + A_2) = R_1 \neq 0$$

which contradicts equation 2 above.

The second case, $\underline{R_1 = -R_2}$, cannot be generally true since this would limit the degrees of freedom for specifying the system by one. Assuming such a constraint, we obtain from equations 1 through 4 above:

$$A_1 = A_2 = A_3 = A_4 = 1/2 \qquad \text{(c)}$$

Thus, any system satisfying this constraint will have a jacobian whose value is zero for the selected measurement functions T_{12} and T_{21}, even though a common reference compartment exists:

$$D = -\begin{vmatrix} \frac{1}{2} & \frac{1}{2} \\ \frac{1}{2} & \frac{1}{2} \end{vmatrix} = 0$$

However, the fact that the jacobian vanishes at a single point does not necessarily imply the irreversibility of the corresponding set of functions. Contrarily, it will now be shown that in fact a unique solution is obtained in the present case.

Consider any two compartment system satisfying the above constraint.

Let,

$$w \equiv A^1_{21}$$

Then by equations 29a , 29b , and constraint c

$$A^1_{22} = -w$$

$$A^2_{11} = 1/4w$$

$$A^2_{12} = -1/4w$$

(Note that since we have assumed that the measured functions are T_{12} and T_{21}, all of the above values as well as the eigenvalue parameters λ_1 and λ_2 would be obtained directly from measurement.)

Equations 34 for the parameters R_1 and R_2 are:

$$(1/4w)(1/R_1) - (1/4w)(1/R_2) = 1$$

$$wR_1 - wR_2 = 1$$

Solving this system, we obtain

$$(R_1 - 1/2w)^2 = 0$$

Therefore,

$$R_1 = 1/2w \quad \text{(double root)}$$

and

$$R_2 = -1/2w \text{ (double root)} \quad .$$

Since there is only one distinct set of values for R_1 and R_2 , and since the value of w is known, a unique solution can be obtained. Assuming no prior knowledge of the constraints, the unknowns A_1 and A_2 can now be determined:

$$A_1 = (1/R_1)\, A_{11}^2 = 1/2$$

$$A_2 = (1/R_2)\, A_{12}^2 = 1/2$$

Employing equation 5 of the text for j=1, we obtain:

$$a = (A^1) \begin{pmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{pmatrix} (A^1)^{-1} = \begin{pmatrix} 1/2 & 1/2 \\ w & -w \end{pmatrix} \begin{pmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{pmatrix} \begin{pmatrix} 1 & 1/2w \\ 1 & -1/2w \end{pmatrix}$$

EXAMPLE 2:

Consider a three compartment system for which the set of measured elements is given by M (compartment 1 is a common injection reference compartment):

$$n=3, \quad m=2, \qquad M = \left\{T_{11},\ T_{21},\ T_{32}\right\}$$

The determinant D given by 40 for this configuration is:

$$D = \begin{vmatrix} A_{11}^{2} & A_{12}^{2} & A_{13}^{2} \\ A_{21}^{2} & A_{22}^{2} & A_{23}^{2} \\ -A_{31}^{1} & -A_{32}^{1} & -A_{33}^{1} \end{vmatrix}$$

Assuming that D=0, equations 52 would be given by:

$$C_1 + C_2 R_k^{12} = -C_3 R_k^{23}, \quad k=1,2,3$$

Thus, a non-trivial solution can only exist for the constants C if the following constraint is satisfied:

$$\begin{vmatrix} 1 & R_1^{12} & R_1^{23} \\ 1 & R_2^{12} & R_2^{23} \\ 1 & R_3^{12} & R_3^{23} \end{vmatrix} = 0$$

Such a constraint would easily be satisfied if, for example,

$$R_k^{12} = R_{k'}^{12} \quad \text{and} \quad R_k^{23} = R_{k'}^{23}$$

for any two values of k and k'. Taking the inverse of each side of the first equation, we have also $R_k^{21} = R_{k'}^{21}$. Consider now the determinant

$$\begin{vmatrix} R_1^{21} & R_2^{21} & R_3^{21} \\ 1 & 1 & 1 \\ R_1^{23} & R_2^{23} & R_3^{23} \end{vmatrix} .$$

In the present case, this determinant would also equal zero, since two of its columns would be identical. If we multiply each column by the corresponding coefficient A_{ik}^2, k=1,2,3 the value of the determinant will, of course, still be zero. Thus,

$$A_i = \begin{vmatrix} A_{i1}^1 & A_{i2}^1 & A_{i3}^1 \\ A_{i1}^2 & A_{i2}^2 & A_{i3}^2 \\ A_{i1}^3 & A_{i2}^3 & A_{i3}^3 \end{vmatrix} = 0$$

However, this implies that the matrix A_i (the coefficient matrix for row i of the transition matrix) is singular. Since this must be true for every row i, it contradicts the assumption of a strongly connected system, as further discussed in Section VI.

SECTION VI

CONCLUSION

It has been shown that the existence of a common reference compartment for a random set of n measured elements of the transition matrix selected so that all n compartments are measured or all n are injected is, in general, a necessary and sufficient condition for completeness for strongly connected systems for which all n^2 transfer rates are initially unknown. Moreover, it has been postulated that the validity of the reference compartment criterion may be more generally extendable to certain non-strongly connected systems assuming that a set of n injection measurement functions exhibiting, as a whole, n distinct eigenvalue parameters is obtainable. In particular, if the eigenvector relations are valid for the columns or rows of T from which the measured elements are selected, then the eigenvector approach for obtaining a solution can be applied. However, as previously discussed, for such relations to be valid, certain topological flow characteristics are obviously essential. The structural implications of some of the basic mathematical assumptions involved in seeking a solution are examined here more closely.

It is evident that if, after initial injection in a compartment j, the labelled material entering (or reentering) a compartment i has no previous access to some of the compartments, then a corresponding number of terms in equation 2 will be zero since the element T_{ij} cannot reflect the existence of all n compartments. Thus, not all eignevalues λ_k will be exhibited in the element T_{ij} by itself since the coefficients A^j_{ik} corresponding to some of the factors $e^{\lambda_k t}$ will be zero. Nevertheless, assuming n distinct eigenvalues exist, the vectors formed from the coefficients A^j_{ik} corresponding to the n elements of an entire column or row of T, given by equations 3 and 16, will still be eigenvectors of α and α^T, respectively, as long as at least one of their components is not zero.

Thus, considering, first, experiments where the measured elements are selected from a set of columns of T, the eigenvector approach can be applied provided that the matrix A^j corresponding to each injected compartment j is not singular. In terms of the system topology, it is usually adequate to assume the existence of either a direct or indirect flow path from each of the injected compartments to all n compartments*, so that the elements T_{ij}

* see footnote on pg. 45 w.r.t. controllability.

in the m columns of T corresponding to the injected compartments are all different from zero. A set of n measurements selected from these columns so that all n compartments are sampled should compositely reflect evidence for the existence of n distinct system components.* Such a network of flow paths implies, of course, that at least the injected compartments must be strongly connected. Similarly, for row type experiments either a direct or indirect flow path must exist from all n compartments to each of the measurement compartments** if the eigenvector approach is to be applicable. Hence, in this case, the measurement compartments must be strongly connected. In either case, however, even if all n compartments are strongly connected, there exist certain cases (mathematically justifiable, though physically unlikely) where one or more eigenvectors may be absent, despite the fact that the structural requirements for the system connectivity are satisfied. The examples given in this section illustrate some of the above observations.

Since the proof of theorem T1 is based on the validity of the eignevector relations, the necessary condition of the reference compartment criterion for completeness is

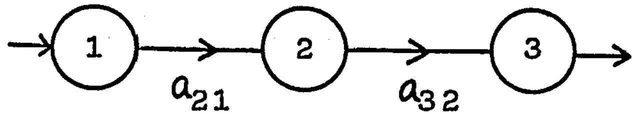
* It has been shown (see pg. 68) that if the eigenvector relations can be applied, then n eigenvalues are obtainable.

** see footnote on pg. 46 w.r.t. observability.

unaltered for those systems with limited flow connectivity for which the n injection-measurement functions can be selected from either columns or rows of the transition matrix which satisfy these relations. (One may extend lemma L1 to include such systems by utilizing eqs.27b or 28b instead of 29a in the proof and by including, respectively, the n(m-1) factors $R_k^{jj'}$ or $R_{ii'k}$ in the set of unknown parameters. The remaining part of the proof of T1 is unchanged.) Similarly, the expression of the Jacobian determinant Δ is algebraically unchanged. Here again, assuming a reference compartment exists, the expression D=0 then represents a general constraint which may not be physically satisfiable (see example 2) and which does not necessarily imply the insufficiency of the reference compartment criterion for any existing systems that may satisfy such a constraint. It may be of interest to note that although for limited flow systems it is implied that certain of the coefficients A_{ik}^j appearing in D must be zero, an entire column or row of D cannot be zero for any system, including limited flow systems, without violating the assumption with respect to the validity of the eigenvector relations for the columns or rows containing the measured elements. For a row (i,j) of zero elements of D, this is evident since it implies that $T_{ij}=0$ for some $j \in J$. For a column (p,k) of zero elements of D, it would be implied that $\underline{A}_k^p = 0$, as can be seen by considering the topological distribution of elements for a column of D (fig. 9) and applying the eigenvector relations to these elements. A precise determination of the extent of the sufficiency of the reference compartment criterion for limited flow systems cannot be presented here.

In the following examples, although a particular system model is examined, it is assumed that the system connectivity is initially unknown. Otherwise, it may not, of course, be necessary to obtain n measurement functions to completely determine the system.

EXAMPLE 1: n=3 Irreversible Catenary System



$$a = \begin{pmatrix} a_{11} & 0 & 0 \\ a_{21} & a_{22} & 0 \\ 0 & a_{32} & a_{33} \end{pmatrix}$$

Assuming that a has n distinct eigenvalues, $\lambda_1 = a_{11}$, $\lambda_2 = a_{22}$, $\lambda_3 = a_{33}$, the transition matrix for this system is*:

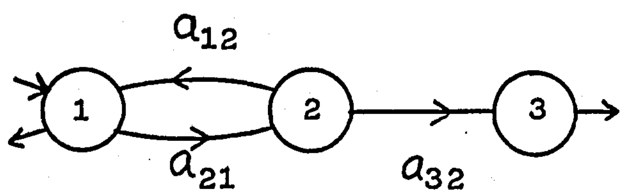
$$T = \begin{pmatrix} e^{\lambda_1 t} & 0 & 0 \\ \frac{a_{21}e^{\lambda_1 t}}{(\lambda_1-\lambda_2)} - \frac{a_{21}e^{\lambda_2 t}}{(\lambda_1-\lambda_2)} & e^{\lambda_2 t} & 0 \\ \frac{a_{21}a_{32}e^{\lambda_1 t}}{(\lambda_1-\lambda_2)(\lambda_1-\lambda_3)} + \frac{a_{21}a_{32}e^{\lambda_2 t}}{(\lambda_2-\lambda_1)(\lambda_2-\lambda_3)} + \frac{a_{21}a_{32}e^{\lambda_3 t}}{(\lambda_3-\lambda_1)(\lambda_3-\lambda_2)} & \frac{a_{32}e^{\lambda_2 t}}{(\lambda_2-\lambda_3)} - \frac{a_{32}e^{\lambda_3 t}}{(\lambda_2-\lambda_3)} & e^{\lambda_3 t} \end{pmatrix}$$

Thus, it is clear that the eigenvector approach can only be (trivially) applied to either the elements of column 1

* easily obtained by Laplace transformation

or row 3 to obtain a solution directly from equations 5 or 14 , respectively. For the remaining columns and rows, the corresponding coefficient matrix A^j or A_i, respectively, is singular. It could be forseen that a hybrid eigenvector approach to obtain a solution from elements chosen from more than one column or more than one row would not be applicable since none of the compartments are reversibly connected.

EXAMPLE 2: n=3



$$a = \begin{pmatrix} a_{11} & a_{12} & 0 \\ a_{21} & a_{22} & 0 \\ 0 & a_{32} & a_{33} \end{pmatrix}$$

Assuming n distinct eigenvalues, the transition matrix is given by

$$T = \begin{pmatrix} \frac{(\lambda_1 - a_{22})e^{\lambda_1 t}}{(\lambda_1-\lambda_2)} - \frac{(\lambda_2 - a_{22})e^{\lambda_2 t}}{(\lambda_1-\lambda_2)} & \frac{a_{12}\, e^{\lambda_1 t}}{(\lambda_1-\lambda_2)} - \frac{a_{12}\, e^{\lambda_2 t}}{(\lambda_1-\lambda_2)} & 0 \\ \frac{a_{21}\, e^{\lambda_1 t}}{(\lambda_1-\lambda_2)} - \frac{a_{21}\, e^{\lambda_2 t}}{(\lambda_1-\lambda_2)} & \frac{(\lambda_1 - a_{11})e^{\lambda_1 t}}{(\lambda_1-\lambda_2)} - \frac{(\lambda_2 - a_{11})e^{\lambda_2 t}}{(\lambda_1-\lambda_2)} & 0 \\ \frac{a_{21}a_{32}e^{\lambda_1 t}}{(\lambda_1-\lambda_2)(\lambda_1-\lambda_3)} + \frac{a_{21}a_{32}e^{\lambda_2 t}}{(\lambda_2-\lambda_1)(\lambda_2-\lambda_3)} + \frac{a_{21}a_{32}e^{\lambda_3 t}}{(\lambda_3-\lambda_1)(\lambda_3-\lambda_2)} & \frac{a_{32}(\lambda_1-a_{11})e^{\lambda_1 t}}{(\lambda_1-\lambda_3)(\lambda_1-\lambda_2)} + \frac{a_{32}(\lambda_2-a_{11})e^{\lambda_2 t}}{(\lambda_2-\lambda_3)(\lambda_2-\lambda_1)} + \frac{a_{32}(\lambda_3-a_{11})e^{\lambda_3 t}}{(\lambda_3-\lambda_1)(\lambda_3-\lambda_2)} & e^{\lambda_3 t} \end{pmatrix}$$

By considering the possible non-singular coefficient matrices A^j, it is seen that the eigenvector approach can be applied to both columns one and two either individually or in a hybrid experiment. Notice that compartments 1 and 2 are reversibly connected and that a flow path exists from each of these compartments to the remaining compartments. However, a row solution can only be obtained for row 3, in agreement with the fact that only compartment 3 has material entry from all other compartments.

For further illustration, consider the measurement set $M = \{T_{11}, T_{21}, T_{32}\}$ where either of the reversibly connected compartments 1 and 2 may be considered a common injection reference compartment:

$$T_{11} = A^1_{11} \cdot e^{\lambda_1 t} + A^1_{12} e^{\lambda_2 t}$$

$$T_{21} = A^1_{21} e^{\lambda_1 t} + A^1_{22} e^{\lambda_2 t}$$

$$T_{32} = A^2_{31} e^{\lambda_1 t} + A^2_{32} e^{\lambda_2 t} + A^2_{33} e^{\lambda_3 t}$$

The system of equations 34 for the parameters $R_1 \equiv R_1^{12}$, $R_2 \equiv R_2^{12}$ and $R_3 \equiv R_3^{12}$ is:

$$R_1 A^1_{11} + R_2 A^1_{12} = 0$$

$$R_1 A^1_{21} + R_2 A^1_{22} = 1$$

$$\frac{1}{R_1} A^2_{31} + \frac{1}{R_2} A^2_{32} + \frac{1}{R_3} A^2_{33} = 0$$

Solving this system, a unique solution is obtained for R_1, R_2, and R_3 and, hence, for a :

$$R_1 = \frac{-A^1_{12}}{A^1_{11}A^1_{22}-A^1_{12}A^1_{21}}, \quad R_2 = \frac{A^1_{11}}{A^1_{11}A^1_{22}-A^1_{12}A^1_{21}}, \quad R_3 = \frac{-A^2_{33}}{\frac{A^2_{31}}{R_1} + \frac{A^2_{32}}{R_2}}$$

$$A^1_{31} = \frac{A^2_{31}}{R_1}, \quad A^1_{32} = \frac{A^2_{32}}{R_2}, \quad A^2_{33} = \frac{A^2_{33}}{R_3}$$

$$a = \begin{pmatrix} A^1_{11} & A^1_{12} & 0 \\ A^1_{21} & A^1_{22} & 0 \\ A^1_{31} & A^1_{32} & A^1_{33} \end{pmatrix} \begin{pmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{pmatrix} \begin{pmatrix} A^1_{11} & A^1_{12} & 0 \\ A^1_{21} & A^1_{22} & 0 \\ A^1_{31} & A^1_{32} & A^1_{33} \end{pmatrix}^{-1}$$

For example, let the measured values be

$$\lambda_1 = -1.27, \quad \lambda_2 = -4.73, \quad \lambda_3 = -3$$

$$A^1_{11} = .79\ , \qquad A^1_{12} = .21$$

$$A^1_{21} = .29\ , \qquad A^1_{22} = -.29$$

$$A^2_{31} = .12\ , \qquad A^2_{32} = -.46\ , \qquad A^2_{33} = .33$$

Then,

$$\frac{1}{R_1} = 1.37\ , \qquad \frac{1}{R_2} = -.37\ , \qquad \frac{1}{R_3} = -1$$

$$A^1 = \begin{pmatrix} .79 & .21 & 0 \\ .29 & -.29 & 0 \\ .17 & .17 & -.33 \end{pmatrix}$$

Thus,

$$\mathcal{a} = (A^1)\,\mathcal{A}\,(A^1)^{-1} = \begin{pmatrix} -2 & 2 & 0 \\ 1 & -4 & 0 \\ 0 & 1 & -3 \end{pmatrix}$$

Note, that even though a hybrid set of measurements are selected, a unique solution is obtained in each such case. That a solution can always be obtained for this system is verified by the fact that the jacobian determinant Δ , evaluated below for the set of measurements $\{T_{11}, T_{21}, T_{32}\}$, can never be zero.

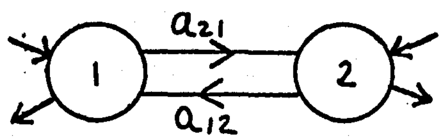
$$\Delta = \frac{1}{R_1R_2R_3}\begin{vmatrix} A_{11}^2 & A_{12}^2 & A_{13}^2 \\ A_{21}^2 & A_{22}^2 & A_{23}^2 \\ -A_{31}^1 & -A_{32}^1 & -A_{33}^1 \end{vmatrix}$$

$$= \frac{a_{12}\, a_{21}\, a_{32}}{R_1R_2R_3(\lambda_1 - \lambda_3)(\lambda_1 - \lambda_2)(\lambda_3 - \lambda_2)} \neq 0$$

As previously indicated in the text, if for a given system the eigenvector relations are valid for all rows and columns of T (i.e., if the coefficient matrix of each row and column is not singular), then no coefficient A^j_{ik} can be zero. (This is evident since if one assumes the contrary, i.e., that one of the coefficients A^j_{ik} is zero, then the eigenvector relations imply that A^j_{ik} must be zero for every j and i for that value of k. Hence, the vectors $\underline{A}^j_k$ and $\underline{A}i_k$ for a constant k would be null vectors for every j and i which contradicts the assumption that they are eigenvectors of a and a^T, respectively.) Assuming that the system in strongly connected usually implies that none of the coefficients A^j_{ik} are zero and, therefore, that the eigenvector relations are applicable for all rows and columns of T. For n=2 there are no exceptions to this case, provided that two distinct eigenvalues $\lambda_1 \neq \lambda_2$ exist, as shown in the following example. On the other hand, for $n > 2$, certain special cases may exist where because of a unique relation among the a_{ij} parameters, some of the coefficients A^j_{ik} may be zero even though the system is strongly connected (see example 4). Thus, the eigenvector relations can always be applied for strongly connected systems in a structural sense only*.

* see footnote on pg.40

EXAMPLE 3: n=2 Strongly Connected System



Expressing the coefficients A_{ik}^{j} in terms of the parameters a_{ij}, we have

$$A_{11}^{1}=\frac{\lambda_1-a_{22}}{\lambda_1-\lambda_2},\quad A_{12}^{1}=-\frac{\lambda_2-a_{22}}{\lambda_1-\lambda_2},\quad A_{11}^{2}=\frac{a_{12}}{\lambda_1-\lambda_2},\quad A_{12}^{2}=-\frac{a_{12}}{\lambda_1-\lambda_2},$$

$$A_{21}^{1}=\frac{a_{21}}{\lambda_1-\lambda_2},\quad A_{22}^{1}=-\frac{a_{21}}{\lambda_1-\lambda_2},\quad A_{21}^{2}=\frac{\lambda_1-a_{11}}{\lambda_1-\lambda_2},\quad A_{22}^{2}=-\frac{\lambda_2-a_{11}}{\lambda_1-\lambda_2}$$

Thus, all A_{ik}^{j} will not be zero provided that

$$a_{12}\neq 0,\quad a_{21}\neq 0,$$

$$\lambda_1\neq a_{11},\ a_{22}$$

$$\lambda_2\neq a_{11},\ a_{22}$$

But, since the eigenvalues λ_1 and λ_2 must satisfy the equation $\lambda^2-(a_{11}+a_{22})+a_{11}a_{22}-a_{12}a_{21}=0$, where $\lambda_1+\lambda_2=a_{11}+a_{22}$ and $\lambda_1\lambda_2=a_{11}a_{22}-a_{12}a_{21}$, the above conditions will all be satisfied if $a_{12}a_{21}\neq 0$ Therefore, if the n=2 system is reversibly connected, the coefficients A_{ik}^{j} will all be different from zero.

EXAMPLE 4: $n=3$ Strongly Connected System for Which Some of the Coefficients A^j_{ik} are Zero

Consider the strongly connected three compartment system whose transfer rate matrix is

$$\mathcal{a} = \begin{pmatrix} -8 & 2 & 5 \\ 1 & -4 & 1 \\ 1 & 3 & -6 \end{pmatrix}$$

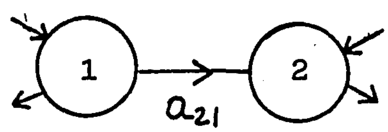
$$\lambda_1 = -1.86, \quad \lambda_2 = -9.14, \quad \lambda_3 = -7$$

The transition matrix T for this system is

$$T = \begin{pmatrix} (.157,\ .843,\ 0)\underline{e} & (.622, .560, -1.182)\underline{e} & (.339, -1.521, 1.182)\underline{e} \\ (.137, -.137,\ 0)\underline{e} & (.546, -.0912, .546)\underline{e} & (.298, .248, -.546)\underline{e} \\ (.137, -.137,\ 0)\underline{e} & (.546, -.091, -.455)\underline{e} & (.298, .248, .455)\underline{e} \end{pmatrix}$$

Thus, even though the system is strongly connected, a solution cannot be obtained from the elements of column 1 since the corresponding coefficient matrix A^1 is singular. However, the eigenvector approach can still be applied to the elements of columns 2 and 3 and to any set of rows of T.

From the previous examples it is evident that the measurement topology must be compatible with the underlying system flow topology if the eigenvector approach is to be applicable. Thus, if a flow path does not exist between two compartments, the corresponding injection measurement function will be zero. However, although the eigenvector approach cannot be applied to a set of n measurement functions which include one or more null measurement functions, such a set may still be complete. For example, for n=2, it can be seen from 25 and 26 and the fact that $a_{ij}=\dot{T}_{ij}(0)^*$, that any pair of measurements that satisfy the reference compartment criterion are complete as long as two distinct eigenvalues can be determined. Thus, e.g., the set $M=\{T_{12},T_{21}\}$ would be complete for the following system,



where,

$$T_{12} = 0\,, \quad T_{21} = \frac{a_{21}}{a_{11}-a_{22}} e^{\lambda_1 t} - \frac{a_{21}}{a_{11}-a_{22}} e^{\lambda_2 t},$$

$$\lambda_1 = a_{11}, \quad \lambda_2 = a_{22}, \quad \lambda_1 \neq \lambda_2$$

even though one of the measurements is a null value. (Note, that the measurement of T_{21} alone would be insufficient since we have assumed that knowledge of the reduced system is established following and not prior to any

* i.e., by applying the Direct Approach

measurement.) However, for the same system, the set $M = \{T_{12}, T_{22}\}$ must be incomplete even though the reference compartment criterion is satisfied, since $T_{12}=0$, $T_{22} = e^{\lambda_2 t}$ and, therefore, both eigenvalues cannot be determined. Similarly, for n=3, in example 4 the set $M = \{T_{11}, T_{21}, T_{31}\}$ is incomplete since all three eigenvalues cannot be determined, even though none of the measurement functions are trivial and even though the reference compartment criterion is satisfied.

Although it is likely that the reference compartment criterion is a more general necessary and sufficient condition for minimal completeness for multicompartment systems for which equation 1 is valid, provided that n distinct eigenvalues can be determined, the proofs presented here are limited to systems for which the eigenvector relations are wholly or in part valid, with some noted exceptions for verifying proof of sufficiency. Of course, in practice the general nature of the system, i.e., whether the measurement topology will be compatible with the underlying structural topology, may not be known a priori. Therefore, one cannot utilize the reference compartment criterion to predetermine with absolute certainty whether a set of measurements will be minimally complete and whether the eigenvector approach will be applicable for obtaining a solution. Indications that the system is not compatible as, for example, null measurements or measurements which do not exhibit the existence of n eigenvalue parameters, may be revealed once the actual process of obtaining measurements has begun or in the analysis of such measurements.

As previously discussed, the distinction between criteria for the theoretical concept of completeness and for the actual determination of a true solution must be made. Further, the complex practical considerations of data aquisition and analysis must be considered by the experimentalist. With respect to the latter, it is suggested that for higher order systems the eigenvector approach may lend itself readily to programming techniques such as Newton's method because of the algebraic simplicity with which the general system of equations can be concisely formated. However, the primary purpose of this study has not been to develop a new experimental approach for obtaining complete solutions but, rather, to examine the fundamental theoretical questions that may arise in the aquisition and analysis of measurements obtained for such a solution. In this respect, the development of theoretical completeness criteria to define the classes of "measurement topologies" that are compatible with a general class of physical systems, may be a concept that is worthy of more broader consideration.

APPENDIX A

Consider the first order differential equation

$$\dot{\underline{x}} = \mathcal{Q}\,\underline{x} \qquad \text{(A-1)}$$

where $\underline{x}$ is an n component vector and $\mathcal{Q}$ is an nxn scalar matrix. Let

$$\underline{x} = A\,\underline{e} \qquad \text{(A-2)}$$

be a solution to equation A-1 , corresponding to arbitrary initial conditions, where A is an nxn scalar matrix and $\underline{e}$ is an n component vector given by

$$\underline{e} = \begin{pmatrix} e^{\lambda_1 t} \\ e^{\lambda_2 t} \\ \vdots \\ e^{\lambda_n t} \end{pmatrix}, \qquad \text{(A-3)}$$

where the eigenvalues λ_k, k=1,2,...,n are distinct.
Then,

$$\dot{\underline{x}} = A\,\dot{\underline{e}} = A\lambda\,\underline{e}\ , \qquad \text{(A-4)}$$

where

$$\lambda = \begin{pmatrix} \lambda_1 & & & 0 \\ & \lambda_2 & & \\ & & \ddots & \\ 0 & & & \lambda_n \end{pmatrix} \qquad \text{(A-5)}$$

Substituting A-2 into A-1 , we obtain

$$\underline{\dot{x}} = \alpha A \, \underline{e} \qquad \text{(A-6)}$$

Therefore, equating A-4 and A-6

$$(\alpha A - A\lambda)\,\underline{e} = 0 \qquad \text{(A-7)}$$

and, since, the eigenvalues λ_k are assumed to be distinct, independent parameters, the following relation must be true:

$$\alpha A = A\lambda \qquad \text{(A-8)}$$

This relation implies that the equation

$$\alpha \underline{A}_k = \lambda_k \underline{A}_k \qquad \text{(A-9)}$$

must always be satisfied for each column vector $\underline{A}_k$ of A. Thus, unless it is a null vector, each column vector of A will be an eigenvector of α, i.e., unless all of its components A_{ik} are zero. In the case that none of the n vectors $\underline{A}_k$, k=1,2,...,n, are null vectors, A will be non-singular, since the eigenvectors of a matrix that has distinct eigenvalues are linearly independent. Hence, it follows from A-8 that the following inverse diagonalization equation* will be valid for A, under this assumption:

$$\alpha = A\lambda A^{-1} \qquad \text{(A-10)}$$

*This equation would, of course, be valid for any matrix that is composed of n independent eigenvectors of α (Gantmacher, 1960) .

APPENDIX B

If in appendix A one specifies initial conditions corresponding to unit initial concentration in a compartment p and zero in all other compartments, then $\underline{x} = \underline{x}^p$ corresponds to a column p of the transition matrix and $A = A^p$ is the corresponding coefficient (A^p_{ik}) matrix. Similar results to those obtained in appendix A , are obtained below for a row p of the transition matrix, represented by the vector $\underline{x}_p$ and the associated coefficient (A^j_{pk}) matrix A_p. (The matrices A^p and A_p are also defined in II)

Let $\underline{x}^p$ and $\underline{x}_p$ be the n dimension vectors corresponding to the elements of column p and row p of T, respectively. Both $\underline{x}^p$ and $\underline{x}_p$ have a common diagonal element $x_{pp} = \sum_k A^p_{pk}\, e^{\lambda_k t}$ and the same* initial vector $\underline{x}^p_o$, whose elements are $x^p_{oi} = \delta_{ip}$. Thus,

$$\underline{x}^p = T\, \underline{x}^p_o = A^p\, \underline{e} \quad , \tag{B-1}$$

$$\underline{x}_p = T^\tau \underline{x}^p_o = A_p\, \underline{e} \quad , \tag{B-2}$$

where A^p and A_p are the coefficient matrices corresponding to the elements of column p and row p of T, respectively, that are mentioned above. Since $\underline{x}^p$ must satisfy equations A-1 and B-1 , we have

$$\dot{\underline{x}}^p = a\underline{x}^p = \dot{T}\, \underline{x}^p_o = a\, T\, \underline{x}^p_o \tag{B-3}$$

* mathematically equivalent, though physically distinct

The last equation states that the p^{th} columns of $\dot{T}$ and of αT are equal. Since p may correspond to any one of the n columns of T, the following well known relation (Bellman, 1970) must be true:

$$\dot{T} = \alpha T \tag{B-4}$$

Thus, also $\quad \dot{T}^{\tau} = T^{\tau}\alpha^{\tau}$

It is also well known that T commutes with α as may be directly observed from the solution to B-4 :

$$T = e^{\alpha t} = I + \alpha t + \frac{\alpha^2 t^2}{2!} + \ldots \tag{B-5}$$

Since T^{τ} must also commute with α^{τ}, we obtain from B-2 and B-4

$$\underline{\dot{x}}_p = \dot{T}^{\tau}\,\underline{x}_o^p = T^{\tau}\alpha^{\tau}\underline{x}_o^p = \alpha^{\tau}T^{\tau}\underline{x}_o^p = \alpha^{\tau}\underline{x}_p \tag{B-6}$$

Thus, $\underline{x}_p$ satisfies an equation analogous to equation A-1 :

$$\underline{\dot{x}}_p = \alpha^{\tau}\underline{x}_p \tag{B-7}$$

Further, from B-2 and B-7 we have

$$\underline{\dot{x}}_p = \alpha^{\tau}A_p\,\underline{e} = A_p\lambda\underline{e} \ , \tag{B-8}$$

and, since the eigenvalues λ_k are distinct, independent parameters, we obtain

$$\alpha^{\tau} A_p = A_p \Lambda \tag{B-9}$$

Thus, in analogy with equation A-9, the column vectors of A_p must be eigenvectors of α^{τ}, unless they are null vectors:

$$\alpha^{\tau} \underline{A}_{p_k} = \Lambda_k \underline{A}_{p_k} , \tag{B-10}$$

where

$$\underline{A}_{p_k} \equiv \begin{pmatrix} A^1_{pk} \\ A^2_{pk} \\ \vdots \\ A^n_{pk} \end{pmatrix} \tag{B-11}$$

Further, if each of the column vectors of A_p is an eigenvector of α^{τ}, i.e., if A_p is not singular, then the following inverse diagonalization relationship, analogous to A-10 , must be true:

$$\alpha^{\tau} = (A_p) \Lambda (A_p)^{-1} \tag{B-12}$$

APPENDIX C

Consider the case where none of the n^2 vectors, $\underline{A}_k^j$, j,k=1,2,...,n , are null vectors. Then in accordance with equation A-9, each vector $\underline{A}_k^j$ must be an eigenvector of $\mathcal{a}$. Further, the factors $R_k^{jj'}$ defined by the equation

$$\underline{A}_k^{j'} = R_k^{jj'} \underline{A}_k^j , \qquad \text{(C-1)}$$

relating any two vectors $\underline{A}_k^j$ and $\underline{A}_k^{j'}$, corresponding to the same eigenvalue λ_k , must be non-zero constants. Such a system must be strongly connected, since, if any element T_{ij} of T is zero, then the corresponding matrix A^j must be singular, which implies the existence of a null component vector, contrary to the above initial assumption. For a fixed j, the factors $R_k^{jj'}$, j',k =1,2,...,n , form an nxn matrix R^j whose j'k$\underline{th}$ element is given by $R_k^{jj'}$:

$$R^j = \begin{pmatrix} R_1^{j1} & R_2^{j1} & \cdots & R_n^{j1} \\ R_1^{j2} & R_2^{j2} & \cdots & R_n^{j2} \\ \vdots & \vdots & & \vdots \\ R_1^{jn} & R_2^{jn} & \cdots & R_n^{jn} \end{pmatrix} \qquad \text{(C-2)}$$

Take any vector $\underline{A}_{i_k}$ as defined by equation B-11 . Then, by B-10 , $\underline{A}_{i_k}$ must satisfy the relation

$$\alpha^{\tau}\underline{A}i_k = \lambda_k \underline{A}i_k \qquad (C-3)$$

It is evident that the vector $\underline{A}i_k$ is equal to the kth column vector of R^j times the coefficient A^j_{ik} for any j :

$$\underline{A}i_k = A^j_{ik}\, \underline{R}^j_k \qquad (C-4)$$

Substituting in C-3 , we have

$$\alpha^{\tau} A^j_{ik}\, \underline{R}^j_k = \lambda_k A^j_{ik}\, \underline{R}^j_k \qquad (C-5)$$

Thus, if A^j_{ik} is not zero, it may be factored from the equation, resulting in the relation

$$\alpha^{\tau} \underline{R}^j_k = \lambda_k \underline{R}^j_k \qquad (C-6)$$

Clearly, the coefficients A^j_{ik} cannot be zero for every i=1,2,...,n, otherwise, the vector $\underline{A}^j_k$ would be a null vector which contradicts the initial assumption. Thus, equation C-6 must be true for every k=1,2,...,n. Hence, for any j, the n vectors $\underline{R}^j_k$, k=1,2,...,n are n independent eigenvectors of α^{τ} . It follows that R^j must be non-singular and the inverse diagonalization relationship

$$\alpha^{\tau} = (R^j)\, \lambda\, (R^j)^{-1} \qquad (C-7)$$

must be true. Hence, the n^2-n factors $R_k^{jj'}$, $j',k=1,2,\ldots,n$, $j' \neq j$ must be independent parameters capable with λ of defining the system. The coefficients A_{ik}^{j} may be expressed in terms of the parameters $R_k^{jj'}$ as follows:

From equations 27b and 29b, interchanging j and j', we obtain

$$\sum_{k=1}^{n} R_k^{jj'} A_{ik}^{j} = \delta_{ij'} , \quad j' = 1,2,\ldots,n \qquad \text{(C-8)}$$

Since the matrix R^j is non-singular, we may solve the linear system C-8 for the factors A_{ik}^{j}. Thus,

$$A_{ik}^{j} = \frac{\mathrm{cof}(R^j)_{ik}}{|R^j|} \qquad \text{(C-9)}$$

Similarly, in an entirely parallel proof, one can show that if the vectors $\underline{A}i'_k$, $i',k=1,2,\ldots,n$, defined by equation B-11, are all eigenvectors of a^{τ}, then the non-zero factors $R_{ii'k}$, relating pairs of vectors having the same eigenvalue λ_k

$$\underline{A}i'_k = R_{ii'k}\, \underline{A}i_k \qquad \text{(C-10)}$$

form an nxn matrix R_i,

$$R_i = \begin{pmatrix} R_{i11} & R_{i12} & \cdots & R_{i1n} \\ R_{i21} & R_{i22} & \cdots & R_{i2n} \\ \vdots & & & \\ R_{in1} & R_{in2} & \cdots & R_{inn} \end{pmatrix} \tag{C-11}$$

whose column vectors $\underline{R}i_k$ are eigenvectors of $\mathcal{a}$:

$$\mathcal{a}\, \underline{R}i_k = \lambda_k\, \underline{R}i_k \tag{C-12}$$

Thus, in this case, the n^2-n factors $R_{ii'k}$, $i',k=1,2,\ldots,n$, must be independent system parameters capable with λ of defining the system:

$$\mathcal{a} = (R_i)\,\lambda\,(R_i)^{-1} \tag{C-13}$$

and,

$$A^j_{ik} = \frac{\text{Cof}(R_i)_{jk}}{|R_i|} \tag{C-14}$$

Finally, if both sets of eigenvector relations C-1 and C-10 are valid for all set of vectors $\underline{A}^j_k$ and $\underline{A}i_k$, respectively, then, of course, all of the above equations must be true. This assumption also implies that none of the n^3 coefficients A^j_{ik} can be zero, since if one such coefficient is zero, then by equations C-4 and the equation

$$\underline{A}_k^j = A_{ik}^j \, \underline{R}i_k \, , \tag{C-15}$$

both vectors $\underline{A}i_k$ and $\underline{A}_k^j$ must be null vectors, contrary to assumption. Utilizing equations C-9 and C-14 , one can then relate the parameters $R_k^{jj'}$ and $R_{ii'k}$ as follows:

$$R_k^{jj'} = \frac{\mathrm{Cof}(R_i)_{j'k}}{\mathrm{Cof}(R_i)_{jk}} \tag{C-16}$$

and,

$$R_{ii'k} = \frac{\mathrm{Cof}(R^j)_{i'k}}{\mathrm{Cof}(R^j)_{ik}} \tag{C-17}$$

APPENDIX D

It has been shown with the aid of the previous appendices, that the equations of section II can be applied to experiments where each initial injection site and each measurement site is a single compartment. Here, it is shown that since we have assumed a linear system, we may more generally extend the applicability of such a set of equations to include experiments where each injection or measurement "site" may involve a multiple set of compartments. Each such multicompartment site may be a single, physically distinct site for which simultaneous input to or output from a set of compartments occurs or it may represent a set of distinct compartments for which injection or measurement is performed simultaneously. In either case, the mathematical analysis is equivalent. For the most general case, both injection and measurement may occur at multicompartment sites, where each site is not necessarily operative for both injection and measurement.

To simplify the analysis, consider first a standard type of experiment where only injection occurs at a multicompartment site g and each of the n compartments i are measured. Assuming that the initial dose D_g in g is instantaneously distributed among the set of the compartments corresponding to the site g, the analysis is a direct application of the results obtained in appendix A .

Thus,

$$D_g = \sum_{j=1}^{n} c_j^g \; V_j \tag{D-1}$$

where c_j^g is the resulting initial concentration in a single compartment j . The initial compartmental concentration vector $\underline{x}(0)$ is, therefore,

$$\underline{x}^g(0) = \begin{pmatrix} c_1^g \\ c_2^g \\ \vdots \\ c_n^g \end{pmatrix} \tag{D-2}$$

Similarly, we may set $\underline{x}(t) = \underline{x}^g(t)$, $A = A^g$, and $\underline{A}_k = \underline{A}_k^g$ in the equations of appendix A , where the elements of the matrix A^g may be denoted by A_{ik}^g. Assuming that none of the vectors $\underline{A}_k^g$ are null, they must be eigenvectors of $\mathcal{a}$ and a complete solution can be obtained from the equation

$$\mathcal{a} = (A^g)\lambda(A^g)^{-1} \tag{D-3}$$

Thus, as is well known (see, e.g., Cobelli and Romanin-Jacur, 1976), the standard method of obtaining a solution is unaltered by assuming injection at a multiple compartment site.

Consequently, for a hybrid type experiment where injection can occur at more than one multiple compartment site and all n compartments are measured, a set of equations similar to equations 27 must apply, assuming independent injection sites.

To further clarify this generalization, we may employ the concept of an "injection space" defined by n basis vectors, $\underline{x}^j(0)$, j=1,2,...,n, corresponding to unit initial concentrations in each of the n compartments, respectively:

$$\begin{pmatrix}1\\0\\ \cdot\\ \cdot\\ \cdot\\0\end{pmatrix}, \begin{pmatrix}0\\1\\ \cdot\\ \cdot\\ \cdot\\0\end{pmatrix}, \ldots, \begin{pmatrix}0\\0\\ \cdot\\ \cdot\\ \cdot\\1\end{pmatrix}$$

(This representation, for an initial input configuration, corresponds to the basic structure of the matrix b employed in the linear system theory representation $\dot{\underline{x}} = a\underline{x} + b\underline{u}$. (Bellman and Astrom, 1970; Cobelli and Jacur, 1975). Since we have a linear system, it is evident that the initial concentration vector $\underline{x}^g(0)$, corresponding to injection at a multicompartment site g, must be a linear combination of the basis vectors:

$$\underline{x}^g(0) = \sum_{j=1}^{n} c_j^g \, \underline{x}^j(0) \qquad \text{(D-4)}$$

Similarly at time t, $\underline{x}^g = \sum_{j=1}^{n} c_j^g \, \underline{x}^j$, as verified by equation 9. For a set of m independent injection sites g_p, p=1,2,...,m, the concentration vectors $\underline{x}^{g_p}$ must be linearly independent. However, for any pair of independent injection sites, the corresponding generalized column eigenvectors $\underline{A}_k^{g_1}$ and $\underline{A}_k^{g_2}$ must be linearly related by a non-trivial constant $R_k^{g_2 g_1}$, k=1,2,...,n:

$$\underline{A}_k^{g_1} = R_k^{g_2 g_1} \, \underline{A}_k^{g_2} \qquad \text{(D-5)}$$

Therefore, an analogous set of equations, similar to equations 27 must be valid for the generalized column eigenvectors $\underline{A}_k^g$. Further, the system of equations 29b may be replaced by the equations:

$$\sum_{k=1}^{n} A_{ik}^{g} = c_i^g \qquad \text{(D-6)}$$

To complete this generalization, one must consider that each measurement is also performed at a multicompartment site s such that the concentration function x_s^g corresponding to each measurement site is a weighted linear sum of the concentrations at each compartment:

$$x_s^g = \sum_{i=1}^{n} w_{si}\, x_i^g \qquad \text{(D-7)}$$

For any set of independent measurement sites, the corresponding weight vectors

$$\begin{pmatrix} w_{s1} \\ w_{s2} \\ \vdots \\ w_{sn} \end{pmatrix} \qquad \text{(D-8)}$$

must be linearly independent. For n such independent measurement sites, s_p, $p=1,2,\ldots,n$ the concentration vector defined by $\underline{x}_S^g$, is given by

$$\underline{x}_S^g = \begin{pmatrix} x_{s_1}^g \\ x_{s_2}^g \\ \vdots \\ x_{s_n}^g \end{pmatrix} . \qquad \text{(D-9)}$$

Clearly, the concentration vector $\underline{x}_S^g$ for measurement at n multicompartment sites is related to the previously defined vector $\underline{x}^g$ corresponding to measurement at n single compartment sites, by the relation

$$\underline{x}_S^g = W \, \underline{x}^g \quad , \qquad \text{(D-10)}$$

where W is an nxn matrix composed of the weighting factors:

$$W = \begin{pmatrix} w_{s_1 1} & w_{s_1 2} & \cdots & w_{s_1 n} \\ w_{s_2 1} & w_{s_2 2} & \cdots & w_{s_2 n} \\ \vdots & \vdots & & \vdots \\ w_{s_n 1} & w_{s_n 2} & \cdots & w_{s_n n} \end{pmatrix} . \qquad \text{(D-11)}$$

Utilizing the above relation and the fact that $\underline{x}^g$ satisfies the system equation

$$\dot{\underline{x}}^g = a \, \underline{x}^g \quad , \qquad \text{(D-12)}$$

we obtain

$$\dot{\underline{x}}_S^g = W \dot{\underline{x}}^g = W a \underline{x}^g = W a W^{-1} W \, \underline{x}^g \quad ,$$

where W cannot be singular since the measurement sites were assumed to be independent. Thus, $\underline{x}_S^g$ satisfies the modified system equation

$$\dot{\underline{x}}_S^g = \langle a \rangle_W \, \underline{x}_S^g \,, \quad \text{where} \quad \langle a \rangle_W = W \, a \, W^{-1} \qquad \text{(D-13)}$$

We can, therefore, conclude that the coefficient vectors $\underline{A}_{S_k}^g$, corresponding to measurement at n multicompartment sites, must be eigenvectors* of $\langle a \rangle_W$ and that the value of $\langle a \rangle_W$ can be obtained by the inverse diagonalization relation:

$$\langle a \rangle_W = (A_S^g) \, \lambda \, (A_S^g)^{-1} \qquad \text{(D-14)}$$

Thus, equations D-5 will still be valid if we set

$$\underline{A}_k^g \rightarrow \underline{A}_{S_k}^g$$

and

$$R_k^{g_2 g_1} \rightarrow R_{S_k}^{g_2 g_1} \, .$$

Note, also, that in the most general case involving both injection and measurement at multicompartment sites, the initial value equations become:

$$\sum_{k=1}^{n} A_{sk}^g = \sum_{i=1}^{n} w_{si} \, \hat{c}_i^g \qquad \text{(D-15)}$$

* assuming that they are not null vectors

Finally, it should be evident that analogous results can be obtained for generalized "row" type experiments involving n independent multicompartment injection sites g_p, p=1,2,...,n and one or more multicompartment measurement sites s. In this case the generalized "row" concentration vector for each s is:

$$\underline{x}_s^G = \begin{pmatrix} x_s^{g_1} \\ x_s^{g_2} \\ \vdots \\ x_s^{g_n} \end{pmatrix} \qquad \text{(D-16)}$$

Thus, in analogy to the equations previously obtained for the generalized "column" type experiment , the following equations are obtained for the generalized "row"type experiment:

$$\dot{\underline{x}}_s^G = \langle a^T \rangle_C \, \underline{x}_s^G \quad , \qquad \text{(D-17)}$$

where

$$\langle a^T \rangle_C = C a^T C^{-1} \quad \text{and} \quad C = \begin{pmatrix} c_1^{g_1} & c_2^{g_1} & \cdots & c_n^{g_1} \\ c_1^{g_2} & c_2^{g_2} & \cdots & c_n^{g_2} \\ \vdots & \vdots & & \vdots \\ c_1^{g_n} & c_2^{g_n} & & c_n^{g_n} \end{pmatrix}$$

Thus,

$$\langle a^T \rangle_C = (A_s^G) \lambda (A_s^G)^{-1} \quad , \qquad \text{(D-18)}$$

assuming that the coefficient vectors $\underline{A}_{s_k}^{G}$ are not null. The above equations are, of course, generalizations of the equations obtained in appendix B for row type experiments.

Thus the eigenvector approach for obtaining a solution can be generally extended for experiments involving multicompartment input sites and weighted measurement sites. It is assumed that the matrices A_S^g or A_s^G corresponding to each input site g or measurement site s must be non-singular for generalized column or row type experiments, respectively, and that the factors w_{si} and c_i^g employed by eq. D-15 are known or obtainable. It seems reasonable to expect that the reference compartment criterion can also be extended to include such experiments in some generalized context. However, a simple generalization is not immediately apparent except for the trivial cases involving single row or column type experiments.

APPENDIX E

Topological concepts are widely used to define properties of network systems that are related to the connectivity of the system and not to the particular system values. The usefulness of such a representation was first recognized by Kirchhoff for electrical networks but have since been applied to various other areas such as mechanical and fluid flow systems.[1-3] In particular with respect to compartmental systems, topological concepts have been used to describe properties related to the system structural connectivity.[4-6] In direct analogy, such topological concepts are employed in this thesis to characterize the "connectivity" of measurement sets as well.

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