DELAYED RESPONSE IN TRACER EXPERIMENTS
AND FRAGMENT-CARRIER APPROACH TO
TRANSIT TIME DISTRIBUTIONS IN NONLINEAR
CHEMICAL KINETICS

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A delayed response tracer experiment is suggested, based on the following constraints: (1) The kinetics of the process can be expressed by local evolution equations without delays, for example by the mass action law. (2) The kinetic isotope effect can be neglected, that is, the rate coefficients for labeled and unlabeled chemicals are the same. (3) The total fluxes of the various chemicals are generally time dependent, but are not modified by the presence of the labeled compounds. (4) The experiment consists in the measurement of the time dependence of the fractions $\beta_u, u = 1, 2, \ldots$ of labeled chemicals in the output fluxes as functionals of the time dependence of the fractions $\alpha_u, u = 1, 2, \ldots$ of labeled chemicals in the input fluxes, which are controlled by the researcher. We show that the output fluxes are related to the input fluxes by a linear delayed superposition theorem: $\beta_u(t) = \sum_{u'} \int \chi_{uu'}(t, t') \alpha_{u'}(t') dt'$, where $\chi_{uu'}(t, t')$, is a delayed susceptibility function, which is related to the probability density of the transit time, that is, the time necessary for a molecular fragment to cross the system. This linear superposition law is not the result of a linearization procedure and holds even if the underlying kinetic equations are highly nonlinear. We establish a relationship between the transit time probability densities and the lifetime distributions of the various species in the system. The law permits extracting information about the mechanism and kinetics of chemical processes from response experiments.

Keywords: Nonlinear kinetics; response experiments.

1. Introduction

Various types of response experiments, qualitative as well as quantitative, have been successfully applied to the study of complex reaction mechanisms, such as electrochemical reactions, homogeneous or heterogeneous catalytic processes, oxidation reactions, and enzymatic reactions in biochemistry. A first group of such approaches is based on the study of the response of a chemical system at equilibrium to small perturbations of the state variables, such as the temperature or pressure. The main feature of this technique, known as the method of chemical relaxation, [Strehlow, 1992, and references therein] is the use of perturbations small enough so that the response of the system is linear, yet large enough
so that the response of the system to the perturbation can be accurately measured experimentally [Mihaliuk & Collab 1999]. Since this type of experiment can be described by linear evolution equations, a systematic study of the kinetics of the process is possible, based on the use of normal mode theory.

A second group of response experiments makes use of radioactive or fluorescent labels [Neiman & Gal, 1971]. The tracer experiments can be used for the qualitative identification of the connections among the various reaction intermediates and of the reaction pathways. The most advanced of the response approaches is the kinetic isotope method suggested by Neiman and Gal [Neiman & Gal, 1971; Boudart, 1968]: this method makes it possible to measure the individual formation and decomposition rates of elementary reaction steps. The kinetic isotope method shares a feature with the relaxation techniques: it is based on the use of perturbations, which modify as little as possible the underlying kinetics of the process. A typical kinetic isotope experiment preserves the total fluxes of chemicals (labeled plus unlabeled) unchanged, and makes use of isotopes which have an atomic mass very close to the ones of the native (unlabeled) isotopes present in the system. Under these circumstances the kinetic isotope effect can be neglected and the rates of the chemical processes occurring in the system are not changed by the presence of the labeled compounds. Similar experiments, which leave the kinetics of the process practically unchanged are based on fluorescent labeling. Despite this analogy between the relaxation methods and the kinetic isotope method, there is an important difference: for the analysis of the kinetic isotope experiments no linearization of the kinetic evolution equations is necessary, as long as the kinetic isotope effect can be neglected. It follows that, unlike the relaxation methods, the kinetic isotope method can be used for the systematic study of the kinetics of nonlinear processes far from equilibrium [Chevalier et al., 1993].

Until now the kinetic isotope method has been used almost exclusively for resolving the total reaction rates of the various reaction intermediates in individual formation and decomposition rates; almost no attention has been paid to time delays between the excitation and the response of the system to an excitation. In this article we enlarge the use of the kinetic isotope method by analyzing what kind of kinetic information can be extracted from such time delays. We consider a response experiment in which the fluxes of various chemicals are generally time dependent and are not modified by the labeled compounds introduced in the system. We only assume that the fractions \( \alpha_u, u = 1, 2, \ldots \) of the labeled input fluxes to the total (labeled and unlabeled) input fluxes can be controlled by the researcher. The experiments consist in the recording of the fractions \( \beta_u, u = 1, 2, \ldots \) of the labeled output fluxes to the total (labeled and unlabeled) output fluxes. By assuming that the kinetic isotope effect is missing we shall prove that the dependence of the output fluxes \( \beta_u, u = 1, 2, \ldots \) on the input fluxes \( \alpha_u, u = 1, 2, \ldots \) is given by a delayed linear superposition law of the type

\[
\beta_u(t) = \sum_{u'} \int \chi_{uu'}(t, t') \alpha_{u'}(t') dt',
\]

where \( \chi_{uu'}(t, t') \) is a delayed susceptibility function which expresses the contribution of the excitation \( \alpha_{u'}(t') \) at time \( t' \) to the response \( \beta_u(t) \) at time \( t \). We emphasize that the linear structure of the response law (1) is not due to any linearization of the underlying evolution equations, but rather to the fact that in its derivation the kinetic isotope effect is neglected. Equation (1) is valid even if the underlying kinetic evolution equations are highly nonlinear and no linearization procedures are involved in its proof.

From Eq. (1) it follows that the main functions which can be extracted from a delayed kinetic isotope experiment are the susceptibility functions \( \chi_{uu'}(t, t') \). Therefore, in order to extract kinetic information from the experimental data we need to elucidate the physical meaning of these susceptibility functions and to establish relationships among these functions and the kinetic parameters of the process. In order to achieve this goal we use the theory of lifetime distributions and transit time distributions developed by us in connection with the theory of metabolic control [Moran et al., 1997; Vlad et al., 1998, 1999, 2000]. Our former theory had a number of limitations, which made it impossible to apply it to the treatment of reaction mechanisms with multiple connections among various pathways. We have to generalize the transit time to the case of an arbitrary reaction mechanism, which may involve multiple branches as well as multiple connections among various pathways. This generalization is based on the investigation of the relationships between a molecular fragment,
which contains the tracer, and the various carrier molecules. By using such a generalized theory we show that the susceptibility functions \( \chi_{uu'}(t, t') \) are related to the probability density of the transit time in the system, that is, to the probability density necessary for a given molecular fragment to cross the system. Once the susceptibility functions are known, the result of any perturbation on the system can be easily calculated.

The outline of the paper is the following. In Sec. 2 we give a general formulation of the problem. In Sec. 3 we derive the general superposition law (1). Sections 3–5 deal with the development of the theories of lifetime distributions and transit time distributions, based on the use of the fragment carrier approach. In Sec. 6 we give a physical interpretation of the susceptibility functions \( \chi_{uu'}(t, t') \), based on the theory of transit time distributions. In Sec. 7 we suggest methods for the experimental measurement of the susceptibility functions from transient as well as frequency response experiments and suggest methods for extracting information about mechanism and kinetics from susceptibility measurements.

2. Formulation of the Problem

We consider a complex chemical system and focus on a set of \( S \) species \( M_u, u = 1, \ldots, S \) which can carry one or more identical molecular fragments which are unchanged during the process; in the following we refer to these species as “carriers”. For simplicity, in this paper we limit ourselves to the case of isothermal, well-stirred, homogeneous systems, for which the concentrations \( c_u = c_u(t), u = 1, \ldots, S \) of the chemicals \( M_u, u = 1, \ldots, S \), are space independent and depend only on time. The theory, however, can be extended to the more complicated case of reaction–convection or reaction–diffusion systems. The deterministic kinetic equations of the process can be expressed in the following form:

\[
\frac{dc_u(t)}{dt} = \mathcal{J}_u^+(t) - \mathcal{J}_u^-(t) + \rho_u^+(c; t) - \rho_u^-(c; t), \quad u = 1, \ldots, S, \tag{2}
\]

where \( \rho_u^\pm(c; t) \) are the rates of formation and consumption of the species \( M_u \), respectively, \( \mathcal{J}_u^\pm(t) \) are the input and output fluxes of \( M_u \), respectively, and

\[
c(t) = [c_u(t)]_{u=1, \ldots, M}, \tag{3}
\]

is the composition vector of the system. In general the functions \( \rho_u^\pm(c; t) \) can be found with the mass action law or other kinetic laws. Together with the initial condition

\[
c(t = t_0) = c_0, \tag{4}
\]

the kinetic equations (2) determine the time evolution (3) of the concentration vector.

We denote by \( z_u \) the number of fragments from one molecule in the carrier species \( u \). We use the notation

\[
F_u = F(M_u), \quad u = 1, \ldots, S, \tag{5}
\]

for a molecular fragment in the carrier \( M_u \). All fragments \( F_u \) in different carriers \( M_u \) have the same structure; the label \( u \) means that a fragment belongs to a given carrier. The concentrations \( f_u(t) \), \( u = 1, \ldots, S \), of the fragments \( F_u \), \( u = 1, \ldots, S \) which belong to different carriers are given by

\[
f_u(t) = z_u c_u(t), \quad u = 1, \ldots, S, \tag{6}
\]

We express the kinetic equations (2) in terms of the concentrations \( f_u(t) \), \( u = 1, \ldots, S \), of the fragment \( F_u \), \( u = 1, \ldots, S \), in the carrier \( u \), resulting in

\[
\frac{df_u(t)}{dt} = J_u^+(t) - J_u^-(t) + R_u^+(c; t) - R_u^-(c; t), \quad u = 1, \ldots, S, \tag{7}
\]

where

\[
R_u^\pm(c; t) = z_u \rho_u^\pm(c; t), \quad u = 1, \ldots, S, \tag{8}
\]

are the rates of formation and consumption of the fragment \( F_u \), in the carrier \( u = 1, \ldots, S \), and

\[
J_u^\pm(t) = z_u \mathcal{J}_u^\pm(t), \tag{9}
\]

are input and output fluxes of fragment \( F_u \), in the carrier \( u = 1, \ldots, S \), respectively.

In the system a fragment is transferred from one carrier to another. These transfer processes involving fragments \( F_u \), \( u = 1, \ldots, S \), among different carriers can be formally represented as:

\[
F_u \xleftarrow{R_{uu'}} F_{u'}, \quad u \neq u', \quad u, u' = 1, \ldots, S, \tag{10}
\]

where \( R_{uu'} = R_{uu'}(c; t) \) is the rate of transport of the fragment \( F_u \) from a carrier \( M_u \) into a carrier \( M_{u'} \). The rates \( R_{uu'} \) are related to the formation
and consumption rates $R^+_u(c; t)$ of the fragment $F_u$ in the carrier $M_u$ by means of the balance equations

$$R^+_u(c; t) = \sum_{u' \neq u} S_u u' R_{u'u}(c; t),$$

$$R^-_u(c; t) = \sum_{u' \neq u} S_u u' R_{u'u}(c; t), \quad u = 1, \ldots, S. \quad (11)$$

If the mechanism and kinetics of the process is known, then the transformation rates $R_{u'u} = R_{u'u}(c; t)$ can be evaluated by using the mass action law or other kinetic laws.

Now we consider a kinetic tracer experiment by assuming that a fraction $\alpha_u$, $u = 1, \ldots, S$ of the “in” flux of the fragment $F_u$ is replaced by a labeled fragment $F_u^*$, and assume that the kinetic isotope effect is missing, that is, the rates of the processes involving labeled compounds are the same as the rates of the processes involving unlabeled chemicals. We assume that the fractions $\beta_u$, $u = 1, \ldots, S$ of the labeled fragments in the output fluxes $J^-_u(t)$, $u = 1, \ldots, S$ can be measured experimentally. We are interested in answering the following questions:

1. If the mechanism and kinetics of the process are known, and if the kinetic isotope effect can be neglected, what is the general relationship between the input fractions $\alpha_u$, $u = 1, \ldots, S$ of labeled fragments and the output fractions $\beta_u$, $u = 1, \ldots, S$ of labeled fragments? The answer to this question is given by Eq. (1).

2. If the kinetics of the process is known, what are the lifetime probability densities of the fragments from different carriers present in the system and the probability densities of the time necessary for a fragment to cross the system? What are the relations among these probability densities and the delayed susceptibility functions $\chi_{uu'}(t, t')$?

3. If the kinetics and the mechanism of the process are unknown and the delayed susceptibility functions $\chi_{uu'}(t, t')$ are known from a tracer experiment, what useful kinetic information can be extracted from these functions?

### 3. Delayed Superposition Law for Response Kinetic Experiments

In order to derive an expression for the response of the system to a tracer experiment we introduce the specific rates of transport of a fragment from one carrier to another and in and out of the system:

$$\omega_{uu'}(t) = \frac{R_{uu'}(t)}{f_{u'}}, \quad (12)$$

$$\Omega^\pm_u = \frac{J^\pm_u(t)}{f_u}. \quad (13)$$

Here $\omega_{uu'}(t) dt = dt R_{uu'}(t)/f_{u'}$ can be interpreted as the infinitesimal probability of transport of a fragment from the carrier $M_{u'}$ to the carrier $M_u$ at a time between $t$ and $t + dt$; similarly $\Omega^\pm_u dt = dt J^\pm_u(t)/f_u$ can be interpreted as the infinitesimal probability that fragment in the carrier $M_u$ enters or leaves the system at a time between $t$ and $t + dt$, respectively. If the kinetic isotope effect is missing the rate of exchange of the labeled fragments in the system can be completely expressed in terms of these infinitesimal probabilities.

We assume that the time dependences of the total rates $R_{u'u} = R_{u'u}(c; t)$ and $J^\pm_u(t) = z_u \mathcal{J}^\pm_u(t)$ attached to the total amounts of fragments from different carriers, labeled and unlabeled, are not changed during the process. We use the notations $f_{u'}(t)$, $u = 1, \ldots, S$, for the concentrations of labeled fragments and $J^\pm_u(t)$, $u = 1, \ldots, S$ for the input and output fluxes of labeled fragments, respectively. By using the kinetic isotope approach in the form suggested Neiman and Gal [Neiman & Gal, 1971; Boudart, 1968] we can derive the balance equations:

$$\frac{df^*_u(t)}{dt} = J^*_u(t) - J^*_u - \sum_{u' \neq u} S_u [R_{u'u}(c; t)

- R_{u'u}(c; t)]

= J^*_u(t) - \Omega^+_u(t) f^*_u(t) + \sum_{u' \neq u} \int \omega_{uu'}(t) f_{u'}(t)

- \omega_{u'u}(t) f^*_u(t)], \quad (14)$$

and

$$J^-_u(t) = \Omega^-_u(t) f^*_u(t). \quad (15)$$

We are interested in establishing a relationship between the fractions of labeled fragments in the input fluxes:

$$\alpha_u(t) = \frac{J^*_u(t)}{J^-_u(t)}, \quad (16)$$
and the fractions of labeled fragments in the output fluxes:

$$\beta_u(t) = \frac{J^{-*}_u(t)}{J^u(t)}. \quad (17)$$

We can derive such a relation by using two different approaches. The first approach consists in solving Eqs. (14), written in matrix form:

$$\frac{df^*(t)}{dt} = J^{**}(t) + K(t)f^*(t), \quad (18)$$

where

$$f^*(t) = \left[F^{**}_{u|u=1,...,S}, J^{**}\right]_{u=1,...,S} \quad (19)$$

and

$$K(t) = \left[1 - \delta_{uu'}\omega_{uu'}(t) - \delta_{uu'}[\Omega_{u'}(t)]
+ \sum_{u''\neq u} \omega_{u'u''}(t) \right] \quad (20)$$

The general solution of Eq. (18) can be formally expressed as

$$f^*(t) = G(t; t_0)f^*(t_0) + \int_{t_0}^{t} G(t; t')J^{**}(t')dt', \quad (21)$$

where

$$G(t, t') = \mathcal{T} \exp \left[\int_{t'}^{t} K(t'')dt''\right], \quad (22)$$

is a matrix Green function which is the solution of the matrix differential equation

$$\frac{d}{dt} G(t, t') = K(t)G(t, t')$$

with

$$G(t = t', t') = I, \quad (23)$$

and $\mathcal{T}$ is the time-ordering chronological operator from quantum field theory. In order to circumvent the difficulties generated by the initial conditions we push the initial condition to minus infinity, $t_0 \to -\infty$, resulting in:

$$f^*(t) = \int_{-\infty}^{t} G(t; t')J^{**}(t')dt'. \quad (24)$$

The transformation $t_0 \to -\infty$ is just a convenient transformation of the frame of reference for measuring time, which is especially useful for studying systems for which a stationary regime does not emerge in the limit of large times. We emphasize that pushing the initial conditions to minus infinity does not limit the range of validity of our results; we use it because it simplifies the analytical representation of the previous history of the process. By using Eqs. (15) and (24), it is easy to express the output fluxes of labeled fragments in terms of the input fluxes of labeled fragments. We have

$$J^{-*}_u(t) = \Omega_{u'}(t)f^*_u(t) = \Omega_{u'}(t) \int_{-\infty}^{t} G_{uu'}(t'; t')J^+_{u'}(t')dt', \quad (25)$$

from which we come to:

$$\beta_u(t) = \sum_{u'=1}^{S} \int_{-\infty}^{t} \chi_{uu'}(t'; t')\alpha_{u'}(t')dt', \quad (26)$$

where

$$\chi_{uu'}(t'; t') = \frac{\Omega_{u'}(t)J^+_{u'}(t')G_{uu'}(t; t')}{J^u(t)}$$

$$= \frac{J^+_{u'}(t')G_{uu'}(t; t')}{\sum_{u'=1}^{S} \int_{-\infty}^{t} G_{uu'}(t; t')J^+_{u'}(t')dt'}, \quad (27)$$

is a generalized susceptibility function, which, according to Eq. (27) fulfills the normalization condition:

$$\sum_{u'=1}^{S} \int_{-\infty}^{t} \chi_{uu'}(t'; t')dt' = 1. \quad (28)$$

In Appendix A we show that the generalized susceptibility function $\chi_{uu'}(t; t')$ is never negative. The non-negativity of the function $\chi_{uu'}(t; t')$, together with the normalization condition (28), suggest that $\chi_{uu'}(t; t')$ may be interpreted as a probability density. This interpretation is discussed in detail in Secs. 4–6.

In order to study the general theoretical properties of the delayed response we need to derive a set of evolution equations for the response functions $\beta_u(t) = J^{-*}_u(t)/J^u(t), \ u = 1, \ldots, S$. By combining Eqs. (14)–(17) we come to:

$$f_u(t) \frac{d}{dt} \beta_u(t) + \beta_u(t) \frac{d}{dt} f_u(t)$$

$$= \alpha_u(t)J^+_u(t) - \Omega^-_u(t)f_u(t)\beta_u(t)$$

$$+ \sum_{u' \neq u} [\omega_{uu'}(t)f_{u'}(t)\beta_u'(t)$$

$$- \omega_{u'u}(t)f_u(t)\beta_u(t)]. \quad (29)$$
Now we express the overall balance equations (7)–(11) in terms of the specific rate coefficients \( \omega_{uu'}(t) \) and \( \Omega^+_u(t) \), resulting in:

\[
\frac{df_u(t)}{dt} = J^+_u(t) - J^-_u(t) + \sum_{u' \neq u} [R^+_u(c;t)]
- R^-_{u'u}(c,t)] = J^+_u(t) - \Omega^-_u(t)f_u(t) + \sum_{u' \neq u} [\omega_{uu'}(t)f_{u'}(t) - \omega_{u'u}(t)f_u(t)].
\]

By combining Eqs. (29) and (30) we can derive a system of evolution equations for the response functions \( \beta_u(t) \), \( u = 1, \ldots, S \):

\[
f_u(t) \frac{d}{dt} \beta_u(t) = [\alpha_u(t) - \beta_u(t)]J^+_u(t)
+ \sum_{u' \neq u} [\omega_{uu'}(t)f_{u'}(t)\beta_u(t)]
- \beta_u(t) \sum_{u' \neq u} \omega_{u'u}(t)f_{u'}(t).
\]

Eqs. (31) can be expressed in a simpler way by using the adjoint specific rates:

\[
\omega_{uu'}(t) = \omega_{uu'}(t) \left[ \frac{f_{u'}(t)}{f_u(t)} \right] = \frac{R_{uu'}(t)}{f_u(t)}.
\]

We have:

\[
\frac{d}{dt} \beta_u(t) = \alpha_u(t)\Omega^+_u(t) - \beta_u(t)\Omega^+_u(t)
+ \sum_{u' \neq u} [\omega_{uu'}(t)\beta_u(t)]
- \omega_{uu'}(t)\beta_u(t).
\]

Equation (33) can be expressed in a matrix form similar to Eq. (18)

\[
\frac{d}{dt} \beta(t) = \Omega^+(t)\alpha(t) + \hat{K}(t)\beta(t),
\] where

\[
\Omega^+(t) = [\Omega^+_u \delta_{uu'}]_{u,u'=1,\ldots,S},
\] and

\[
\hat{K}(t) = \left[ (1 - \delta_{uu'})\omega_{uu'}(t) - \delta_{uu'} \Omega^+_u(t) \right]_{u,u'=1,\ldots,S}
+ \sum_{u' \neq u} \omega_{uu'}(t). \]

The general solution of Eq. (34) can be expressed in a form similar to Eq. (21)

\[
\beta(t) = \hat{G}(t; t_0)\beta(t_0)
+ \int_{t_0}^t \hat{G}(t; t')\Omega^+(t')\alpha(t')dt',
\]

where

\[
\hat{G}(t, t') = \mathcal{T} \exp \left[ \int_{\tau}^t \hat{K}(t'')dt'' \right],
\]

is an adjoint matrix Green function which is the solution of the matrix differential equation

\[
\frac{d}{dt} \hat{G}(t, t') = \hat{K}(t)\hat{G}(t, t')
\]

with \( \hat{G}(t = t', t') = I \).

By pushing the initial condition in Eq. (37) to minus infinity, \( t_0 \to -\infty \), we recover the response theorem (26), where the susceptibility function \( \chi_{uu'}(t, t') \) is given by

\[
\chi_{uu'}(t, t') = \hat{G}_{uu'}(t; t')\Omega^+_u(t').
\]

The response theorem (26) serves as a basis for the development of tracer experiments for the investigation of the reaction kinetics of complex chemical networks. In Sec. 7 we analyze the kinetic information which can be extracted from such experiments.

The results obtained in this section are strongly dependent on the fact that the kinetic isotope effect is neglected that is, the rate coefficients for labeled and unlabeled species are the same. In general this is a reasonable assumption for systems for which the molecular weights of the labeled and unlabeled species are close to each other. For example, we mention an experimental report of recent oxygen-18 kinetic isotope effect studies of the tyrosinehydroxylase reaction [Francisco et al., 1998]; for this reaction the isotope effect is very close to unity, \( 1.0174 \pm 0.0019 \).

In conclusion, in this section we have shown that, if the kinetic isotope effect can be neglected, the response of the system to an input of labeled
fragments, which preserves the total fluxes and the total reaction rates in this system, can be expressed by a time-inhomogeneous linear response law of the type (26). We emphasize that Eq. (26) is valid even if the underlying kinetics of the process is highly nonlinear. The linearity of Eq. (26) is not due to the assumption that the kinetics of the process is linear, but rather because in the derivation of Eq. (26) the kinetic isotope effect has been neglected.

4. Transit Time Distributions

In this section we introduce the transit time probability densities attached to a fragment crossing the system and investigate their connections with the susceptibility functions $\chi_{uu'}(t,t')$ from Eq. (1). We define these probability densities in terms of the following density functions:

$$\zeta_{uu}(\theta; t) d\theta \text{ with } \sum_{j} \int_{0}^{\infty} \zeta_{uj}(\theta; t) d\theta = f_{u}(t).$$

(41)

$\zeta_{uj}(\theta; t) d\theta$ is the concentration of fragments in the carrier $M_u$ present in the system at time $t$ which have entered the system in a carrier molecule of type $j$ and have a transit (residence) time in the system between $\theta$ and $\theta + d\theta$. In terms of these density functions we can introduce the following probability densities:

$$\Psi_{uu'}(\theta; t) d\theta = \frac{\zeta_{uu'}(\theta; t) \Omega_{u}^+ d\theta}{\sum_{u} \Omega_{u}^- \int_{0}^{\infty} \zeta_{uu'}(\theta; t) d\theta} \text{ with } \sum_{u} \int_{0}^{\infty} \Psi_{uu'}(\theta; t) d\theta = 1,$$

(42)

$$\Phi_{uu'}(\theta; t) d\theta = \frac{\zeta_{uu'}(\theta; t) d\theta}{\sum_{u} \int_{0}^{\infty} \zeta_{uu'}(\theta; t) d\theta} \text{ with } \sum_{u} \int_{0}^{\infty} \Phi_{uu'}(\theta; t) d\theta = 1,$$

(43)

$$\psi_{uu'}(\theta; t) d\theta = \frac{\zeta_{uu'}(\theta; t) \Omega_{u}^- d\theta}{\sum_{u} \Omega_{u}^+ \int_{0}^{\infty} \zeta_{uu'}(\theta; t) d\theta} \text{ with } \sum_{u} \int_{0}^{\infty} \psi_{uu'}(\theta; t) d\theta = 1,$$

(44)

$$\varphi_{uu'}(\theta; t) d\theta = \frac{\zeta_{uu'}(\theta; t) d\theta}{\sum_{u'} \int_{0}^{\infty} \zeta_{uu'}(\theta; t) d\theta} \text{ with } \sum_{u'} \int_{0}^{\infty} \varphi_{uu'}(\theta; t) d\theta = 1,$$

(45)

$\Phi_{uu'}(\theta; t) d\theta$ is the probability that a fragment which enters the system in a carrier of given type, $u'$, is present in a system at time $t$ in a carrier of type $u$ and has a transit (residence) time between $\theta$ and $\theta + d\theta$. Similarly, $\Psi_{uu'}(\theta; t) d\theta$ is the probability that a fragment which enters the system in a carrier of given type, $u'$, leaves the system at time $t$ in a carrier of type $u$ and has a transit (residence) time between $\theta$ and $\theta + d\theta$. The other two functions, $\varphi_{uu'}(\theta; t) d\theta$ and $\psi_{uu'}(\theta; t) d\theta$, have similar meanings, with the difference that the initial type, $u'$, of the carrier is random, and the current type, $u$, of the carrier is known. $\varphi_{uu'}(\theta; t) d\theta$ is the probability that a fragment which is present in the system at time $t$, in a known carrier of type $u$, has a random transit (residence) time between $\theta$ and $\theta + d\theta$ and entered the system in a carrier of random type $u'$. $\psi_{uu'}(\theta; t) d\theta$ is the probability that a fragment which is leaving the system at time $t$, in a known carrier of type $u$, has a random transit (residence) time between $\theta$ and $\theta + d\theta$ and entered the system in a carrier of random type, $u'$. Note that, according to Eqs. (44) and (45), we have

$$\psi_{uu'}(\theta; t) d\theta = \varphi_{uu'}(\theta; t) d\theta.$$

(46)

The equality (46) of the probabilities $\varphi_{uu'}(\theta; t) d\theta$ and $\psi_{uu'}(\theta; t) d\theta$, is due to the fact that, according to the mass action law or other local kinetic laws, the specific exit rates $\Omega_{u}(t)$ depend only on time and concentrations and are independent of the residence time $\theta$. 

Delayed Response in Tracer Experiments
For the density functions $\zeta_{uj}(\theta; t)$ we can derive the following balance equations:

$$
\left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \theta} \right) \zeta_{uj}(\theta; t)
= \sum_{u' \neq u} \omega_{uu'}(t) \zeta_{u'j}(\theta; t)
- \left[ \Omega_u^-(t) + \sum_{u' \neq u} \omega_{u'u}(t) \right] \zeta_{uj}(\theta; t),
$$

(47)

with the boundary conditions

$$
\zeta_{uj}(\theta = 0; t) = \delta_{uj} J_u^+(t).
$$

(48)

Equation (42) and (43) can be integrated along the characteristics, by considering the initial conditions:

$$
\zeta_{uj}(\theta; t = t_0) = \zeta_{uj}^{(0)}(\theta).
$$

(49)

In matrix notation Eqs. (47)–(49) become

$$
\left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \theta} \right) \zeta(\theta; t) = K(t) \zeta(\theta; t),
$$

(50)

$$
\zeta(\theta = 0; t) = J^+(t),
$$

(51)

and

$$
\zeta(\theta; t = t_0) = \zeta^{(0)}(\theta).
$$

(52)

The solution of these equations can be expressed in terms of the Green function $G(t, t')$, Eq. (22), introduced in Sec. 3. After some calculations we come to:

$$
\zeta(\theta; t) = h(t - t_0 - \theta) G(t, t - \theta) J^+(t - \theta)
+ h(\theta - t + t_0) G(t, \theta - t) \zeta^{(0)}(\theta)
- t + t_0),
$$

(53)

where $h(t)$ is the Heaviside step function. By following the procedure introduced in Sec. 3 we push the initial condition to minus infinity, $t_0 \to -\infty$, resulting in

$$
\zeta(\theta; t) = \lim_{t_0 \to -\infty} \zeta(\theta; t)
= G(t, t - \theta) J^+(t - \theta).
$$

(54)

Under these circumstances the probabilities $\varphi_{uu'}(\theta; t) d\theta$ and $\psi_{uu'}(\theta; t) d\theta$ can be easily evaluated

$$
\varphi_{uu'}(\theta; t) = \psi_{uu'}(\theta; t) = \frac{\zeta_{uu'}(\theta; t) \Omega_u^-}{\Omega_u^+ J_0^\infty \zeta_{uu'}(\theta; t) d\theta}
= G_{uu'}(t, t - \theta) J_u^+(t - \theta)
= \chi_{uu'}(t; t' = t - \theta).
$$

(55)

It follows that the probability densities $\varphi_{uu'}(\theta; t)$ and $\psi_{uu'}(\theta; t)$ are equal to the susceptibility function $\chi_{uu'}(t; t')$ evaluated for an initial time $t' = t - \theta$. Vice versa, the susceptibility function $\chi_{uu'}(t; t')$ is equal to the probability densities $\varphi_{uu'}(\theta; t)$ and $\psi_{uu'}(\theta; t)$ evaluated for a transit (residence) time equal to $\theta = t - t'$:

$$
\chi_{uu'}(t; t') = \varphi_{uu'}(\theta = t - t'; t)
= \psi_{uu'}(\theta = t - t'; t).
$$

(56)

Equation (56) is the main result of this section: this equation establishes a relationship among the susceptibility function $\chi_{uu'}(t; t')$ and the probability densities $\varphi_{uu'}(\theta; t)$ and $\psi_{uu'}(\theta; t)$ of the transit time of a fragment crossing the system.

For the study of the general theoretical properties of the probability densities $\varphi_{uu'}(\theta; t)$ and $\psi_{uu'}(\theta; t)$ we need to derive an adjoint equation for these functions, similar to the evolution equation (33) derived in Sec. 3 for the description of the response experiments. The main steps of the derivation are outlined in Appendix B. We can derive the following evolution equations for the probability densities $\varphi_{uu'}(\theta; t)$ and $\psi_{uu'}(\theta; t)$:

$$
\left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \theta} \right) \varphi_{uj}(\theta; t)
= \sum_{u' \neq u} \hat{\omega}_{uu'}(t) \varphi_{u'j}(\theta; t)
- \left[ \Omega_u^+(t) + \sum_{u' \neq u} \hat{\omega}_{uu'}(t) \right] \varphi_{uj}(\theta; t),
$$

(57)

with the boundary and initial conditions

$$
\varphi_{uj}(\theta = 0; t) = \delta_{uj} \Omega_u^+(t),
$$

(58)

$$
\varphi_{uj}(\theta; t = t_0) = \varphi_{uj}^{(0)}(\theta).
$$

(59)
The solution of Eqs. (57)–(59) can be expressed in a matrix form similar to Eq. (53)
\[ \hat{G}^*(z, t; t') = \mathcal{G}(t; t') + z \int_{t'}^{t} \mathcal{G}(t; t'') \hat{K}_{\text{eff}}(t'') \hat{G}^*(z, t''; t') dt'', \]
where \( \hat{G}(t, t') \) is the adjoint matrix Green function defined by Eq. (39).

By pushing the initial condition to minus infinity, \( t_0 \to -\infty \), we get a new expression for the probability densities \( \varphi_{uu'}(\theta; t) \) and \( \psi_{uu'}(\theta; t) \):
\[ \varphi_{uu'}(\theta; t) = \psi_{uu'}(\theta; t) = \hat{G}_{uu'}(t, t - \theta) \Omega^+_{uu'}(t - \theta). \]

By comparing Eq. (40) with Eq. (61) we recover Eqs. (55) and (56).

Both methods of computing the probability densities \( \varphi_{uu'}(\theta; t) \) require the repeated integration of matrix differential equations with time-dependent coefficients, necessary for the evaluation of the direct or adjoint Green functions, \( G(t; t') \) or \( \hat{G}(t; t') \), for different initial times \( t' = t'_1, t' = t'_2, \ldots \). In general this integration cannot be done by using analytical methods. In the particular case of a stationary process, however, the matrix elements \( \omega_{uu'}, \hat{\omega}_{uu'} \) and \( \Omega^+_{uu'} \) are time independent and the Green functions \( G(t; t') \) and \( \hat{G}(t; t') \) become translationally invariant
\[ G(t; t') = G(t - t'); \hat{G}(t; t') = \hat{G}(t - t'). \]

In this case the matrix differential equations (23) and (39) can be solved analytically, by using the Sylvester theorem. The Green functions \( G(t - t') \) and \( \hat{G}(t - t') \) depend on the eigenvalues of the matrices \( K \) and \( \hat{K} \), respectively, which satisfy the secular equations:
\[ \det [K - sI] = 0, \det [\hat{K} - sI] = 0. \]

From the definitions (20), (32) and (36) of the matrices \( K, \hat{K} \) and the adjoint specific rates \( \hat{\omega}_{uu'} \), it follows that the matrices \( K \) and \( \hat{K} \) can be derived from each other by means of a similarity transformation and therefore their eigenvalues are identical. We notice that for a stationary process the argument \( t - t' \) of the Green functions \( G(t - t') \) and \( \hat{G}(t - t') \) is the transit time \( t - t' = \theta \) and introduce the Laplace transforms:
\[ \overline{G}(s) = \int_{0}^{\infty} \exp(-s\theta) G(\theta) d\theta, \]
\[ \overline{\hat{G}}(s) = \int_{0}^{\infty} \exp(-s\theta) \hat{G}(\theta) d\theta, \]
where \( s \) is the Laplace variable conjugate to the transit time \( \theta \). By applying the Laplace transform, Eqs. (20) and (36) lead to:
\[ \overline{G}(s) = [K - Is]^{-1}, \overline{\hat{G}}(s) = [\hat{K} - Is]^{-1}. \]

If the secular equations (63) have the roots \( s_1, s_2, s_3, \ldots \) with the multiplicities \( m_1, m_2, m_3, \ldots \) respectively, then the stationary Green functions are given by linear combinations of exponential terms of the form \( \exp(s_\beta \theta) \), \( \beta = 1, 2, 3, \ldots \) modulated by polynomials in the transit time. For computing the explicit expression for the matrix of stationary Green functions, \( G(\theta) \) and \( \hat{G}(\theta) \), we must compute the matrices on the right-hand side of Eqs. (65) and evaluate the inverse Laplace transform of the resulting matrices. The calculations, based on Heaviside’s second expansion theorem, are lengthy but standard. The final expressions for \( G(\theta) \) and \( \hat{G}(\theta) \) are:
\[ G(\theta) = \sum_{\beta} \sum_{b=1}^{m_\beta} \frac{\theta^{b-1} \exp(s_\beta \theta)}{(b-1)!(m_\beta - b)!} G_{\beta b}, \]
\[ \hat{G}(\theta) = \sum_{\beta} \sum_{b=1}^{m_\beta} \frac{\theta^{b-1} \exp(s_\beta \theta)}{(b-1)!(m_\beta - b)!} \hat{G}_{\beta b}, \]
where \( G_{\beta b} \) and \( \hat{G}_{\beta b} \) are constant matrices determined by the relationships
\[ G_{\beta b} = \frac{d^{m_\beta - b}}{ds^{m_\beta - b}} \left\{ \frac{\text{Adj}(K - Is)}{\det [K - Is]} (s - s_\beta)^{m_\beta} \right\} \bigg|_{s = s_\beta}, \]
\[ \hat{G}_{\beta b} = \frac{d^{m_\beta - b}}{ds^{m_\beta - b}} \left\{ \frac{\text{Adj}(\hat{K} - Is)}{\det [\hat{K} - Is]} (s - s_\beta)^{m_\beta} \right\} \bigg|_{s = s_\beta}. \]
Equations (66)–(69) can be used in two different ways. If the kinetics of the process is known then they can be used for the theoretical computation of the probability densities of the transit times. If the rate coefficients are unknown but the probability densities of the transit time are known from experiment, then Eqs. (66)–(69) can be used for the evaluation of the rate coefficients.

In conclusion, in this section we have introduced four sets of the transit time probability densities of a fragment in the system. We have shown that two of these four probability densities are related to the susceptibility function from the delayed superposition law (26). We have shown that the susceptibility functions are related to one of these four probability densities of the transit time. We have suggested two theoretical methods for the computation of the probability densities of the transit times from the kinetics data. For nonstationary processes both methods involve the repeated numerical integration of systems of matrix differential equations with time-dependent coefficients. For stationary processes, however, the problem can be solved completely by using only analytical methods. These results have important implications for the design of delay tracer experiments: they suggest that, whenever possible, it is preferable to carry out the response experiments for chemical systems operated in stationary conditions.

5. Lifetime Distributions

In this section we introduce a different type of time distribution which plays a major role in the understanding of the physical meaning of the response theorem (26). For the fragments \( F_u, \ u = 1, \ldots, S \) from different carriers \( M_u, \ u = 1, \ldots, S \) we introduce the probability densities

\[
\xi_{uj}(\tau; t),
\]

which obey the normalization conditions:

\[
\sum_{j=0}^{S} \int_{0}^{\infty} \xi_{uj}(\tau; t) \, d\tau = 1.
\]

Here \( \tau \) is the lifetime of a fragment in a carrier \( M_u \), that is, the time spent by a fragment in the carrier \( M_u \), from its transport from a previous carrier \( M_{u'} \) until now. \( \xi_{u'uj}(\tau; t) \, d\tau \) is the probability that a fragment present in a carrier \( M_u \) at time \( t \) has been present in this carrier without interruption for a time interval between \( \tau \) and \( \tau + d\tau \), and that the type of its previous carrier was \( u' \).

In order to compute the transit time distributions of the fragments of different types in the system we introduce a set of density functions, similar to the density functions (41) introduced in Sec. 4:

\[
\eta_{uj}(\tau; t) \, d\tau = \sum_{j} \int_{0}^{\infty} \eta_{uj}(\tau; t) \, d\tau = f_u(t). \quad (72)
\]

Here \( \eta_{uj}(\tau; t) \, d\tau \) is the concentration of fragments from the carrier \( M_u \) of type \( u \) present in the system at time \( t \) which has been present in this carrier without interruption in the time interval between \( \tau \) and \( \tau + d\tau \) and which has been transported in the carrier \( M_j \) to the carrier \( M_u \). We can express the probability density \( \xi_{uj}(\tau; t) \) as:

\[
\xi_{uj}(\tau; t) \, d\tau = \frac{\eta_{uj}(\tau; t) \, d\tau}{\sum_{u'} \int_{0}^{\infty} \eta_{u'u'}(\tau; t) \, d\tau} = \frac{\eta_{uj}(\tau; t) \, d\tau}{f_u(t)}. \quad (73)
\]

The density functions \( \eta_{uj}(\tau; t) \) obey a system of balance equations similar to Eqs. (47)–(49) derived in Sec. 4 for \( \xi_{uj}(\theta; t) \)

\[
\left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \tau} \right) \eta_{uj}(\tau; t)
= - \left[ \frac{\Omega^-_u(t)}{2} + \frac{\omega_{u'u}(t)}{2} \right] \eta_{uj}(\tau; t)
= - K_{uu}(t) \eta_{uj}(\tau; t),
\]

with the boundary conditions

\[
\eta_{uj}(\tau = 0; t) = \left[ (1 - \delta_{uj}) \omega_{uj}(t) + \delta_{uj} \Omega^-_u(t) \right] f_j(t), \quad (75)
\]

and the initial conditions

\[
\eta_{uj}(\tau; t = t_0) = \eta_{uj}(0)(\tau). \quad (76)
\]
The solution of Eqs. (74)–(76) is:

\[
\eta_{uj}(\tau; t) = h(t - t_0 - \tau)[(1 - \delta_{uj})\omega_{uj}(t - \tau) + \delta_{uj}\Omega_u^+(t - \tau)]f_j(t - \tau)\exp\left[-\int_{t-\tau}^{t} K_{uu}(t')dt'\right] 
\]

\[
+ h(\tau - t + t_0)\eta_{uj}^{(0)}(\tau - t + t_0)\exp\left[-\int_{t_0}^{t} K_{uu}(t')dt'\right].
\]

The lifetime probability density \(\xi_{uj}(\tau; t)\) can be easily evaluated from Eqs. (73) and (77). An alternative approach is to derive a set of evolution equations for \(\xi_{uj}(\tau; t)\). The computations are similar to the ones developed in Sec. 4 for the transit time probability density. By combining Eqs. (7) and (73)–(77) we come to:

\[
\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial \tau}\right)\xi_{uj}(\tau; t) = -K_{uu}(t)\xi_{uj}(\tau; t),
\]

with the boundary conditions

\[
\xi_{uj}(\tau = 0; t) = (1 - \delta_{uj})\hat{\omega}_{uj}(t) + \delta_{uj}\Omega_u^+(t),
\]

and the initial conditions

\[
\xi_{uj}(\tau; t = t_0) = \xi_{uj}^{(0)}(\tau).
\]

Equations (78)–(80) have the solution:

\[
\xi_{uj}(\tau; t) = h(t - t_0 - \tau)[(1 - \delta_{uj})\hat{\omega}_{uj}(t - \tau) + \delta_{uj}\Omega_u^+(t - \tau)]\exp\left\{-\int_{t-\tau}^{t} \Omega_u^+(t'') + \sum_{u'' \neq u} \hat{\omega}_{uu''}(t'') dt''\right\}
\]

\[
+ h(\tau - t + t_0)\xi_{uj}^{(0)}(\tau - t + t_0)\exp\left\{-\int_{t_0}^{t} \Omega_u^+(t'') + \sum_{u'' \neq u} \hat{\omega}_{uu''}(t'') dt''\right\}.
\]

By pushing the initial condition to minus infinity, \(t_0 \to -\infty\) Eq. (81) reduces to:

\[
\xi_{uj}(\tau; t) = [(1 - \delta_{uj})\hat{\omega}_{uj}(t - \tau) + \delta_{uj}\Omega_u^+(t - \tau)]\exp\left\{-\int_{t-\tau}^{t} \Omega_u^+(t'') + \sum_{u'' \neq u} \hat{\omega}_{uu''}(t'') dt''\right\}.
\]

In the particular case where the chemical process studied is in a stationary regime, the matrix elements \(K_{uu}\), \(\hat{\omega}_{uj}\) and \(\Omega_u^+\) are independent of time and the lifetime probability density (82) reduces to an exponential

\[
\xi_{uj}(\tau) = [(1 - \delta_{uj})\hat{\omega}_{uj} + \delta_{uj}\Omega_u^+]\times\exp\left[-\tau\left(\Omega_u^+ + \sum_{u'' \neq u} \hat{\omega}_{uu''}\right)\right].
\]

The lifetime probability densities introduced in this section play a central role in the physical interpretation of the transit time probability densities; this physical interpretation is discussed in detail in the next section.

6. Physical Interpretation of the Transit Time Distributions and Delayed Superposition Law

In order to clarify the physical meaning of the transit time probability densities \(\varphi_{uu'}(\theta; t)\) we introduce an additional random variable, the number of transport events \(q\) from one carrier to another of a given fragment present in the system. We introduce the probability density:

\[
\varphi_{uu'}^{tr}(q, \theta; t),
\]

with the normalization condition:

\[
\sum_{q=1}^{\infty} \sum_{u'} \int_{0}^{\infty} \varphi_{uu'}^{tr}(q, \theta; t)d\theta = 1.
\]
\( \varphi_{u'u'}^{tr}(q, \theta; t) d\theta \) is the probability that a fragment which is present in the system at time \( t \), in a known carrier, of type \( u \), has a random transit (residence) time between \( \theta \) and \( \theta + d\theta \), entered the system in a carrier of random type, \( u' \) and has undergone \( q \) transport events during its stay in the system. By using the method developed in Sec. 4 we can derive a chain of equations for \( \varphi_{u'u'}^{tr}(q, \theta; t) \) similar to Eqs. (57)–(59) derived in Sec. 4,

\[
\left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \theta} \right) \varphi_{u'u'}^{tr}(0, \theta; t) = - \left[ \Omega_u^+(t) + \sum_{u' \neq u} \hat{\omega}_{u'u'}(t) \right] \varphi_{u'u'}^{tr}(0, \theta; t),
\]

\( q = 1, 2, \ldots, \)

(86)

\[
\left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \theta} \right) \varphi_{u'u'}^{tr}(q, \theta; t) = \sum_{u' \neq u} \hat{\omega}_{u'u'}(t) \varphi_{u'u'}^{tr}(q - 1, \theta; t) - \left[ \Omega_u^+(t) + \sum_{u' \neq u} \hat{\omega}_{u'u'}(t) \right] \varphi_{u'u'}^{tr}(q, \theta; t),
\]

(87)

with the boundary and initial conditions

\[
\varphi_{u'u'}^{tr}(q, \theta; t) = 0, \quad \varphi_{u'u'}^{tr}(0, \theta; t) = 0,
\]

(88)

\( q = 1, 2, \ldots, \)

(89)

In order to solve Eqs. (86)–(89) we introduce a partial generating function, defined as the \( z \)-transform of \( \varphi_{u'u'}^{tr}(q, \theta; t) \) with respect to the number \( q \) of transport events

\[
\varphi_{u'u'}^{tr}(z, \theta; t) = \sum_{q=0}^{\infty} z^q \varphi_{u'u'}^{tr}(q, \theta; t), \text{ with } |z| \leq 1,
\]

(90)

where \( |z| \leq 1 \) is a complex \( z \)-variable, conjugated to the number \( q \) of transport events. By applying the \( z \)-transform to Eqs. (86)–(89) we come to:

\[
\left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \theta} \right) \varphi_{u'u'}^{tr}(z, \theta; t) = \sum_{j=1}^{S} \hat{K}_{u'j}^*(z, t) \varphi_{u'u'}^{tr}(z, \theta; t),
\]

(91)

\[
\varphi_{u'u'}^{tr}(z, \theta; t) = \delta_{u'u'} \Omega_u^+(t),
\]

(92)

\[
\varphi_{u'u'}^{tr}(z, \theta; t) = \varphi_{u'u'}^{(0)tr}(z, \theta),
\]

(93)

where

\[
\varphi_{u'u'}^{(0)tr}(z, \theta; t) = \sum_{q=0}^{\infty} z^q \varphi_{u'u'}^{tr}(q, \theta; t) = 1,
\]

(94)

\[
\hat{K}_{u'j}^*(z, t) = \hat{K}_{off}(t) + z \hat{K}_{diag}(t)
\]

(95)

and \( \hat{K}_{off}(t) \) and \( \hat{K}_{diag}(t) \) are the off-diagonal and the diagonal components of the adjoint matrix \( \hat{K}(t) \) defined by Eq. (36):

\[
\hat{K}_{off}(t) = [(1 - \delta_{u'u'}) \hat{\omega}_{u'u'}(t)]_{u,u'=1,...,S},
\]

(96)

\[
\hat{K}_{diag}(t) = \left[ - \delta_{u'u'} \left[ \Omega_u^+(t) + \sum_{u' \neq u} \hat{\omega}_{u'u'}(t) \right] \right]_{u,u'=1,...,S}.
\]

(97)

The solution of Eqs. (91)–(93) is:

\[
\varphi_{u'u'}^{tr}(z, \theta; t) = h(t - t_0 - \theta) \hat{G}^*(z, t; \theta) \Omega_u^+(t - \theta)
\]

\[
+ h(\theta - t + t_0) \hat{G}^*(z, \theta; t - t_0)
\]

\[
- t \varphi_{u'u'}^{(0)tr}(z, \theta - t + t_0),
\]

(98)

where \( \hat{G}^*(z, t; t') \) is a modified adjoint Green function which is the solution of the matrix differential equation:

\[
\frac{d}{dt} \hat{G}^*(z, t; t') = \hat{K}_{off}(t) \hat{G}^*(z, t; t') + z \hat{K}_{diag}(t) \hat{G}^*(z, t; t')
\]

(99)

By pushing the initial conditions to minus infinity, Eq. (98) turns into a form similar to Eq. (61):

\[
\varphi_{u'u'}^{tr}(z, \theta; t) = \hat{G}_{u'u'}^*(z, t; \theta - t) \Omega_u^+(t - \theta).
\]

(100)

From the definition (90) of the \( z \)-transform \( \varphi_{u'u'}^{tr}(z, \theta; t) \) and Eq. (100) we can derive the following expression for the joint probability density \( \varphi_{u'u'}^{tr}(q, \theta; t) \)

\[
\varphi_{u'u'}^{tr}(q, \theta; t) = \frac{1}{q!} \frac{d^q}{dz^q} [\hat{G}_{u'u'}^*(z, t; t - \theta)]_{z=0} \Omega_u^+(t - \theta).
\]

(101)
In order to apply Eq. (101) we derive a Lippmann–Schwinger expansion for the adjoint Green matrix $\mathbf{G}(t; t')$. We solve Eq. (99) formally by considering that $z\mathbf{K}_{\text{off}}(t)\mathbf{G}^*(z; t; t')$ is a source term. We come to:

$$
\hat{\mathbf{G}}^*(z, t; t') = \mathbf{G}(t; t') + z \int_t^{t'} \mathbf{G}(t; t'')\hat{\mathbf{K}}_{\text{off}}(t'')\hat{\mathbf{G}}^*(z, t'', t')dt'',
$$

(102)

where $\mathbf{G}(t; t')$ is a diagonal Green matrix which is the solution of:

$$\varphi^{(1)}_{uu'}(\theta; t) = \varphi^{(2)}_{uu'}(\theta; t) \text{ with } \mathbf{G}(t = t'; t') = \mathbf{I}.
$$

Equation (103) can be integrated term by term, resulting in

$$
\mathbf{G}(t; t') = \left[ \exp \left[ -\int_t^{t'} \left( \Omega^+_u(t'') + \sum_{u' \neq u} \hat{\omega}_{uu'}(t'') \right) dt'' \right] \delta_{uu'} \right]_{u, u' = 1, \ldots, S},
$$

(104)

$$
\hat{\mathbf{G}}^*(z, t; t') = \mathbf{G}(t; t') + z \int_t^{t'} \mathbf{G}(t; t_1)\hat{\mathbf{K}}_{\text{off}}(t_1)\mathbf{G}(t_1; t')dt_1
$$

$$
+ z^2 \int_t^{t'} \int_{t_1}^{t_2} \mathbf{G}(t; t_2)\hat{\mathbf{K}}_{\text{off}}(t_2)\mathbf{G}(t_2; t_1)\hat{\mathbf{K}}_{\text{off}}(t_1)\mathbf{G}(t_1; t')dt_2dt_1 + \cdots
$$

$$
+ z^q \int_t^{t'} \int_{t_1}^{t_2} \cdots \int_{t_{q-1}}^{t_2} \mathbf{G}(t; t_q)\hat{\mathbf{K}}_{\text{off}}(t_q)\mathbf{G}(t_q; t_{q-1})\cdots\hat{\mathbf{K}}_{\text{off}}(t_1)\mathbf{G}(t_1; t')dt_q \cdots dt_1 + \cdots
$$

(105)

From Eqs. (101) and (105) we get the following expression for the joint probability density $\varphi^{tr}_{uu'}(q, \theta; t)$:

$$
\varphi^{tr}_{uu'}(q, \theta; t) = \sum_{u_q} \cdots \sum_{u_1} \int_t \int_{t-q-1} \cdots \int_{t-\theta} \mathbf{G}(t; t_1)\mathbf{G}(t_1; t_2)\mathbf{G}(t_2; t_3)\cdots \mathbf{G}(t_{q-1}; t_q)\mathbf{G}(t_q; t_\theta)\mathbf{G}(t_\theta; t)dt_q \cdots dt_1.
$$

(106)

In Eq. (106) we express the diagonal Green matrix $\mathbf{G}(t; t')$ in terms of the lifetime probability densities $\xi_{uu'}(\tau; t)$ [see Eqs. (82) and (104)], get rid of the sums over various Kronecker symbols and introduce the new integration variables

$$
\tau_1 = t - t_1 \quad \text{for} \quad q = 1
$$

(107)

$$
\tau_q = t - t_q, \tau_q - u = t_q - u - t_q - u - 1;
$$

$$
\quad \text{for} \quad q = 2, 3, \ldots \quad \text{for} \quad q = 2, 3, \ldots
$$

(108)

After lengthy algebraic manipulations we come to

$$
\varphi^{tr}_{uu'}(0; \theta; t) = \xi_{uu'}(\theta; t).
$$

(109)

$$
\varphi^{tr}_{uu'}(1; \theta; t) = \sum \xi_{uu'}(\tau_1; t)\xi_{uu'}(\theta - \tau_1; t - \tau_1)d\tau_1,
$$

(110)

In order to clarify the physical meaning of Eqs. (109)–(111) we consider a succession of transport events of a fragment:

$$
u_0' \rightarrow u_1' \rightarrow \cdots \rightarrow u_q' \rightarrow u,
$$

(112)

and introduce a joint probability density

$$
\xi_{uu'q\ldots u_q'\ldots u_0'}(\tau_{q+1}, \ldots, \tau_1; t)d\tau_{q+1} \cdots d\tau_1,
$$

(113)
with the normalization condition
\[ \sum_{u_0'} \cdots \sum_{u_q'} \int_0^\infty \cdots \int_0^\infty \xi^{(q)}_{u_0' \cdots u_q'}(\tau_{q+1}, \ldots, \tau_1; t) d\tau_{q+1} \cdots d\tau_1. \] (114)

\( \xi^{(q)}_{u_0' \cdots u_q'}(\tau_{q+1}, \ldots, \tau_1; t) d\tau_{q+1} \ldots d\tau_1 \) is the probability that a fragment present in the system at time \( t \) in a known carrier of type \( u \), \( q+1 \) steps before was in a carrier of type \( u_0' \), was transported to a carrier \( u_1' \) and its lifetime in the carrier \( u_1' \) was between \( \tau_1 \) and \( \tau_1 + d\tau_1 \), then it was transported to a carrier of type \( u_2' \) and its lifetime in this carrier is between \( \tau_2 \) and \( \tau_2 + d\tau_2 \), \ldots, and then finally it was transported to the state \( u \) and its lifetime in the current state \( q \) is between \( \tau_{q+1} \) and \( \tau_{q+1} + d\tau_{q+1} \). This joint probability can be expressed as
\[ \xi^{(q)}_{u_0' \cdots u_q'}(\tau_{q+1}, \ldots, \tau_1; t) d\tau_{q+1} \ldots d\tau_1 = \xi_{u_0'}(\tau_{q+1}; t) d\tau_{q+1} \xi_{u_0'u_{q-1}'}(\tau_q; t - \tau_{q+1}) d\tau_q \times \xi_{u_1'u_0'}(\tau_1; t - \tau_{q+1} - \cdots - \tau_2) d\tau_1. \] (115)

In terms of \( \xi^{(q)}_{u_0' \cdots u_q'}(\tau_{q+1}, \ldots, \tau_1; t) d\tau_{q+1} \ldots d\tau_1 \) we can express the joint probability density \( \varphi^{tr}_{u_0'}(q, \theta; t) \) as a path average:
\[ \varphi^{tr}_{u_0'}(q, \theta; t) = \left\langle \delta \left[ \theta - \sum_{q'=1}^{q+1} \tau_{q'} \right] \right\rangle. \] (116)

where \( \left\langle \cdots \right\rangle \) is a path average taken over all possible transitions \( u_0' \to u_1' \to \cdots \to u_q' \to u \) and all possible intermediate states \( u_1', \ldots, u_q \) and lifetimes \( \tau_1, \ldots, \tau_{q+1} \):
\[ \left\langle \delta \left[ \theta - \sum_{q'=1}^{q+1} \tau_{q'} \right] \right\rangle = \sum_{u_1'} \cdots \sum_{u_q'} \int_0^\infty \cdots \int_0^\infty \delta \left[ \theta - \sum_{q'=1}^{q+1} \tau_{q'} \right] \times \xi^{(q)}_{u_0' \cdots u_q'}(\tau_{q+1}, \ldots, \tau_1; t) d\tau_{q+1} \ldots d\tau_1. \] (117)

By taking into account that the transit time probability density \( \varphi^{tr}_{u_0'}(q, \theta; t) \) of the transit time can be expressed as a marginal distribution, in terms of \( \varphi^{tr}_{u_0'}(q, \theta; t) \)
\[ \varphi^{tr}_{u_0'}(q, \theta; t) = \sum_{q=0}^{\infty} \varphi^{tr}_{u_0'}(q, \theta; t), \] (118)
we come to:
\[ \varphi^{tr}_{u_0'}(\theta; t) = \psi^{tr}_{u_0'}(\theta; t) = \xi_{u_0'}(\theta; t) + \sum_{q=1}^{\infty} \left\langle \delta \left[ \theta - \sum_{q'=1}^{q+1} \tau_{q'} \right] \right\rangle. \] (119)

Equation (119) is the main result of this paper, which clarifies the physical meaning of the probability densities \( \varphi^{tr}_{u_0'}(\theta; t) = \psi^{tr}_{u_0'}(\theta; t) \) of the transit time \( \theta \). We notice that the probability density of transit times can be interpreted as a path sum, which is a discrete analog of a path integral, and which expresses the individual contributions of various successions of transformations of different lengths of the type \( u_0' \to u_1' \to \cdots \to u_q' \to u \), to the transport of a fragment within the system. Similarly, a random realization of the transit time \( \theta \) is the sum of the various lifetimes \( \tau_1, \ldots, \tau_{q+1} \) of the states \( u_1', \ldots, u_q', u \)
\[ \theta = \sum_{q'=1}^{q+1} \tau_{q'}. \] (120)

Equation (120) looks similar to a relationship derived by Easterby [1989, 1990] for a linear reaction sequence of \( n \) intermediate species (metabolites), \( I_1, \ldots, I_n \) operated in stationary conditions
\[ S \rightarrow I_1 \rightleftharpoons I_2 \rightleftharpoons \cdots \rightleftharpoons I_n \rightarrow P, \] (121)
for which the average transit time \( \langle \theta \rangle \) can be expressed as a sum of average lifetimes \( \langle \tau_1 \rangle, \ldots, \langle \tau_n \rangle \) corresponding to the intermediates \( I_1, \ldots, I_n \):
\[ \langle \theta \rangle = \sum_{j=1}^{n} \langle \tau_j \rangle = \sum_{j=1}^{n} \frac{[I_j]}{J^{st}}, \] (122)
where \([I_1]^{st}, \ldots, [I_n]^{st}\) are the stationary concentrations of the species \( I_1, \ldots, I_n \), \( J^{st} \) is the stationary value of the reaction flux along the linear reaction sequence (121) and \( \langle \tau_1 \rangle, \ldots, \langle \tau_n \rangle \) are the average lifetimes of the species \( I_1, \ldots, I_n \):
\[ \langle \tau_j \rangle = \frac{[I_j]^{st}}{J^{st}}, \quad j = 1, \ldots, n. \] (123)

Despite the apparent similar structure, between Eqs. (120) and (122), there is a fundamental difference: in Eq. (120) all quantities are fluctuating; they are random realizations of various
different random variables, which are generally correlated, whereas Easterby equation (122) is a
deterministic relation connecting the average values of these random variables in the particular
case of a linear reaction sequence operated in sta-
tionary conditions. According to Eq. (115) for
the time-dependent joint probability distribution
ξ(τ)

(q)
uq′...u′...u0q′(τq+1,...,τ1)duq+1...dq1 of the transit
times, the stochastic dependence among the various
lifetimes is generally non-Markovian and a simple
relationship similar to the Easterby’s equation (120)
does not exist.

In the particular case of a stationary regime the
stochastic dependence among the random variables
attached to the different carriers becomes Marko-
vian and our formalism becomes much simpler. In
Eq. (115) the long memory effects due to the time
dependence of the lifetime probability densities dis-
appear. We have:

\[ \xi_{uu′...u′...u0}(τq+1,...,τ1)duq+1...dq1 = \xi_{u0}(τq+1)dτ_q+1\xi_{u′q′}(τ_q)dτ_q...\xi_{u′1u0}(τ_1)dτ_1, \]  

and the integrals in the path summation formulas
derived above can be expressed in terms of mul-
tiple convolution products. After some algebraic
manipulations we can rewrite Eq. (119) in a com-
 pact matrix form:

\[ \varphi(θ) = ψ(θ) = ξ(θ) + \sum_{q=1}^{∞} \xi(θ) ⊗...⊗\xi(θ), \]  

where ⊗ denotes the temporal convolution product.
By using Eq. (125) we can compute the average
value of the transit time,

\[ ⟨θ⟩_u = \sum_{u′} \int_{0}^{∞} θμu′(θ)dθ, \]  

in terms of the marginal averages

\[ ⟨τ⟩_uj = \int_{0}^{∞} τξ(uj)(τ)dτ \]

\[ = \int_{0}^{∞} τ[(1 - δ_{uj} ‾ξ_{uj} + δ_{uj} Ω_{u}^+) e^{-τ(Ω_{u}^+ + \sum_{u′≠u} Ω_{u}^+ + \hat{ω}_{u′})(τ)}dτ \]

\[ = (1 - δ_{uj} ‾ξ_{uj} + δ_{uj} Ω_{u}^+) \Omega_{u}^+ + \sum_{u′≠u} Ω_{u}^+ + \hat{ω}_{u′}. \]  

We have:

\[ ⟨θ⟩_u = \sum_{u′} ⟨τ⟩_uu′ + \sum_{q=1}^{∞} \sum_{u_q′} \cdots + ⟨τ⟩_uu_0′ . \]  

Eq. (128) is a generalization of Easterby’s equation
(120). In the particular case of a linear reaction
sequence of the type (121), Eq. (128) reduces to
the original Easterby relation (120).

In conclusion, in this section we have clarified
the relationships between the transit times and the
lifetimes of the different species present in the sys-
tem. We have shown that a transit time is a sum
of different lifetimes corresponding to different re-
action pathways. We have proven that this physical
definition of the transit time leads to chains of in-
tegral equations for the probability densities of the
transit time, which are equivalent with the integro-
differential balance equations derived in the pre-
vius sections of the article. We have shown that
in the particular case of a time-invariant system
our definition of the transit time is consistent with
Easterby’s definition [Easterby, 1989, 1990].

7. Extracting Kinetic Information
from Experimentally Determined
Susceptibilities

In this section we discuss various methods for the
experimental evaluation of the dynamic suscepti-
bilities from the linear response law (1). We also
examine what one can learn about the reaction
mechanism and kinetics from response experiments.

In order to extract kinetic information from
tracer experiments, we express the response the-
orem (26) in terms of the probability density
φ(u; t′). We have:

\[ β_u(t) = \sum_{u′=1}^{∞} \int_{0}^{∞} φ(u; t′)α_{u′}(t - t′)dθ. \]  

We consider two different types of response ex-
periments: (1) Transient experiments, for which
the excitation functions α_{u′}(t) have the form of a
unitary impulse or as a Heaviside step function.
(2) Frequency response experiments, for which
the excitation functions α_{u′}(t) are periodic.

An example of the first type of transient re-
response experiment consists in the generation of an
impulse in the input channel $u_0$, at different initial times $t_0 = t_0^{(1)}, t_0^{(2)}, \ldots$:

$$\alpha_u(t) = A_{u0} \delta_{uu0} \delta(t - t_0), \quad (130)$$

where $A_{u0}$ is an amplitude factor. According to Eq. (129) the response to the excitation (130) is:

$$\beta_u(t)|_{t_0} = \sum_{u'}^{\infty} \int_0^\infty \varphi_{uu'}(\theta; t) A_{u0} \delta_{uu'0} \delta(t - t_0 - \theta) d\theta$$

$$= A_{u0} \varphi_{uu0}(t - t_0; t), \quad (131)$$

from which we get the following expression for the probability density $\varphi_{uu0}(\theta; t)$:

$$\varphi_{uu0}(\theta; t) = \frac{\beta_u(t)|_{t_0=t-\theta}}{A_{u0}}. \quad (132)$$

We notice that, in order to evaluate the probability density $\varphi_{uu0}(\theta; t)$ it is necessary to carry out many response experiments, for which the excitation of the system takes place at different initial times $t_0 = t_0^{(1)}, t_0^{(2)}, \ldots$. The method simplifies considerably if the system is operated in a stationary regime. In this case due to the property of temporal invariance, we have:

$$\beta_u(t)|_{t_0} = \beta_u(t - t_0), \quad (133)$$

and Eq. (132) reduces to:

$$\varphi_{uu0}(\theta) = \frac{\beta_u(\theta)}{A_{u0}}, \text{ independent of } t_0. \quad (134)$$

Here a single set of response experiments is enough for the evaluation of the probability density $\varphi_{uu0}(\theta; t)$ of the transit time.

The generation of an impulse with the shape of a Dirac delta function is a difficult experimental problem. It is usually easier to generate a response having the shape of a step function of the type:

$$\alpha_u(t) = B_{u0} \delta_{uu0} h(t - t_0), \quad (135)$$

where $B_{u0}$ is another amplitude factor and $h(t - t_0)$ is the unitary Heaviside step function. The response to the excitation (135) is:

$$\beta_u(t)|_{t_0} = B_{u0} \sum_{t_0}^{\infty} \varphi_{uu0}(x; t) dx, \quad (136)$$

from which we get the following expressions for the probability density $\varphi_{uu0}(\theta; t)$:

$$\varphi_{uu0}(\theta; t) = -\frac{1}{B_{u0}} \frac{\partial}{\partial t_0} [\beta_u(t)|_{t_0}]|_{t_0=t-\theta}. \quad (137)$$

In particular, if the system is operated in a stationary regime, the response is expressed by Eq. (133), and Eq. (137) turns into a simpler form:

$$\varphi_{uu0}(\theta) = \frac{1}{B_{u0}} \frac{\partial}{\partial \theta} \beta_u(\theta). \quad (138)$$

For a typical frequency response experiment the excitation function $\alpha_u(t)$ is a periodic function characterized by a single harmonic, corresponding to the carrier $u_0$:

$$\alpha_u(t) = [\ddot{\alpha}_{u0} + \Delta \alpha_{u0} \exp(i\omega t)]\delta_{uu0}, \quad (139)$$

where $\omega$ is a characteristic frequency, and $\alpha_{u'}$ and $\Delta \alpha_{u'}$ are the temporal average and the amplitude of the excitation function, respectively. By combining Eqs. (129) and (139) we come to:

$$\beta_u(t) = \ddot{\alpha}_{u0} \Xi_{uu0}(\omega = 0; t)$$

$$+ \Delta \alpha_{u0} \Xi_{uu0}(\omega; t) \exp(i\omega t), \quad (140)$$

where

$$\Xi_{uu'}(\omega; t) = \int_0^\infty \varphi_{uu'}(\theta; t) \exp(-i\omega \theta) d\theta, \quad (141)$$

is a frequency-dependent susceptibility function given by the one-sided Fourier transform of the transit time distribution $\varphi_{uu'}(\theta; t)$. The frequency response of the system, expressed by Eq. (140) can be rather complicated: for example, if the underlying kinetics of the process generates a stable limit cycle, it is made up of the superposition of two different oscillatory processes, an external oscillation given by the excitation (139) and an internal oscillation, represented by the complex susceptibility $\Xi_{uu'}(\omega; t)$, which, like the transit time probability density, $\varphi_{uu'}(\theta; t)$, is a periodic function of time. The interaction between these two oscillations may lead to interesting effects, which can be studied by using Eqs. (140) and (141). We do not deal with this problem here. The main implication of Eqs. (140) and (141) considered in this article is the possibility of evaluating the time and frequency dependence of the susceptibility function $\Xi_{uu'}(\omega; t)$ from a set of frequency response experiments corresponding to different input frequencies. If the experimental data are sufficiently accurate then from the susceptibility function $\Xi_{uu'}(\omega; t)$ it is possible to evaluate the transit time probability density, $\varphi_{uu'}(\omega; t)$, by
means of numerical inverse Fourier transformation. If the experimental data are not very accurate then it is still possible to evaluate the first two or three moments and cumulants of the transit time. Since the mean transit time is non-negative it follows that
\[ \varphi_{uu'}(\theta < 0; t) = 0, \]  
(142)
and thus the characteristic function of the probability density \( \varphi_{uu'}(\theta; t) \) is identical with the susceptibility function in the frequency domain, \( \Xi_{uu'}(\omega; t) \), experimentally accessible from Eqs. (140) and (141). It follows that the moments \( \langle \theta^m(t) \rangle_u \), \( m = 1, 2, \ldots \) and the cumulants \( \langle \langle \theta^m(t) \rangle_u \), \( m = 1, 2, \ldots \) of the transit time can be evaluated by computing the derivatives of \( \Xi_{uu'}(\omega; t) \) and for zero frequency, respectively
\[
\langle \theta^m(t) \rangle_u = i^m \sum_{u' = 1}^S \frac{d^m}{d\omega^m} \Xi_{uu'}(\omega = 0; t),
\]
\[ m = 1, 2, \ldots, \]  
(143)
\[
\langle \langle \theta^m(t) \rangle_u = i^m \sum_{u' = 1}^S \frac{d^m}{d\omega^m} \ln \Xi_{uu'}(\omega = 0; t),
\]
\[ m = 1, 2, \ldots. \]  
(144)

We notice a formal analogy between the description of the tracer experiments suggested in this article and the linear response theory of Kubo [Toda & Collab., 1991] for nonequilibrium systems with memory. From this point of view Eq. (129), and its particular case (140) corresponding to a frequency response experiment, is the analog of the force-flux relationships for systems with memory. This analogy is only superficial because Kubo’s theory is limited to linear systems whereas the underlying dynamics of the chemical processes studied in this article are nonlinear. The linear structure of Eqs. (129) and (140) is generated by the particular experiment suggested in this article, i.e. the fact that the total influx concentrations are kept constant and only the proportion of tracer molecules is variable. If the total influx concentrations are not kept constant the linear relationships (129) and (140) do not hold anymore. Even when the total influx concentrations are constant, and Eqs. (7.1) and (7.12) are valid, the underlying nonlinear dynamics of the process generates some features which are missing from the linear systems described by the Kubo’s theory. The susceptibility function \( \Xi_{uu'}(\omega; t) \) is given by a Fourier transform with respect to the transit time of a fragment and not with respect to the time variable. For this reason, unlike in the case of Kubo’s theory, in our approach the susceptibility function \( \Xi_{uu'}(\omega; t) \) depends both on frequency and on time. For example, for a system with a limit cycle the time dependence is periodic and determined by the underlying nonlinear dynamics of the process.

Despite the fact that the susceptibility function in the frequency domain \( \Xi_{uu'}(\omega; t) \) is both frequency- and time-dependent some general features of Kubo’s theory are preserved in the case of our approach. An important consequence is generated by the causality principle which leads to a general relationship of the Kramers–Kronig type between the real and imaginary parts of the susceptibility function. We have already derived Kramers–Kronig relations of this type in the particular case of lifetime distributions [Vlad et al., 1998]. The derivation from [Vlad et al., 1998] can be easily extended to the case of transit time distributions.

The qualitative and quantitative analyses of the probability densities of the transit time may lead to interesting hints concerning the reaction mechanism and the kinetics of the process. This is an important problem and we intend to present it in detail elsewhere. Here we present only a simple illustration based on the study of the response of a time-invariant (stationary) system.

In general, in the response law Eq. (1) the non-linearity of the underlying kinetics leads to a non-stationary, time-dependent regime, for which the delay functions \( \chi_{uu'}(t, t') \) depend on two different times. In this case, the investigation of the reaction mechanisms and the kinetics is rather difficult. However if the chemical process can be operated in a stationary regime then the response experiment can be described by stationary time series and the delay function \( \chi_{uu'}(t, t') \) depends only on the difference, \( t - t' \), of the entrance and exit times.

\[
\chi_{uu'}(t, t') = \chi_{uu'}(t - t') = \varphi_{uu'}(\theta),
\]
(145)
where \( \varphi_{uu'}(\theta) \) is the probability density of the transit time \( \theta = t - t' \). For a stationary process the probability density of the transit time \( \varphi_{uu'}(\theta) \) can be expressed in terms of a stationary transport matrix \( K = |K_{uu'}| \), where \( K_{uu'} \) is the rate of transport of a molecular fragment from a carrier \( u \) to
a carrier \( u' \). In this case the investigation of reaction mechanisms by means of tracer experiments is much simpler.

The qualitative examination of the shape of the response curve may lead to interesting conclusions concerning the reaction mechanism. A simple procedure consists in the examination of the shape of a log–log plot of the effective decay rate \( k_{\text{eff}}(\theta) = -\partial \ln \varphi_{uu'}(\theta) / \partial \theta \) versus the transit time, \( \log k_{\text{eff}}(\theta) \) versus \( \log \theta \). The number of horizontal regions displayed by such a log–log plot give a limit for the minimum number of reaction steps involved in the process. Such a method works only if the time scales corresponding to the different reaction steps have different orders of magnitude. An alternative approach, which works if the time scales of the various chemical steps are close to each other, is based on the fitting of the delay function \( \chi_{uu'}(t - t') = \varphi_{uu'}(\theta) \) by a superposition of exponential factors

\[
\varphi_{uu'}(\theta) = \sum_m A_m \exp(-\lambda_m \theta) \quad (146)
\]

The minimum number of exponential factors in Eq. (146) necessary for fitting the experimental data provides a lower limit for the number of chemical steps involved in the process.

Important information about the reaction mechanisms can be obtained by investigating the concentration dependence of the fitted eigenvalues \( \lambda_m \) from Eq. (146). The dependence of the eigenvalues on the concentrations can be extremely complicated. In order to circumvent this complication we suggest to analyze the concentration dependence of a set of the tensor invariants [Aris, 1990] constructed from the eigenvalues \( \lambda_m \)

\[
J_w = \sum_{v_1} \cdots \sum_{v_w} \lambda_{v_1} \cdots \lambda_{v_w} \quad (147)
\]

The tensor invariants depend on the concentrations of the different species present in the system. In most cases the dependence \( J_w = J_w \) (concentration) can be expressed by a polynomial, where the coefficients of the concentrations depend on the rate coefficients of the process. Starting from different assumed reaction mechanisms we can derive theoretical expressions for the dependence on the invariants of the concentrations. By checking the validity of these dependences for the experimental data we can test the validity of the assumed reaction mechanisms.

In conclusion, in this section we have suggested that the linear response law and the response experiments can be applied to the study of dynamic behavior of complex chemical systems. We have shown that the response experiments make it possible to evaluate the susceptibility functions from transient as well as frequency response experiments. We have shown that the susceptibility functions bear important information about the mechanism and kinetics of complex chemical processes. We have suggested a method, based on the use of tensor invariants, which may be used for extracting information about reaction mechanism and kinetics from susceptibility measurements in time-invariant systems.

8. Conclusions

In this article we have suggested a new type of response experiments for nonlinear chemical systems, based on the assumption that least one of the chemical species present in the system can exist in two forms, labeled and unlabeled, respectively and that for the labeled species the kinetic isotope effect can be neglected. Under these circumstances it is possible to design an experiment for which the response of the system to an excitation can be represented by a linear superposition law. The linear response law is valid even for highly nonlinear systems and is due to the fact that the kinetic isotope effect for the labeled species is assumed to be negligible.

We have shown that the dynamic susceptibility function from the response law can be interpreted as the probability density of the transit time of a molecular fragment crossing the system. We have suggested methods for extracting information about the mechanism and kinetics of complex chemical processes from response experiments.

The approach presented in this article can be easily extended for a broad class of transport-interaction processes from physics, chemistry and biology. The following conditions are necessary: (1) at least one of the interacting species from the system exists in two forms, labeled and unlabeled and (2) the labeled species obeys a "neutrality condition", that is its kinetic and transport properties are identical with the corresponding properties of the unlabeled species. An interesting application of such an approach is related to the study of time and space propagation of neutral genes borne by the \( Y \) chromosome during human evolution. Work on this problem is in progress.
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References


Appendix A

We express the matrix \( \mathbf{K}(t) \) as a sum of off-diagonal and diagonal contributions:

\[
\mathbf{K}(t) = \mathbf{K}_{\text{off}}(t) + \mathbf{K}_{\text{diag}}(t),
\]

where \( \mathbf{K}_{\text{off}}(t) = [(1 - \delta_{uu'})\omega_{uu'}(t)]_{u,u'=1,...,S} \), \( \mathbf{K}_{\text{diag}}(t) = \left[ -\delta_{uu'} \left[ \Omega_u^- (t) + \sum_{u'' \neq u} \omega_{u'u''} (t) \right] \right]_{u,u'=1,...,S} \).

We insert Eq. (A.1) into Eq. (23) and integrate the resulting equation by assuming that the term \( \mathbf{K}_{\text{off}}(t) \mathbf{G}(t,t') \) is known. We come to a Lippmann–Schwinger equation similar to Eq. (102)

\[
\mathbf{G}(t,t') = \mathbf{F}(t,t') + \int_{t'}^{t} \mathbf{F}(t,t'') \times \mathbf{K}_{\text{off}}(t'') \mathbf{G}(t'',t') dt'',
\]

where

\[
\mathbf{F}(t,t') = \left[ \exp \left[ -\int_{t'}^{t} \left[ \Omega_u^+ (t'') \right] \right] + \sum_{u'' \neq u} \omega_{uu''} (t'') dt'' \right]_{u,u'=1,...,S}.
\]

By expressing the solution of Eq. (A.4) as a Lippmann–Schwinger series, we come to:

\[
\mathbf{G}(t,t') = \mathbf{F}(t,t') + \int_{t'}^{t} \mathbf{F}(t,t_1) \mathbf{K}_{\text{off}}(t_1) \mathbf{F}(t_1,t') dt_1 + \int_{t'}^{t} \int_{t'}^{t_2} \mathbf{F}(t,t_2) \mathbf{K}_{\text{off}}(t_2) \mathbf{F}(t_2,t_1) \times \mathbf{K}_{\text{off}}(t_1) \mathbf{F}(t_1,t') dt_2 dt_1 + \int_{t'}^{t} \int_{t'}^{t_{q-1}} \cdots \int_{t'}^{t_q} \mathbf{F}(t,q) \mathbf{K}_{\text{off}}(q) \times \mathbf{F}(t_q,t_{q-1}) \cdots \mathbf{K}_{\text{off}}(t_1) \mathbf{F}(t_1,t') dt_q \cdots dt_1 + \cdots
\]
We notice that according to their definitions, all matrix elements in the r.h.s of Eq. (A.6) are non-negative. By taking into account the structure of Eq. (A.6), it follows that all matrix elements \( G_{uu'}(t; t') \) are non-negative. Since the fluxes \( J_u^+(t) \) are also non-negative by definition, from Eq. (27) it follows that the susceptibility functions \( \chi_{uu'}(t; t') \) are non-negative.

**Appendix B**

In order to derive a system of evolution equations for the probability densities of the transit time, \( \varphi_{uu'}(\theta; t) \) and \( \psi_{uu'}(\theta; t) \), we express Eqs. (47)–(49) for the density functions \( \zeta_{uj}(\theta; t) \), in terms of \( \varphi_{uu'}(\theta; t) \) and \( \psi_{uu'}(\theta; t) \). From Eqs. (47)–(49) we come to:

\[
f_u(t) \left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \theta} \right) \varphi_{uu'}(\theta; t) + \varphi_{uu'}(\theta; t) \frac{df_u(t)}{dt} = \sum_{u' \neq u} \omega_{uu'}(t) \varphi_{u'j}(\theta; t) - \varphi_{uu'}(\theta; t) \left\{ \Omega_u^+ + \sum_{u'' \neq u} \omega_{uu''} \frac{f_{u''}(t)}{f_u(t)} \right\},
\]

(B.1)

In Eq. (B.1) we compute the time derivatives \( df_u(t)/dt \) from the kinetic equations (7), resulting in:

\[
\left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \theta} \right) \varphi_{uu'}(\theta; t) = \sum_{u' \neq u} \omega_{uu'}(t) \frac{f_{u'}(t)}{f_u(t)} \varphi_{u'j}(\theta; t)
\]

\[-\varphi_{uu'}(\theta; t) \left\{ \Omega_u^+ + \sum_{u'' \neq u} \omega_{uu''} \frac{f_{u''}(t)}{f_u(t)} \right\} \]  

(B.4)

By expressing Eqs. (B.2)–(B.4) in terms of the adjoint rates \( \dot{\omega}_{uu'}(t) \) defined by Eqs. (32) we come to Eqs. (57)–(59).

\[
\varphi_{uj}(\theta = 0; t) = \delta_{uj} \Omega_u^+(t), \quad \text{(B.2)}
\]

\[
\varphi_{uj}(\theta; t = t_0) = \varphi_{uj}^{(0)}(\theta), \quad \text{(B.3)}
\]