Kinetic laws, phase-phase expansions, renormalization group, blood coagulation and INR calibration

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Abstract

We introduce systematic approaches to chemical kinetics based on the use of phase-phase expansions. The approaches follow from the observation that mass action law kinetics displays a linear dependence in a phase-phase (log-log) representation, which is a first order approximation of a regular (or functional) series expansion. For slow processes, such a representation leads to a corrected form of the mass-action law, where the concentrations are replaced by kinetic activities. For fast reactions delay expressions are derived. A generic mechanism is introduced for the occurrence of a generalized mass-action law as a result of self-similar recycling. The approaches are applied to a biological problem of major medical interest, blood coagulation. We show that our self-similar recycling model applied to the first stage of in vitro coagulation reproduces the empirical equations for the International Normalized Ratio calibration, (*INR*), as well as the Watala, Golanski and Kardas relation (WGK) for dependence of the *INR* on the concentrations of coagulation factors. Conversely, experimental results, without use of a theoretical model, show that the calibration equation for the *INR*, combined with the empirical WGK relation, leads to a generalized mass-action type kinetic law for the onset of 'in vitro' blood coagulation.

Key words: chemical kinetics, phase-phase expansions, renormalization group, blood coagulation, international normalized ratio.

1. Background

The mechanisms of chemical and biochemical reaction systems have been traditionally guessed or hypothesized, and then tested experimentally. We have been concerned for nearly twenty years with the design of experiments and theories from which reaction mechanisms can be deduced [1,2]. Our first studies were on oscillatory reactions ([1], Chaper 3). A second approach concentrated on the determination of direct connectivities of chemical species by chemical reactions ([1], Chapter 5), and this simple method was shown to be useful in experiments on a test system, a part of glycolysis ([1], Chapter 6). A third study focused on correlation functions of concentrations of reacting species ([1], Chapter 7) as determined from measurements. The interpretations of such experiments by means of multidimensional scaling analysis leads to a skeletal diagram of the reaction mechanism and sites of strong control within the reaction network. This method was also tested on a part of glycolysis, and has been used on systems with hundreds of genes [3], [4]. Lifetime distributions of reacting species and general response experiments are discussed in [1], Chapter 12. Yet other approaches based on optimization methods, such as genetic algorithms, of the performance of a task assigned to a reaction mechanism have led to interesting results [5],[6]. In this article we continue our research on the kinetics of complex systems in a study of kinetic laws and phase – phase expansions with application to the problem of blood coagulation.

2. Introduction

Developments in biochemistry, genetics, genomics and molecular biology require the development of suitable tools for describing the overall kinetics of complex processes. Blood coagulation can be used as a test case for the description of such complicated phenomena: it involves not only biochemical reactions, but it also has genetic, physiological and demographic components. Our study focuses on the kinetic analysis of the prothrombin time, a clinical laboratory test commonly used to adjust the dosage of the oral anticoagulants, such as warfarin. Readers interested only in blood coagulation may skip Sections 3 and 4 and proceed directly to Section 5.

In this paper we use the phase-phase expansion as a useful tool for representing rate equations in chemical kinetics; our starting point is our previous study of nonlinear functional response laws [7]. Then

we derive a renormalization group approach for the global kinetics of complex systems with recycling, that is, with the regeneration and reuse of some of the reagents, for example enzymes. The self-similar recycling reaction model is applied to the initial stage of 'in vitro' blood coagulation. The model succeeds in deriving theoretically the empirical equations for the coagulation time presented in the literature. Conversely, the experimental results, without use of a theoretical model, lead to a generalized mass-action kinetic law.

3. Phase-phase expansion and kinetic laws

The mass action-law is a central paradigm of chemical kinetics. According to this law the rate of a chemical reaction u, r_u, is proportional to the product of the concentrations $c_1, c_2, \ldots$, of the different reagents, raised to different powers, $r_u = k_u \prod_{u'} \left(c_{u'}\right)^{\nu^{(1)}_{uu'}}$; here k_u are rate coefficients and $\nu^{(1)}_{uu'}$ are reaction orders. Strictly speaking, this form of mass action law is assumed to hold only for elementary reactions for which the reaction orders $\nu^{(1)}_{uu'}$ are integers. More complicated rate equations are derived from the mass-action law for complex reactions, which are made up of successions of elementary reactions; the resulting rate laws are more complicated, such as hyperbolic (Michaelis-Menten, Langmuir kinetics) and polynomical fractions or ratios of fractal laws (cooperative, Hill kinetics). Generalizations of the mass-action laws can also hold for complex reaction, where the reaction orders can be fractions (rate-determining step models) or arbitrary real numbers (generalized power law, fractional kinetics [8]).

Even for elementary reactions, the validity of the mass action law is limited: it only holds for slow processes at local equilibrium occurring in ideal gases and dilute solutions. An important problem is the development of systematic methods for representing the deviations from the ideal case represented by the mass action law. Our approach is based on the assumption that the dependences of the logarithms of the reaction rates, $\ln r_u$, on the logarithms of the concentrations of the different species, $\ln c_{u'}$, are analytic in the vicinity of the logarithms of a set of reference concentrations $\ln c^0_{u'}$; we can represent $\ln r_u$ by a Taylor series

$$\ln r_u(\mathbf{c}) = \ln r_u\left(\mathbf{c}^0\right) + \sum_{m=1}^{\infty} \frac{1}{m!} \sum_{u'_1 \ldots u'_m} \left(\frac{\partial^m \ln r_u\left(\mathbf{c}^0\right)}{\partial \ln c^0_{u'_1} \ldots \partial \ln c^0_{u'_m}} \right) \prod_{\alpha=1}^{m} \ln\left(\frac{c_{u'_\alpha}}{c^0_{u'_\alpha}} \right), u = 1,2,\ldots. \qquad (1)$$

We notice that a kinetic law of the mass-action law type, $r_u(\mathbf{c}) = k_u \prod_{u'} (c_{u'})^{\nu^{(1)}_{uu'}}$, is obtained from Eq.(1) if we only keep the first order terms, where $k_u = r_u(\mathbf{c}^0) \prod_{u'} (c^0_{u'})^{-\nu^{(1)}_{uu'}}$ and $\nu^{(1)}_{uu'} = \left(\partial \ln r_u(\mathbf{c}^0) \big/ \partial \ln c^0_{u'}\right)$. In this case the reaction orders $\nu^{(1)}_{uu'}$ are given by the derivatives of the logarithms of the reaction rates with respect to the logarithms of the concentrations of the different species evaluated at the reference concentration vector $\mathbf{c}^0$. The reaction orders $\nu^{(1)}_{uu'}$ can be arbitrary real numbers, positive or negative, rational or irrational. The kinetic law obtained by keeping the first-order terms in Eq. (1) is a generalized mass-action law, which includes the classical mass-action law for elementary reactions as well as for rate-determining step kinetics (reaction orders positive or negative, integer or fractions) and general power law kinetics (arbitrary real reaction orders) as particular cases.

Eq. (1) makes it possible to express the kinetic laws in two alternative ways in terms of concentration-dependent reaction orders or of kinetic activities. After a few elementary algebraic manipulations, Eq.(1) can be rewritten in the following form:

$$r_u(\mathbf{c}) = k_u(\mathbf{c}^0) \prod_{u'} (c_{u'})^{\mu^{(1)}_{uu'}(\mathbf{c}^0, \mathbf{c})}, \tag{2}$$

where $\mu^{(1)}_{uu'_1}(\mathbf{c}, \mathbf{c}^0) = \nu^{(1)}_{uu'_1}(\mathbf{c}^0) + \delta\nu^{(1)}_{uu'_1}(\mathbf{c}, \mathbf{c}^0)$ are effective reaction orders that depend both on the reference concentration vector $\mathbf{c}^0$ and on the current concentration vector $\mathbf{c}$; the functions $\delta\nu^{(1)}_{uu'_1}(\mathbf{c}, \mathbf{c}^0)$ are correction factors, which depend on both concentration vectors, the current vector $\mathbf{c}$ and the reference vector $\mathbf{c}^0$

$$\delta\nu^{(1)}_{uu'_1}(\mathbf{c}, \mathbf{c}^0) = \sum_{m=2}^{\infty} \frac{1}{m!} \sum_{u'_2 \ldots u'_m} \nu^{(m)}_{uu'_1 u'_2 \ldots u'_m}(\mathbf{c}^0) \prod_{\alpha=2}^{m} \ln\left(\frac{c_{u'_\alpha}}{c^0_{u'_\alpha}}\right); \tag{3}$$

and

$$\nu^{(m)}_{uu'_1 \ldots u'_m}(\mathbf{c}^0) = \frac{\partial^m \ln r_u(\mathbf{c}^0)}{\partial \ln c^0_{u'_1} \ldots \partial \ln c^0_{u'_m}}, \tag{4}$$

are reaction orders of different orders that depend only on the reference concentration vector $\mathbf{c}^0$. Systematic corrections to the generalized mass action law $r_u(\mathbf{c}) = k_u \prod_{u'} (c_{u'})^{\nu^{(1)}_{uu'}}$ can be expressed by considering concentration dependent corrections to the reaction orders, $\delta\nu^{(1)}_{uu'_1}(\mathbf{c}, \mathbf{c}^0)$, which are expressed as series of the logarithms of concentrations, Eq.(3). Corrections of the kinetic laws can be also expressed in terms of

kinetic activities [9],[10]. The approach of kinetic activities is based on the assumption that, for non-ideal systems, the reaction rates can be expressed as $r_u \sim \prod_{u'} (a_{u'})^{\nu^{(1)}_{uu'}}$, where $a_{u'}$ are kinetic activities attached to the different species; the kinetic activities are functions of the concentration vector, which tend toward the corresponding concentrations for infinite dilution: they are similar, although not necessarily identical to the thermodynamic activities. Our theory shows that in general for each reaction we have to define a different set of kinetic activities. Eq.(2) can be rewritten as:

$$r_u(\mathbf{c}) = k_u(\mathbf{c}^0) \prod_{u'} \left[a_{uu'}\right]^{\nu^{(1)}_{uu'}(\mathbf{c}^0)}. \tag{5}$$

Here the terms $a_{uu'} = c_{u'} \left(c_{u'} / c^0_{u'}\right)^{\delta\nu^{(1)}_{uu'}(\mathbf{c},\mathbf{c}^0)/\nu^{(1)}_{uu'}(\mathbf{c}^0)}$ play the role of kinetic activities; in general they are not only species specific, but also reaction specific, depending both on the reaction label u as well as on the species label u'. The kinetic activities are only species-specific and not reaction specific for systems for which the ratios $\delta\nu^{(1)}_{uu'}(\mathbf{c},\mathbf{c}^0)/\nu^{(1)}_{uu'}(\mathbf{c}^0)$ are independent of the reaction label u. Kinetic activities make it possible to express the rate laws for non-ideal systems in a form similar to the reference kinetic laws. It is possible to develop methods for evaluating kinetic activities from experimental data.

Our considerations can be easily extended for fast reactions and/or inhomogeneous systems, for which the reaction rates depend on the time and/or space histories of the variation of the concentration field. In this case the concentration vector $\mathbf{c}$ is a field that depends on a position vector $\boldsymbol{\rho}$, which can represent the time, $\boldsymbol{\rho} = (t)$, the position in real space $\boldsymbol{\rho} = (\mathbf{r})$, or the position in space-time continuum $\boldsymbol{\rho} = (\mathbf{r}, t)$. Similarly, the reaction rates $r_u\left[\mathbf{c}(\boldsymbol{\rho})\right]$ are functionals of the concentration field $\mathbf{c}(\boldsymbol{\rho})$. By using a functional phase-phase (log-log) expansion we can give the following representation for the reaction rates:

$$r_u\left[\mathbf{c}(\boldsymbol{\rho})\right] =$$

$$r_u\left[\mathbf{c}^0(\boldsymbol{\rho}^0)\right] \exp\left\{\sum_{m=1}^{\infty} \frac{1}{m!} \sum_{u'_1,\ldots,u'_m} \int_{\boldsymbol{\rho}'_1} \cdots \int_{\boldsymbol{\rho}'_m} \eta^{(m)}_{uu'_1,\ldots,u'_m}(\boldsymbol{\rho};\boldsymbol{\rho}'_1,\ldots,\boldsymbol{\rho}'_m) \prod_{v=1}^{m} \ln\left[\frac{c_{u'_v}(\boldsymbol{\rho}'_v)}{c^0_{u'_v}\left(\boldsymbol{\rho}^{(0)}\right)}\right] d\boldsymbol{\rho}'_1, \ldots d\boldsymbol{\rho}'_m\right\}, \tag{6}$$

where the influence functionals $\eta^{(m)}_{uu'_1,\ldots,u'_m}(\boldsymbol{\rho};\boldsymbol{\rho}'_1,\ldots,\boldsymbol{\rho}'_m)$ are generalized susceptibilities defined as the functional derivatives of the logarithms of the reaction rates with respect to the logarithms of concentrations:

$$\eta^{(m)}_{uu'_1\ldots u'_m}(\boldsymbol{\rho};\boldsymbol{\rho}'_1,\ldots,\boldsymbol{\rho}'_m)=\frac{\delta^m}{\delta\ln\left[\dfrac{c_{u'_1}(\boldsymbol{\rho}'_1)}{c^0_{u'_1}\left(\boldsymbol{\rho}^{(0)}\right)}\right]\ldots\delta\ln\left[\dfrac{c_{u'_m}(\boldsymbol{\rho}'_m)}{c^0_{u'_m}\left(\boldsymbol{\rho}^{(0)}\right)}\right]}\ln\left\{\frac{r_u\left[\mathbf{c}(\boldsymbol{\rho})\right]}{r_u\left[\mathbf{c}^0\left(\boldsymbol{\rho}^0\right)\right]}\right\}. \tag{7}$$

The susceptibilities $\eta^{(m)}_{uu'_1,\ldots,u'_m}(\boldsymbol{\rho};\boldsymbol{\rho}'_1,\ldots,\boldsymbol{\rho}'_m)$ can be interpreted as densities of apparent reaction orders. This equation includes the mass-action law as a particular case, where only the linear terms are considered and full locality is assumed, that is, $\eta^{(1)}_{uu'}(\boldsymbol{\rho};\boldsymbol{\rho}')=\nu^{(1)}_{uu'}\delta(\boldsymbol{\rho}-\boldsymbol{\rho}')$.

The first order functional kinetic law analog to the mass-action law (2) is:

$$r_u\left[\mathbf{c}(\boldsymbol{\rho})\right]=r_u\left[\mathbf{c}^0\left(\boldsymbol{\rho}^0\right)\right]\exp\left\{\sum_{u'}\int_{\boldsymbol{\rho}'}\eta^{(1)}_{uu'}(\boldsymbol{\rho};\boldsymbol{\rho}')\ln\left[\frac{c_{u'_1}(\boldsymbol{\rho}')}{c^0_{u'}\left(\boldsymbol{\rho}^{(0)}\right)}\right]d\boldsymbol{\rho}'\right\}, \tag{8}$$

Eq.(2) is a functional fractal response law [7]. Non-ideality corrections to Eq.(8) can be expressed in a form similar to Eq.(2). We have:

$$r_u\left[\mathbf{c}(\boldsymbol{\rho})\right]=r_u\left[\mathbf{c}^0\left(\boldsymbol{\rho}^0\right)\right]\exp\left\{\sum_{u'}\int_{\boldsymbol{\rho}'_1}\sigma^{(1)}_{uu'}\left[\mathbf{c}(\boldsymbol{\rho}),\mathbf{c}^0\left(\boldsymbol{\rho}^0\right);\boldsymbol{\rho};\boldsymbol{\rho}'\right]\ln\left[\frac{c_{u'}(\boldsymbol{\rho}')}{c^0_{u'}\left(\boldsymbol{\rho}^{(0)}\right)}\right]d\boldsymbol{\rho}'\right\}, \tag{9}$$

where $\sigma^{(1)}_{uu'}\left[\mathbf{c}(\boldsymbol{\rho}),\mathbf{c}^0\left(\boldsymbol{\rho}^0\right);\boldsymbol{\rho};\boldsymbol{\rho}'\right]=\eta^{(1)}_{uu'}\left[\mathbf{c}^0\left(\boldsymbol{\rho}^0\right);\boldsymbol{\rho};\boldsymbol{\rho}'\right]+\delta\eta^{(1)}_{uu'}\left[\mathbf{c}(\boldsymbol{\rho}),\mathbf{c}^0\left(\boldsymbol{\rho}^0\right);\boldsymbol{\rho};\boldsymbol{\rho}'\right]$ are effective densities of reaction orders which depend both on the reference concentration field $\mathbf{c}^0\left(\boldsymbol{\rho}^0\right)$ as well as on the current concentration field $\mathbf{c}(\boldsymbol{\rho})$; the functionals $\delta\eta^{(1)}_{uu'}\left[\mathbf{c}(\boldsymbol{\rho}),\mathbf{c}^0\left(\boldsymbol{\rho}^0\right);\boldsymbol{\rho};\boldsymbol{\rho}'\right]$ are correction factors which depend on both concentration fields

$$\delta\eta^{(1)}_{uu'}\left[\mathbf{c}(\boldsymbol{\rho}),\mathbf{c}^0\left(\boldsymbol{\rho}^0\right);\boldsymbol{\rho};\boldsymbol{\rho}'\right]=$$

$$\sum_{m=2}^{\infty}\frac{1}{m!}\sum_{u'_2,\ldots,u'_m}\int_{\boldsymbol{\rho}'_1}\ldots\int_{\boldsymbol{\rho}'_m}\eta^{(m)}_{uu'u'_2,\ldots,u'_m}(\boldsymbol{\rho};\boldsymbol{\rho}',\boldsymbol{\rho}'_2,\ldots,\boldsymbol{\rho}'_m)\prod_{v=2}^{m}\ln\left[\frac{c_{u'_v}(\boldsymbol{\rho}'_v)}{c^0_{u'_v}\left(\boldsymbol{\rho}^{(0)}\right)}\right]d\boldsymbol{\rho}'_2,\ldots d\boldsymbol{\rho}'_m. \tag{10}$$

We can also derive a functional generalization of Eq.(5):

$$r_u\left[\mathbf{c}(\boldsymbol{\rho})\right]=r_u\left[\mathbf{c}^0\left(\boldsymbol{\rho}^0\right)\right]\exp\left\{\sum_{u'}\int_{\boldsymbol{\rho}'}\eta^{(1)}_{uu'}\left[\mathbf{c}^0\left(\boldsymbol{\rho}^0\right);\boldsymbol{\rho};\boldsymbol{\rho}'\right]\ln\left[\frac{a_{uu'}(\boldsymbol{\rho}')}{c^0_{u'}\left(\boldsymbol{\rho}^{(0)}\right)}\right]d\boldsymbol{\rho}'\right\}, \tag{11}$$

where

$$a_{uu'}(\boldsymbol{\rho}') = c_{u'}(\boldsymbol{\rho}')\left[c_{u'}(\boldsymbol{\rho}')/c^0_{u'}\left(\boldsymbol{\rho}^{(0)}\right)\right]^{\delta\eta^{(1)}_{uu'}\left[\mathbf{c}(\boldsymbol{\rho}),\mathbf{c}^0(\boldsymbol{\rho}^0);\boldsymbol{\rho};\boldsymbol{\rho}'\right]/\eta^{(1)}_{uu'}\left[\mathbf{c}^0(\boldsymbol{\rho}^0);\boldsymbol{\rho};\boldsymbol{\rho}'\right]}, \qquad (12)$$

are species-specific and reaction specific kinetic activities. We reach the same conclusion as for localized processes, that is, in general the activity fields $a_{uu'}(\boldsymbol{\rho}')$ depend on two labels, u and u'; that is, they are both species specific and reaction specific. They are species specific and reaction independent only if the ratios $\delta\eta^{(1)}_{uu'}\left[\mathbf{c}(\boldsymbol{\rho}),\mathbf{c}^0(\boldsymbol{\rho}^0);\boldsymbol{\rho};\boldsymbol{\rho}'\right]/\eta^{(1)}_{uu'}\left[\mathbf{c}^0(\boldsymbol{\rho}^0);\boldsymbol{\rho};\boldsymbol{\rho}'\right]$ are independent of the reaction label u.

This field theory includes the localized theory as a particular case. For localized processes, the densities of reaction orders contain products of delta functions $\eta^{(m)}_{uu'_1,\ldots,u'_m}(\boldsymbol{\rho};\boldsymbol{\rho}'_1,\ldots,\boldsymbol{\rho}'_m) = \nu^{(m)}_{uu'_1,\ldots,u'_m}\prod_\alpha \delta\left(\boldsymbol{\rho}-\boldsymbol{\rho}_{u'_\alpha}\right)$ and Eqs.(8)-(12) lead to Eqs.(1)-(5).

In conclusion, in this section we have shown that phase-phase (log-log) expansions provide a systematic way of representing kinetic laws. The first order expansions leads to generalized mass-action laws with real reaction orders.

4. Recycling processes and generalized mass-action law

Many processes may be represented by a generalized mass action law, where the reaction orders are arbitrary real numbers, not only integers or fractions. The formalism of chemical kinetics is a meta-language [11], which can be used for describing various time-dependent phenomena, not only in chemistry but also in other natural and social sciences, ranging from physics, chemistry and biology to demography and economics.

In this section we show that there is a connection between complex recycling phenomena, and generalized mass action laws with arbitrary, real, apparent reaction orders. Although our approach is motivated by the study of blood coagulation kinetics, which will be analyzed in the next section, recycling processes are a generic mechanism for the emergence of generalized mass-action kinetic laws. We consider a process which can be represented by a single overall rate, and study the contributions to this overall rate by a number of species, with concentrations $c_1,\ldots,c_n$. Each of these species is involved in a recycling process, which takes place over and over, with different efficiencies. Other chemical species may be involved in the process, but they are not explicitly considered in our analysis; we assume that their concentrations are kept constant throughout the process. In a reaction network, recycling can take place in

different ways. In biochemical reactions, enzymes, after facilitating a catalytic process, are generally released in free forms ready to enter another reaction cycle. Theoretically recycling can be complete; in practice, due to enzyme degradation and other reactions, recycling is not complete and from one cycle to the next lower quantities of the initial amount of enzyme are recycled. In 'in vivo' systems the body tries to keep constant concentrations by compensating the losses with newly produced enzymes. The processes can be further complicated by the arrangement of the reactions in cascades, where the product of a reaction enters another reaction and in these cascades different species are subjected to recycling. Another type of recycling involves amplification, not reduction. This is the case of a branched chain reaction, where one active intermediate can produce two or more active intermediates and the possible losses are outweighed by this growth phenomenon.

We denote by $\psi_{q_1 \ldots q_n}$ the probability that the species c_u is involved in q_u recycling processes, where $u = 1, \ldots, n$. The probability $\psi_{q_1 \ldots q_n}$ can be determined from the detailed kinetics of the process and typically is the product of geometric (Pascal) probabilities. From the cycle $q_{u-1} - 1$ to the cycle q_u the concentration of the species u is changed (reduced or amplified) by a factor $b_u^{(q_u)}$; these factors can be also determined from the detailed kinetics of the process. We denote by $r(c_1, \ldots, c_n)$ the overall reaction rate in the case where no recycling processes occur and by $\tilde{r}(c_1, \ldots, c_n)$ the reaction rate for a system with recycling; $\tilde{r}$ can be expressed as an average over all possible numbers of recycling processes:

$$\tilde{r}(c_1, \ldots, c_n) = \sum\nolimits_{q_1 \ldots q_n} \psi_{q_1 \ldots q_n} r\left(\frac{c_1}{\prod_{q_1'=1}^{q_1} b_1^{(q_1')}}, \ldots, \frac{c_n}{\prod_{q_n'=1}^{q_n} b_n^{(q_n')}} \right). \tag{13}$$

We express both rates r and $\tilde{r}$ in the form (2) that is $r = k \prod_{u'} (c_{u'})^{\mu_{u'}^{(1)}}$ and $\tilde{r} = \tilde{k} \prod_{u'} (c_{u'})^{\tilde{\mu}_{uu'}^{(1)}}$, where in general both sets of apparent stoichiometric coefficients, $\mu_{u'}^{(1)}$ and $\tilde{\mu}_{u'}^{(1)}$, are concentration dependent. From Eq.(13) we come to:

$$\tilde{\mu}_u^{(1)} = \partial \ln \tilde{r} / \partial \ln c_u = \left\langle \mu_u^{(1)}(c_1, .., c_u c_u / B_u, \ldots c_n) / B_u \right\rangle, \tag{14}$$

where $B_u = \prod_{q_u'=1}^{q_u} b_u^{(q_u')}$ are instantaneous overall change (reduction or amplification) factors attached to the species u and the average $\langle \ldots . \rangle$ is taken over all numbers of recycling steps q_u and evaluated in terms of a scaled, renormalized probability for the number q_u of recycling events attached to the species u:

$$\widetilde{\psi}_{q_u}^{(u)} = \frac{\sum_{q_1'\ldots q_n' \neq q_u} \psi_{q_1'\ldots q_n'} r\left(c_1 / B_1^{(q_1')}, \ldots, c_n / B_n^{(q_n')}\right)}{\sum_{q_1'\ldots q_n'} \psi_{q_1'\ldots q_n'} r\left(c_1 / B_1^{(q_1')}, \ldots, c_n / B_n^{(q_n')}\right)}, \text{ with } \sum_{q_u} \widetilde{\psi}_{q_u}^{(u)} = 1, \tag{15}$$

Eq.(14) is quite general and independent of the details of the kinetic process. It shows that recycling processes can easily produce arbitrary, real reaction orders. An interesting particular case of Eq.(14) is that for which the initial kinetics obeys the classical mass action law and thus the initial reaction orders $\mu_u^{(1)}$ are concentration independent. In this case we have $\widetilde{\mu}_u^{(1)} = \mu_u^{(1)} \left\langle B_u^{-1} \right\rangle$ with $\left\langle B_u^{-1} \right\rangle = \sum_{q_u} \widetilde{\psi}_{q_u}^{(u)} / B_u^{(q_u)}$.

A simple case is the one where a constant probability p_u for the occurrence of a step in the recycling process is attached to the species u, where $u = 1, \ldots, n$; p_u is assumed to be the same for all steps of a given recycling process but varies from process to process. Similarly, the step-by-step change factors, $b_u^{(q_u')}$, are assumed constant for a given recycling process u, that is $b_u^{(q_u')} = b_u$. If these conditions are fulfilled each step of a recycling process has exactly the same behavior as any other step and the recycling processes are self-similar. Under these circumstances $\psi_{q_1\ldots q_n} = \prod_u \left[(p_u)^{q_u} (1 - p_u) \right]$ and Eq.(13) becomes

$$\widetilde{r}(c_1, \ldots, c_n) = \sum_{q_1\ldots q_n} \prod_u \left[(p_u)^{q_u} (1 - p_u) \right] r\left(c_1 (b_1)^{-q_1}, \ldots, c_n (b_n)^{-q_n}\right), \tag{16}$$

The series expansion (16) can be put in the form of a renormalization group equation (RG, [12]). We multiply each term of Eq.(16) by $\prod_u p_u$ and make the substitutions $c_u \to c_u / b_u$; finally we add a number of terms on both sides of the resulting equation, so that we can identify an expansion equal to $\widetilde{r}(c_1, \ldots, c_n)$ on the left side. The final result is the following:

$$\begin{aligned} &\widetilde{r}(c_1, \ldots, c_n) \\ &= \prod_u p_u \widetilde{r}\left(\frac{c_1}{b_1}, \ldots, \frac{c_n}{b_n}\right) + \sum_{\substack{1 \geq q_1 \ldots q_n \geq 0 \\ \sum q_u \leq n-1}} \prod_u \left[(p_u)^{q_u} (1 - p_u) \right] r\left(c_1 (b_1)^{-q_1}, \ldots, c_n (b_n)^{-q_n}\right). \end{aligned} \tag{17}$$

The RG equation (17) is a functional equation for the rate $\widetilde{r}(c_1, \ldots, c_n)$, which can be solved by using the method of Mellin transformation [13]. An alternative approach is the use of the Poissonian summation formula [14] for evaluating the expansion (16). In either case the behavior of $\widetilde{r}$ for medium to large concentrations is given by:

$$\widetilde{r}(c_1, \ldots, c_n) = \Xi\left[\ln(c_1 / c_1^0), \ldots, \ln(c_n / c_n^0)\right] \prod_{u=1}^{n} (c_u)^{\mu_u}, \tag{18}$$

where $\mu_u = \ln p_u / \ln b_u$ are fractal exponents and $\Xi\left[\ln\left(c_1/c_1^0\right),\ldots,\ln\left(c_n/c_n^0\right)\right]$ is a periodic function of $\ln\left(c_1/c_1^0\right),\ldots,\ln\left(c_n/c_n^0\right)$ with periods $\ln b_1,\ldots,\ln b_n$, respectively. We obtain a generalized mass action law modulated by a slowly varying, periodic function of the logarithms of concentrations. Such logarithmic oscillations occur commonly in RG theory; although usually they are mathematical artifacts, in some cases they do exist in experimental situations; even if they do exist, in general they are very slow and hard to observe and can be neglected. Since p_u are probabilities, $\ln p_u$ are negative or zero and thus the signs of the effective reaction orders are determined by change factors b_u: for amplification, $b_u > 1$ the effective reaction orders are negative, whereas for reduction, $b_u < 1$, they are positive.

In conclusion, in this section we have shown that recycle mechanisms may lead to generalized mass-action laws with real, arbitrary reaction orders. An explicit solution was given in the particular case of self-similar recycling processes. These results will be applied in the following section to the kinetics of blood coagulation [15].

5. Application to experimental data on blood coagulation kinetics.

Blood coagulation is the body response to tissue injury, by producing clots formed of fibrin, platelets and other biological materials; bleeding is stopped and the way for tissue repair is opened. The biochemical part of blood coagulation consists of chains of enzymatic reactions, in which inactive proenzymes are activated to become reactive enzymes in the next reaction in the cascade [16]-[17]. The proenzymes and enzymes involved in the process are called coagulation factors. Ultimately these cascades of reactions lead to the transformation of fibrinogen into fibrin. The detailed kinetic analysis of blood coagulation is beyond the scope of the present paper. We point out recent studies that deal both with the analysis of experimental data, as well as with kinetic simulations [16]-[17].

The medical treatment with oral anticoagulants is of major clinical importance; it is applied to about 1% of the general population [18]. Its purpose is to control undesired coagulation processes, which may lead to thromboembolic events, even death. If it is not properly controlled, such a treatment may lead to serious secondary hemorrhagic events. The procedure is the following: blood is collected from the patients, the coagulation process is chemically inhibited, through decalcification, typically using citrate. The blood plasma samples are brought to testing laboratories, where the coagulation is tested ‘in vitro’,

upon addition of factor III or tissue thromboplastin and recalcification. The measured prothrombin time equals the duration of the first phase of exogenic blood coagulation, i.e. the time that it takes to convert prothrombin to thrombin up to the occurrence of the first fibrin clots. The results for the prothrombin time depend on several factors and vary from laboratory to laboratory. The main factor of variability is the source and concentration of thromboplastin used by different laboratories. Extensive research has been carried out for calibrating the results of these measurements. An international normalized ratio was introduced, (INR), [19], which is equal to the ratio between the measured coagulation time, t, and a standard coagulation time, t_c, raised to a calibration exponent α which is positive,

$$INR = \left(t/t_c\right)^{\alpha}. \tag{19}$$

Numerous experimental and statistical studies have shown that the definition (19) provides a proper calibration for the experimental data. Although very successful, the theoretical meaning of the INR equation (19) is not clear.

Statistical studies of kinetic coagulation data have shown that the first stage of the exogenic coagulation can be described by an allometric relation between the INR and the concentrations c_u of several plasma proteins:

$$INR = \eta \prod\nolimits_u \left(c_u\right)^{\beta_u} = \prod\nolimits_u \left(c_u / c_u^0\right)^{\beta_u}, \text{ where } \eta = \prod\nolimits_u \left(c_u^0\right)^{-\beta_u}, \tag{20}$$

Eq.(20) was introduced by Watala, Golanski and Kardas (WGK, [20]). In most cases, an equation with as few as five or even two concentrations of plasma proteins (typically the factors II and VII) will provide a satisfactory description of the experimental data. The exponents in Eq.(20) are negative.

Our approach to coagulation kinetics is twofold. a) We carry out a theoretical analysis of the initial stage of 'in vitro' blood coagulation, based on the self-similar recycling model introduced in the preceding section and show that the experimental, empirical relations (19)-(20) can be derived theoretically from our model. b) Conversely, we carry out a direct analysis of the experimental equations (19)-(20) without using a theoretical model and show that these two equations yield an overall kinetic equation, which is of the generalized mass-action type.

a. Theoretical analysis. We start out by neglecting the slow logarithmic oscillations in Eq.(18) and write the overall, renormalized, kinetic equation in the form $\tilde{r}\left(c_1,\ldots,c_n\right) = \tilde{k}\prod_{u=1}^{n}\left(c_u\right)^{\mu_u}$. The

renormalized rate $\widetilde{k}$ is the constant term in the multiperiodic function Ξ, which is also the average of Ξ with respect to all possible values of $\ln c_u$; the generalized reaction orders are given by $\mu_u = \ln p_u / \ln b_u$. The recycling processes occur with losses ($b_u < 1$), due to other reactions and enzyme degradation, and thus the apparent reaction orders μ_u are positive,. Since in the first stage of the coagulation the concentrations of the relevant plasma proteins are practically constant, the reduction factors b_u are close to one, $b_u = 1 - \varepsilon_u$, where, ε_u the fraction of losses, is close to zero. Similarly, for small losses, the number of recycling processes tends to be high and thus the probability p_u of the occurrence of a recycling event is close to one, $p_u = 1 - \pi_u$, where π_u, the probability that a recycling process stops, is close to zero. According to the self-similar model we have:

$$\mu_u = \ln(1-\pi_u)/\ln(1-\varepsilon_u) \approx \pi_u/\varepsilon_u \approx 1/\varepsilon_u \langle q_u \rangle , \tag{21}$$

where $\langle q_u \rangle = \sum_u q_u (1-p_u)(p_u)^{q_u} = 1/\pi_u - 1 \approx 1/\pi_u$, for π_u close to zero, is the average number of recycling events of the species u. It follows that the bigger the efficiency of the recycling, the higher the effective reaction orders and the coagulation rate, and the smaller the coagulation time. Of course, since the self-similar model is idealized, these results are only qualitative.

We can show that the empirical calibration law (19) is a consequence of the generalized kinetic law (18). We consider coagulation experiments on the same blood sample, carried out in many different laboratories, of which one laboratory, marked by the label 0, is used for calibration of all other laboratories that are involved in clinical practice and work directly with the patients. Due to different working conditions and reagents, especially the type and concentration of thromboplastin, the reaction rates for the same blood sample differ among these laboratories. However, if the experiments are carried out correctly, there should be an explicit, bijective relation between the reaction rates from any pair of two laboratories. We consider the reference laboratory 0 with the rate r_0 and another laboratory w, with the rate r_w. There should be a one-to-one correspondence between these two rates, expressed by a functional relation $r_w = \Phi_{w0}(r_0)$; such relations should exist for any pair of laboratories, but it is enough to analyze only one pair. We have $r_0 = \widetilde{k}_0 \prod_u (c_u)^{\mu_u^0}$ and $r_w = \widetilde{k}_w \prod_u (c_u)^{\mu_u^w}$; since we deal with the same blood sample in both cases the concentrations are the same. The functional relation between the two rates can be rewritten as

$\Gamma_0 = \Psi_{0w}(\Gamma_w)$, where $\Psi_{0w}(x) = \Phi_{0w}(k_w x)/k_0$, and $\Gamma_0 = \prod_u (c_u)^{\mu_u^0}$ and $\Gamma_w = \prod_u (c_u)^{\mu_u^w}$ are Horn complexions with physical dimensions $[\text{concentration}]^{\sum \mu_u^0}$ and $[\text{concentration}]^{\sum \mu_u^w}$, respectively. By applying the Pi theorem from dimensional analysis [21] it follows that the relation $\Gamma_0 = \Psi_{0w}(\Gamma_w)$ can be expressed in terms of a single adimensionalized variable $\Gamma_0^{1/\sum \mu_u^0}/\Gamma_w^{1/\sum \mu_u^w}$, or any real power of it different from zero. It follows that $\Gamma_0^{1/\sum \mu_u^0}/\Gamma_w^{1/\sum \mu_u^w} = \text{Constant}$ and thus $\Gamma_0 \sim \Gamma_w^{\sum \mu_u^0/\sum \mu_u^w}$, from which we come to $\tilde{r}_0 \sim \tilde{r}_w^{\sum \mu_u^0/\sum \mu_u^w}$. Since for the initial stage of 'in vitro' coagulation the relevant plasma proteins are practically constant, the reaction rates $\tilde{r}_0$ and $\tilde{r}_w$ are also practically constant; they are inversely proportional to the corresponding reaction times, t_0 and t_w, $\tilde{r}_0 = 1/t_0$, $\tilde{r}_w = 1/t_w$ and therefore. $t_0 \sim (t_w)^{\alpha_{0w}}$, with $\alpha_{0w} = \sum \mu_u^0/\sum \mu_u^w$. Thus, the coagulation times measured in different laboratories, raised to different powers, $\alpha_{0w} = \sum \mu_u^0/\sum \mu_u^w$, $,w = 1,2,\ldots$ are proportional to the coagulation time measured in the reference laboratory 0. Up to a proportionality factor, the theoretical calibration law $t_0 \sim (t_w)^{\alpha_{0w}}$ is identical to the empirical calibration law (19); Eq.(19) is derived by introducing a proportionality factor $t_{c0}/(t_{cw})^{\alpha_{0w}}$, expressed in terms of two characteristic times, t_{c0} and t_{cw}, attached to the laboratories 0 and w, respectively, and defining the *INR* as a dimensionless normalized prothrombin time, corresponding to the reference laboratory 0, $INR = t_0/t_{c0}$.

The WGK equation (20), which establishes a relation between the *INR* and the concentrations of the coagulation factors, can be derived in a similar way. We consider the laboratory w and take into account that the prothrombin time t_w is inversely proportional to the coagulation rate, $t_w \sim 1/\tilde{r}_w \sim \prod_u (c_u)^{-\mu_u^w}$. Combining this equation with the INR calibration equation (19) we obtain $INR \sim (t_w)^{\alpha_{0w}} \sim \prod_u (c_u)^{\beta_u^w}$, where $\beta_u^w = -\mu_u^w \sum \mu_{u'}^0/\sum \mu_{u'}^w$. We have derived the WGK equation (20) and also obtained expressions for the scaling exponents β_u^w in terms of the apparent reaction orders attached to the reference laboratory 0 and to the working laboratory w.

b. Analysis of the experimental data. Here we show that the experimental information contained in the calibration equation (19) and the WGK equation (20) can be used for determining an overall kinetic equation; in our analysis here we do not use a theoretical model. We assume that the coagulation rate r,

expressed by the rate of thrombin formation, is a function of the concentrations of the coagulation factors $c_1, c_2, \ldots$. Since in the first stage of exogenic blood coagulation there is practically no consumption of relevant plasma proteins, the rate is constant and the coagulation rate is inversely proportional to the coagulation rate, $r \sim 1/t$. From Eqs.(19)-(20) it follows that

$$r \sim 1/t \sim (INR)^{-1/\alpha} \sim \prod_u (c_u)^{-\beta_u/\alpha}, \tag{22}$$

that is, a generalized mass action law with arbitrary real exponents. By evaluating the proportionality factor Eq.(22) can be written as:

$$r = k\prod_u (c_u)^{\mu_u}, \text{ with } \mu_u = -\beta_u/\alpha \text{ and } k = \frac{\Delta T_h}{t_c}\prod_u \left(c_u^0\right)^{-\mu_u} = \frac{\Delta T_h}{t_c \eta^{1/\alpha}}, \tag{23}$$

where ΔT_h is the amount of thrombin formed in the first coagulation stage. Since the scaling exponents in the WGK Eq.(20) are negative, the effective reaction orders in Eq.(23) are positive. We notice that our derivation can be carried out backwards; without using a theoretical model. Starting from a generic law of the type, $\tilde{r} = \tilde{k}\prod_{u=1}^{n} (c_u)^{\mu_u}$, seen as an empirical law, we can derive Eqs.(19)-(20).

Our analysis clarifies the theoretical meaning of the *INR* calibration equation (19) and of the WGK equation (20). These equations express the self-similarity, that is, the fractal scaling properties of the initial stage of 'in vitro' coagulation, induced by the generalized mass-action kinetic law, which describes the process.

c. Comparison among theory, experiments and simulations. In addition to the comparison between theory and experiment, we also did a quick comparison with a simplified simulation of the coagulation process, based on the Hockin and Mann model [16]. We focused on the INR as a function of the coagulation factors II and VII. The integration of the kinetic equations was carried out repeatedly for different concentrations of the coagulation factors II and VII, F_2 and F_7, and the results were stored in a database. According to Hockin and Mann, we follow a standard procedure and express the concentrations F_2 and F_7 as fractions from a set reference 'normal' values [16]. We have also assumed that the coagulation time is the duration necessary for the production of a concentration of 20 nanomoles/liter of factor II activated, IIa, by starting from a concentration of factor II of 1400 nanomoles/liter [16]. The reference time necessary for computing the INR is the coagulation time corresponding to the reference normal values of

the factors II and VII. A regression analysis of the integration results leads to $\ln INR = -0.181 \ln F_2 - 0.153 \ln F_7 + 1.516$, which is similar to the two-variable version of the WGK equation [20]. $\ln INR = -0.251 \ln F_2 - 0.296 \ln F_7 + 1.168$. Our regression equation is characterized by a very good coefficient of correlation between the linear log – log fit and the real values, $R = 0.97$. The log – log plots of the INR versus the values of F_2 for different values of F_7, display almost straight lines. Similar results are obtained if F_2 is replaced by F_7 and vice versa. Our theory does not make any specific numeric, predictions regarding the values of the exponents in the WGK equations; any differences between the WGK fit and our fit are due to the limitations of the Hockin and Mann model [16]. Improved prediction of the scaling exponents can be made by using a more detailed model of coagulation, for example the one from [17].

6. Conclusions

In this article we have developed a method for the systematic development of kinetic laws based on the use of phase – phase (log – log) expansions. We have shown that a generalized mass-action law may emerge for complex systems that involve self-similar recycling. For such systems a stochastic renormalization group approach is developed, which can be dealt with analytically.

As an illustration for our approaches we have studied the kinetics of the first step of exogenic, 'in vitro' blood coagulation. In clinical practice blood tests are calibrated with the scaling equations (18) in order to obtain the International Normalized Ratio (INR), which is a standardized measure for the prothrombin time. Although this equation for the INR has been used for decades in thousands of laboratories all around the world, for millions of tests, until now it was only an empirical law without scientific meaning. Our treatment provides a theoretical derivation for this equation and clarifies its scientific meaning.

The methods developed in this paper provide useful tools for the kinetic analysis of complex biochemical reactions. Our work on blood coagulation may serve as a basis for applied biomedical research, resulting in better software programs for treatment planning with oral anticoagulants [15].

A surprising feature of our treatment for blood coagulation is the relative simplicity of the kinetic laws for an extremely complicated process, that involves not only hundreds of biochemical reactions, but biological processes as well. This apparent simplicity is due to the fact that we study only the first stage of

exogenic coagulation; the later stages of blood coagulation are more complicated and 'in vivo' coagulation is even more complicated.

Recycling is a common feature for many enzymatic reactions, where a molecule of enzyme may participate in many reaction cycles and, thus, our approach may be used for describing the kinetics of the initial stages of other complex biochemical processes.

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References

[1] Ross, J.; Schreiber, I.; Vlad, M. O. **(2006)** *Determination of Complex Reaction Mechanisms: Analysis of Chemical, Biological, and Genetic Networks*; Oxford University Press, New York.

[2] Ross, J. **(2008)** From the Determination of Complex Reaction Mechanisms to Systems Biology, *Annual Reviews of Biochemistry*, *77*, 479-494.

[3] Schmitt, W. M. Jr.; Raab, R. M.; Stephanopoulos, **(2008),** G. *Genome Res*., 14:1654-63.

[4] Remondini, D.; O'Connell, B.; Intrator, N.; Sedivy, J. M; Neretti, N.; Castellani, G. C.; Cooper, L. N. **(2005)** Targeting c-Myc-activated genes with a correlation method: detection of global changes in large gene expression network dynamics. *Proc. Natl. Acad. Sci. USA, 102*, 6902-6906.

[5] Gilman, A; Ross, J. **(1995)** Genetic-algorithm selection of a regulatory structure that directs flux in a simple metabolic model. *Biophys. J. 69*, 1321-1333.

[6] Tsuchiya M.; Ross, **(2003)** J. Advantages of external periodic events to the evolution of biochemical oscillatory reactions. *Proc. Natl. Acad. Sci. USA 100*, 9691-9695.

[7] Marcel O. Vlad, F. Moran, Vlad, T. Popa, Stefan E. Szedlacsek and J. Ross, **(2007)** Functional, fractal nonlinear response with application to rate processes with memory, allometry, and population genetics, *Proc. Natl. Acad. Sci. USA***,** *104*, 4798-4803.

[8] Savageau, M.A. **(1998).** Development of fractal kinetic theory for enzyme-catalysed reactions and implications for the design of biochemical pathways, *BioSystems*, 47, 9-36.

[9] Brønsted, J N. **(1922)** On the theory of chemical reaction rate (Transladted from German, 'Zur Theorie der chemischen Reaktiongeschwindigkeit') , *Zeit. Phys. Chem. 102*, 169-79

[10] Logan, S. R. **(1966)** Theory of kinetic salt effects in diffusion-controlled reactions, *Trans Faraday Soc., 62*, 34163422.

[11] Erdi, P., Toth, J. **(1989),** Mathematical Models of Chemical Reactions: Theory and Applications of Deterministic and Stochastic models, Manchester University Press, Manchester.

[12] Shlesinger, M.F., and Hughes,B.D., **(1981)** *Analogs of renormalization group transformation in random processes, Physica A 109*, 597-608.

[13] Vlad, M.O., Tsuchyia, M., Ofner, P., Ross, J. **(2001)** Bayesian analysis of systems with random chemical composition: Renormalization-group approach to Dirichlet distributions and the statistical theory of dilution*, Phys. Rev. E., 65*, 011112, 1-8.

[14] West, B.J. **(1990)** Sensing Scaled Scintillations, *J.Opt.Soc.Am.* 7, 1074-1100

[15] Vlad, M.O., Corlan, A.D., Morán, F., Oefner P., and Ross,J. **(2008)** '*Incremental parameter evaluation from incomplete data with application to the population pharmacology of anticoagulants'*, *Proc. Natl. Acad. Sci. USA*, 105, 4627-4632.

[16] Hockin , M.F.,, Jones, K.C., Everse, S.J., and Mann, K.G. **(2002)** A Model for the Stoichiometric Regulation of Blood Coagulation, *J. Biol. Chem., 277*, 18322-18333

[17] Luan D, Zai, M., Varner, J.D. **(2007),** Computationally derived points of fragility of a human cascade are consistent with current therapeutic strategies, *Plos Computational Biology*, 3, 1347-1359.

[18] A.K. Hamberg, M.L. Dahl, M. Barban, M.G. Scordo, M. Wadelius, V. Pengo, R. Padrini, E.N. Jonsson, **(2007)** A PK-PD model for predicting the impact of age, CYP2C9, and VKORC1 genotype on individualization of warfarin therapy*, Clin. Pharmacol. Ther.,* 81, 529-538.

[19] Van der Besselaar, A.M.H.P., Barrowcliffe, T.W., Houbouyan-Reveillard, L.L., Jespersen, J., Johnson, M., Poller, L., and Tripodi, A. **(2004)** Guidelines on preparation, certification and use of certified plasmas for ISI calibration and INR determination, *J. Thrombosis and Haemostasis*, 2, 1946-1953

[20] Watala, C, Golanski, J., Kardas, P. **(2003)** Multivariate relationships between international normalized ratio and vitamin K-dependent coagulation-derived parameters in normal healthy donors and oral anticoagulant therapy patients. *Thromb. J.* 1, 7, 1-10.

[21] Kline, S.J., **(1986)** *Similitude and Approximation Theory*, Springer, New York.