J Math Chem (2014) 52:1253–1291
DOI 10.1007/s10910-014-0308-2

ORIGINAL PAPER

Repair of irradiated cells by Michaelis–Menten enzyme catalysis: the Lambert function for integrated rate equations in description of surviving fractions

Dževad Belkić

Received: 28 December 2013 / Accepted: 19 January 2014 / Published online: 11 February 2014

Abstract Michaelis–Menten second-order chemical kinetics is used to describe the three main mechanisms for surviving fractions of cells after irradiation. These are a direct yield of lethal lesions by single event inactivation, metabolic repair of radiation lesions and transformation of sublethal to lethal lesions by further irradiations. The mass action law gives a system of time-dependent differential equations for molar concentrations of the invoked species that are the DNA substrates as lesions, enzyme repair molecules, the product substances, etc. The approximate solutions of these coupled rate equations are reduced to the problem of finding all the roots of the typical transcendental equation $axe^{-bx} = c$ with $x \geq 0$ being a real variable, where a, b and c are real constants. In the present context, the unique solution of this latter equation is given by $x = (1/b)W_0(bc/a)$ where W_0 is the principal-branch real-valued Lambert function. Employing the concept of Michaelis–Menten enzyme catalysis, a new radiobiological formalism is proposed and called the "Integrated Michaelis–Menten" (IMM) model. It has three dose-range independent parameters ingrained in a system of the rate equations that are set up and solved by extracting the concentration of lethal lesions whose time development is governed by the said three mechanisms. The indefinite integral of the reaction rate is given by the Lambert W_0 function. This result is proportional to the sought concentration of lethal lesions. Such a finding combined with the assumed Poisson distribution of lesions yields the cell surviving fraction after irradiation. Exploiting the known asymptotes of the Lambert W_0 function, the novel dose-effect curve is found to exhibit a shoulder at intermediate doses preceded by the exponential cell kill with a non-zero initial slope and followed by the exponential decline with the reciprocal of the D_0 or D_{37} dose as the final slope. All three dose

Dž. Belkić (✉)
Department of Oncology and Pathology, Karolinska Institute, P.O. Box 260,
171 76 Stockholm, Sweden
e-mail: Dzevad.Belkic@ki.se

regions are universally as well as smoothly covered by the Lambert function and, hence, by the ensuing cell surviving fractions. The outlined features of the proposed IMM model stem from a comprehensive mechanistic description of radiation-lesion interactions by means of kinetic rate equations. They are expected to be of critical importance in new dose-planning systems for high doses per fraction where the conventional linear-quadratic radiobiological modeling is demonstrably inapplicable.

Keywords Cell repair · Michaelis–Menten enzyme catalysis · Lambert function · Chemical kinetics · Rate equations · Dose planning systems · Hypofractionated radiotherapy

1 Introduction

This study is on mechanistic biophysical models for cellular radiobiology and hypofractionated radiotherapy, which administers a few large doses in shorter time intervals. Such radiobiological models are of paramount importance for dose-planning systems for treatment of patients with cancer. When radiation doses are progressively increased, measurements of surviving fractions of damaged cells demonstrate a purely exponential inactivation e^{-D/D_0}. This pathway of cell death is ignored in the linear-quadratic (LQ) model, which predicts a dominant Gaussian inactivation $\mathrm{e}^{-\beta D^2}$ at high doses. As a result, in computing the needed conversions of administered physical doses to its biological counterparts, such as cell surviving fraction S_F, biologically effective dose (BED), full effect (Fe), tumor control probability (TCP), etc., the LQ model incurs errors that increase as the absorbed dose becomes larger. These errors yield unrealistic estimates of the effectiveness of the absorbed high-dose per fraction and are, therefore, responsible for inaccuracies of treatment plans for hypofractionated radiotherapy.

This difficulty is explicit in the probability expression for the surviving fraction in the LQ model, $S_\mathrm{F} = \mathrm{e}^{-\alpha D-\beta D^2}$, as well as in the corresponding $\mathrm{Fe} = -(1/D)\ln S_\mathrm{F} = \alpha + \beta D$, where α and β are the two radiosensitivity parameters. Here, at large doses D, the asymptote $\mathrm{e}^{-\beta D^2}$ of the surviving fraction S_F in the LQ model is much smaller that the corresponding experimentally measured quantity, which usually behaves as e^{-D/D_0}, where D_0 is the mean lethal dose. Such a smaller cell survival given by the LQ model corresponds to a situation where many more tumor cells are predicted to be killed by radiation than in reality as recorded by measurements. A direct consequence of the absence the exponential cell kill mode from the LQ model at high doses is the fact that the expression $\alpha + \beta D$ for the Fe linearly increases indefinitely without a bound as dose D is augmented. This pattern is opposed to customary measurements where the experimental Fe linearly increases with dose only at low doses, but gradually saturates to a constant value attaining a plateau at very high doses. Such a behavior of the measured Fe, recorded as being leveled off, signifies the lack of biological benefit from radiation when the high-dose region is approached. In fact, the same conclusion also holds true by drawing a linear-linear plot with dose as the abscissa and surviving fraction as the ordinate. Therein, after a sufficiently high value of D, any further increase in dose would make only a negligi-

ble biological difference because of the occurrence of the nearly zero survival in the tail of S_F. All these facts jointly confirm the fact that the high-dose prediction of the biological effectiveness of radiation is inadequate in the LQ model which is, as such, unsuited for hypofractionated radiotherapy.

To systematically overcome these obstacles in the LQ model, we presently propose a different biophysical theory of radiation damage based upon the Michaelis–Menten mechanism of enzyme catalysis for cell repair. This chemical reaction is comprised of two steps: (i) formation and (ii) destruction of an intermediate molecular compound built from free enzymes and lesions. The essential substances targeted by radiation are usually taken to be deoxyribonucleic acid (DNA) molecules. The most critical damages (lesions) are single and double strand breaks (SSB, DSB) of DNA. The exit channel of enzyme catalysis contains the products as repaired lesions and free unaltered enzymes that continue further bindings to other sublethal lesions for the purpose of repairing them from radiation injury. The ensuing biophysical description of the studied radiation-lesion interactions is called the Integrated Michaelis–Menten (IMM) model because it uses the integrated form of the Michaelis–Menten equation [1–3]. This is an alternative to the Differential Michaelis–Menten (DMM) model, or equivalently, the Padé linear-quadratic (PLQ) model [4–9] which defines the repair function by the differential form of the Michaelis–Menten equation, through the dependence of the initial velocity v_0 of enzymes on dose D by way of the well-known rectangular hyperbola.

The cell surviving fraction in the IMM model is given by the concise analytical expression in terms of the explicit principal-branch Lambert W_0 function. The independent variable of the W_0 function contains the physically absorbed dose D. This new radiobiological model is applicable to all doses. It predicts the existence of a shoulder situated between the low- and high-dose regions, both of which are described as being dominated by the exponential cell kill modalities, that are also encountered in the majority of the corresponding measurements. In the small-dose limit, the IMM model is reduced to the DMM or PLQ model, which at still lower doses coincides with the LQ model. In the limit of very high doses, the IMM model yields the surviving fraction S_F in the form $n\mathrm{e}^{-D/D_0}$, which is reminiscent of the associated observable from the multi-target and single hit model, where n is the extrapolation number. In the IMM model, the extrapolation number n is related to the product of the maximal enzyme velocity $v_{\max}$ and repair time t_R through the relation $\ln n = v_{\max} t_R$.

In the development of treatment schedules for hypofractionated radiotherapies (e.g. stereotactic radiosurgery, stereotactic body radiotherapy and high-dose rate brachytherapy) as well as in the phase I dose-finding studies, it is necessary to carry out the accompanying long and involved processes. This is typically continued by a gradual progression from the preclinical to clinical trial steps until reaching a stage which would permit a broader implementation of the fractionation regimens in clinical practice. The efficacy of these procedures is critically influenced by biophysical models that play a pivotal role in designing clinical trials and in analyzing as well as interpreting the obtained results. The presently proposed radiobiological model possesses a more predictive and interpretative power than its competitors, as it involves cell repair by means of the Michaelis–Menten mechanism for enzyme catalysis, which has passed the test of time in biochemistry and enzymology. Therefore, the IMM model is

expected to accelerate the mentioned long process, to make them more cost-effective and, most importantly, to enable radiation oncologists to design the new and more adequate radiation dose schedules.

2 Chemical kinetics for enzyme catalysis

Here, we shall consider repair by the mechanism of enzyme catalysis [1–3]. This kind of chemical reaction implies a temporary creation and breakage of an intermediate unstable compound structure between the irradiated cell molecules and enzymes. The products of this chemical reaction are repaired lesions and enzyme molecules, as symbolized by:

$$[\mathrm{E}] + [\mathrm{S}] \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [\mathrm{ES}] \xrightarrow[k_2]{} [\mathrm{E}] + [\mathrm{P}]\,. \tag{2.1}$$

In the general nomenclature, labels $[\mathrm{E}](t)$, $[\mathrm{S}](t)$, $[\mathrm{ES}](t)$ and $[\mathrm{P}](t)$ denote the time-dependent concentrations of the free enzyme molecules, substrate, enzyme-substrate complex and product, respectively. Hereafter, whenever the independent variable t of concentrations is not explicitly shown, it will be understood that the following convention holds:

$$[\mathrm{S}] \equiv [\mathrm{S}](t), \quad [\mathrm{E}] = [\mathrm{E}](t), \quad [\mathrm{ES}] = [\mathrm{ES}](t), \quad [\mathrm{P}] = [\mathrm{P}](t)\,. \tag{2.2}$$

In (2.1), we have the simplest enzyme catalysis involving only a single substrate [S] with no inhibitors, co-inhibitors, etc. Here, k_1 is the rate coefficient for formation of the intermediate complex molecule [ES]. This complex can be destroyed with either the rate constant k_{-1} or k_2 in the backward or forward reaction, retrieving the initial [E] + [S] or creating the final [E] + [P] reactants, respectively. Here, only k_1 is a bimolecular rate constant, whereas k_{-1} and k_2 are unimolecular rate constants. Unimolecular or monomolecular reactions are those chemical reactions (or subreactions, i.e. different paths of a given reaction) in which only one reactant undergoes alteration of its mass. By contrast, in bimolecular reactions, both reactants change their masses. The basic mechanism behind reaction (2.1) is that the free enzymes [E] are reversibly bound to substrate [S], thus forming a temporary intermediate complex [ES], which is unstable and, therefore, prone to decay. After decay, enzyme [E] is set free and emerges unaltered from reaction (2.1), whereas substrate [S] is irreversibly converted into the product [P]. In this way, enzyme [E] becomes again free for further binding with another substrate.

To determine the rate of the conversion of [S] to [P] via reaction (2.1) and to find the time evolution of the invoked concentrations $[\mathrm{E}](t)$, $[\mathrm{S}](t)$, $[\mathrm{ES}](t)$ and $[\mathrm{P}](t)$, it is common to assume that the initial substrate concentration is much larger than that of the enzyme:

$$[\mathrm{S}]_0 \gg [\mathrm{E}]_0\,, \tag{2.3}$$

which, in practice, reads as, for example:

$$\frac{[\mathrm{E}]_0}{[\mathrm{S}]_0} \leq \frac{1}{100} = 0.01\,. \tag{2.4}$$

Here, $[\mathrm{E}](0) \equiv [\mathrm{E}]_0$ and $[\mathrm{S}](0) \equiv [\mathrm{S}]_0$ are the respective initial values of the enzyme and substrate concentrations at $t = 0$. Approximation (2.3) is ordinarily adequate for in vitro, but it is usually not satisfied for in vivo intracellular media with high enzyme concentrations [10–12]. Repair by way of enzyme catalysis for in vivo substances will be considered later on in a separate publication.

The overall goal is to determine the initial reaction velocity v_0 for (2.1), i.e. the rate of enzymatic catalysis at which the complex [ES] irreversibly decomposes itself to [E] and [P]. The first step towards this end is to evoke the law of mass action, which states that reaction rates are proportional to concentrations of the reactants. Since the intermediate complex [ES] is labile, it will decay to an enzyme and a product. Thus, the initial rate of fragmentation of [ES] to [E] + [P] must be directly proportional to the concentration of the complex:

$$v_0 \sim [\mathrm{ES}]\,. \tag{2.5}$$

A proportionality constant is needed to pass to the equality sign in (2.5). Such a constant can be identified by reference to the dissociation part $[\mathrm{ES}] \xrightarrow[k_2]{} [\mathrm{E}] + [\mathrm{P}]$ of reaction (2.1), so that:

$$v_0 = k_2[\mathrm{ES}]\,. \tag{2.6}$$

This relation, however, is not useful in practice for determination of v_0, since [ES] is unknown and, moreover, it cannot be directly measured in experiments. Nevertheless, there ought to be an alternative way to approximately determine [ES] by expressing it in terms of some other observables (experimentally measurable quantities). This would render expression (2.6) useful in practice. This is indeed possible within four settings, the Michaelis and Menten (MM) [1] quasi-equilibrium (QE), the van Slyke and Cullen (SC) time summation (TS) [2], the Briggs and Haldane (BH) [3] quasi-steady state (QSS) [3] and the presently proposed halved harmonic mean (HHM) formalisms. They are based on three different interpretations of the same assumption stating that after an initial, short, transient time, the reactant concentrations will vary slowly. Thus, the QE formalism [1] supposes that there is a quasi-equilibrium between formation and destruction of [ES]. The TS formalism [2] for the irreversible version of (2.1) with $k_{-1} = 0$ is based on adding two different times spent first on forming the intermediate complex [ES] and then on destroying it with the emergence of free enzyme [E] and product [P]. This latter time sum is inversely proportional to enzyme velocity v_0. In the QSS formalism [3], which relies on the earlier original concept of Bodenstein [13], the state of the complex [ES] is viewed as a quasi-steady state or a pseudo steady state (PSS) which is prone to decay. In the HHM formalism for the reversible chemical reaction (2.1), enzyme velocity v_0 is identified as the effective velocity v_{eff} proportional to the halved harmonic mean of the two limiting velocities for reversible formation and dissociation of the intermediate molecular compound [ES]. The proportionality factor in v_0 is the probability $k_2/(k_2 + k_{-1})$ which represents the

branching fraction for product formation and the total dissociation rate. The QE, QSS and HHM formalisms are quantitatively equivalent to each other, since they all stem from the same reversible reaction (2.1) of enzyme catalysis thus yielding the identical analytical formula for the initial velocity v_0. Moreover, this latter joint formula for v_0 is reduced to the corresponding expression from the TS formalism of van Slyke and Cullen [2] by setting $k_{-1} = 0$, which applies only to irreversible enzyme catalysis.

2.1 A quasi-equilibrium between formation–destruction of the transient compound (the Michaelis–Menten theory)

We shall first show how an estimate of [ES] can be made using the concept of quasi-equilibrium for formation-destruction of the intermediate complex. In the QE framework, the intermediate complex is in a quasi-equilibrium with the reactants. This means that the reaction of the compound formation via $[\mathrm{E}]+[\mathrm{S}] \xrightarrow[k_1]{} [\mathrm{ES}]$ is in quasi-equilibrium with the two pathways of the compound destruction or breakdown through $[\mathrm{E}]+[\mathrm{S}] \xleftarrow[k_{-1}]{} [\mathrm{ES}]$ and $[\mathrm{ES}] \xrightarrow[k_2]{} [\mathrm{E}]+[\mathrm{P}]$. Thus, the QE hypothesis can be formulated as the following rate or velocity matching condition:

$$\left.\begin{array}{l}\text{Rate of formation of complex [ES]} \approx \text{Rate of destruction of complex [ES]} \\ \left\{\dfrac{\mathrm{d[ES]}}{\mathrm{d}t}\right\}_{\text{formation}} \approx \left\{\dfrac{\mathrm{d[ES]}}{\mathrm{d}t}\right\}_{\text{destruction}} \qquad (\text{Quasi}-\text{equilibrium, or QE})\end{array}\right\}, \tag{2.7}$$

where,

$$\left.\begin{array}{c}[\mathrm{E}]+[\mathrm{S}] \xrightarrow[k_1]{} [\mathrm{ES}] \\ \left\{\dfrac{\mathrm{d[ES]}}{\mathrm{d}t}\right\}_{\text{formation}} = k_1[\mathrm{E}]\,[\mathrm{S}]\end{array}\right\}, \tag{2.8}$$

and,

$$\left.\begin{array}{c}[\mathrm{E}]+[\mathrm{S}] \xleftarrow[k_{-1}]{} [\mathrm{ES}] \quad \& \quad [\mathrm{ES}] \xrightarrow[k_2]{} [\mathrm{E}]+[\mathrm{P}] \\ \left\{\dfrac{\mathrm{d[ES]}}{\mathrm{d}t}\right\}_{\text{destruction}} = (k_{-1}+k_2)[\mathrm{ES}]\end{array}\right\}. \tag{2.9}$$

Inserting (2.8) and (2.9) into (2.7) yields:

$$0 \approx \{(\mathrm{d}/\mathrm{d}t)[\mathrm{ES}]\}_{\text{formation}} - \{(\mathrm{d}/\mathrm{d}t)[\mathrm{ES}]\}_{\text{destruction}} = k_1[\mathrm{E}]\,[\mathrm{S}] - (k_{-1}+k_2)[\mathrm{ES}]\,.$$

This gives the following relation:

$$[\mathrm{ES}] \approx \frac{[\mathrm{E}]\,[\mathrm{S}]}{K_{\mathrm{M}}} \qquad (\text{Consequence of the the equilibrium}), \tag{2.10}$$

where K_M is the Michaelis–Menten rate constant:

$$K_M = \frac{k_{-1} + k_2}{k_1}. \tag{2.11}$$

On the other hand, quantity [E] from (2.10) is equal to the difference between the concentrations $[E]_0$ and [ES] of free and bound enzyme molecules, respectively:

$$[E] = [E]_0 - [ES]. \tag{2.12}$$

Inserting [E] from (2.12) into (2.10) yields the expression $[ES] = ([E]_0 - [ES])[S]/K_M$, which gives concentration [ES] as:

$$[ES] = \frac{[E]_0[S]}{K_M + [S]}. \tag{2.13}$$

Finally, by substituting (2.13) into (2.6), it follows $v_0 = k_2[ES] = k_2[E]_0[S]/(K_M + [S])$, or equivalently:

$$v_0 = \frac{v_{max}[S]}{K_M + [S]}, \tag{2.14}$$

where v_{max} is the maximal enzyme velocity,

$$v_{max} = k_2[E]_0. \tag{2.15}$$

This is the Michaelis–Menten equation. If the substrate concentration from Eq. (2.14) is varied in such a way that [S] could approximately acquire the fixed value K_M :

$$[S] \approx K_M, \tag{2.16}$$

then Eq. (2.14) would give:

$$v_0 \approx \frac{1}{2} v_{max} \quad \text{at} \quad [S] \approx K_M. \tag{2.17}$$

Hence K_M is seen as the concentration of substrate [S] for which the reaction velocity v_0 attains one half of its maximum value v_{max}. Likewise, with the setting (2.16), we have the special case of Eq. (2.13):

$$[ES] \approx \frac{1}{2}[E]_0 \quad \text{at} \quad [S] \approx K_M. \tag{2.18}$$

Therefore, if during a continued variation of the substrate concentration [S], one of its particular values becomes equal to the MM constant (fixed by the enzyme catalysis under study), $[S] = K_M$, then the concentration [ES] of the intermediate complex will coincide with one half of the initial enzyme concentration $[E]_0$. Thus, for a given

$[E]_0$, although we said earlier that no values of [ES] could be directly measured due to the small lifetime of the intermediate complex, we nevertheless see from (2.18) that a special value $[ES] \approx [E]_0/2$ can be inferred for $[S] \approx K_M$, under the assumed validity of the MM kinetics.

The result (2.14) shows that the decline in the approximate velocity v_0 during the enzyme catalyzed reaction (2.1) is due solely to depletion of the substrate concentration [S]. It is seen in (2.14) that the enzyme reaction velocity v_0 *increases* with augmentation of the substrate concentration [S] at a *decelerating* rate until the point at which the asymptotic speed v_{max} is attained. Specifically, at $v_0 \approx v_{max}$, the enzyme [E] is fully *saturated* with the substrate [S] in the sense that any further increase in [S] has no effect whatsoever on the enzyme velocity v_0. In other words, after reaching the limit value v_{max}, velocity v_0 levels off as a function of [S], i.e. v_0 reaches a plateau, $v_0 \approx v_{max}$ as a sign of the absence of further binding of [E] to [S]. This type of behavior in (2.14) plotted as v_0 versus [S] gives a shape which is called a rectangular hyperbola. Similar rectangular hyperbolae are also encountered in the dependence of the relative radiosensitivity of bacteria as a function of concentration of either oxygen [14–16] or glycerin [17]. Moreover, the MM-type rectangular hyperbolae appear in other research problems across interdisciplinary fields e.g. the Langmuir adsorption equation in surface physics for variation of adsorption with pressure, the Monod function in resource competition theory, the Holling type II functional response in predator-prey dynamics, the Beverton-Holt stock-recruitment function in fish biology, etc. The MM Eq. (2.14) has only two parameters, v_{max} and K_M that need to be extracted from the analyzed experimental data. Such parameters have a key biophysical significance in enzyme kinetics. This huge advantage of mechanistic data analyses on enzyme catalysis (2.1) is especially important relative to curve fitting techniques that use freely adjustable parameters with some assumptions made to minimize the squared errors (squared difference between a model and experimental data), but without any mechanistic backing.

2.2 Quasi-steady states of the intermediate complex (the Briggs-Haldane theory)

2.2.1 Conventional derivation of enzyme velocity

The quasi-steady state concept of Bodenstein [13] was employed by Briggs and Haldane [3] for enzyme catalysis (2.1). This was motivated by the realization that the QE hypothesis of Michaelis and Menten [1] is unnecessarily restrictive and, as such, could be replaced by a more general assumption. Alternatively, one can suppose that the intermediate complex [ES] is in a quasi-steady or quasi-stable state. With this setup, the complete time evolution of the studied system is described by a kinetic system of coupled non-linear differential equations. Four such rate equations are needed from the onset because enzyme catalysis (2.1) involves four substances $[E](t)$, $[S](t)$, $[ES](t)$ and $[P](t)$:

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES] \tag{2.19}$$

$$\frac{\mathrm{d[E]}}{\mathrm{d}t} = -k_1[\mathrm{E}]\,[\mathrm{S}] + (k_{-1} + k_2)[\mathrm{ES}] \tag{2.20}$$

$$\frac{\mathrm{d[ES]}}{\mathrm{d}t} = k_1[\mathrm{E}]\,[\mathrm{S}] - (k_{-1} + k_2)[\mathrm{ES}] \tag{2.21}$$

$$\frac{\mathrm{d}[P]}{\mathrm{d}t} = k_2[\mathrm{ES}], \tag{2.22}$$

with the initial conditions at $t = 0$:

$$[\mathrm{S}](0) = [\mathrm{S}]_0, \quad [\mathrm{E}](0) = [\mathrm{E}]_0, \quad [\mathrm{ES}](0) = [\mathrm{ES}]_0, \quad [\mathrm{P}](0) = [\mathrm{P}]_0. \tag{2.23}$$

Even though the independent variable t is not written in (2.19)–(2.22), the displayed concentrations are time-dependent according to the convention (2.2). The sought initial velocity v_0 is defined as the rate of formation of product the [P] :

$$v_0 \equiv \frac{\mathrm{d[P]}}{\mathrm{d}t}\,, \tag{2.24}$$

in accordance with (2.6) and (2.22). This is also the speed by which the enzyme from the labile complex [ES], then sets itself free and expels the product [P] via the catalysis reaction (2.1). Note that on account of relation $v_0 = k_2[\mathrm{ES}]$ from (2.6), the 4th equation $\mathrm{d[P]}/\mathrm{d}t = k_2[\mathrm{ES}]$ in the system of kinetic rate Eqs. (2.19)–(2.22) can be rewritten as $\mathrm{d[P]}/\mathrm{d}t = v_0$, which is the definition (2.24) of the enzyme velocity v_0. Hence consistency.

Not all the equations from the system (2.19)–(2.22) are independent. Certain obvious inter-relationships among these rate equations can be exploited to reduce the original system to a simpler, but nevertheless still exact form. For example, by adding together Eqs. (2.19) and (2.20), it follows:

$$\frac{\mathrm{d[E]}}{\mathrm{d}t} + \frac{\mathrm{d[ES]}}{\mathrm{d}t} = 0\,, \tag{2.25}$$

which upon integration gives:

$$[\mathrm{E}] + [\mathrm{ES}] = C_1\,. \tag{2.26}$$

The boundary conditions from (2.23) can be used to determine the integration constant C_1 as:

$$C_1 = [\mathrm{E}]_0\,. \tag{2.27}$$

With this, Eq. (2.26) is recognized as the enzyme mass conservation law:

$$[\mathrm{E}](t) + [\mathrm{ES}](t) = [\mathrm{E}]_0\,. \tag{2.28}$$

This has also been used in the QE formalism via (2.12). Relation (2.28) explicitly shows that at any time t, the sum of the concentrations $[\mathrm{E}](t)$ and $[\mathrm{ES}](t)$ for the free

and bound enzymes is constant and equal to the enzyme concentrations at the initial time $[\mathrm{E}](0) = [\mathrm{E}]_0$, which is also called the *total* enzyme concentration $[\mathrm{E}]_{\mathrm{tot}}$:

$$[\mathrm{E}](0) = [\mathrm{E}]_0 \equiv [\mathrm{E}]_{\mathrm{tot}} \,. \tag{2.29}$$

Likewise, the sum of (2.19), (2.21) and (2.22) is the following differential equation:

$$\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t} + \frac{\mathrm{d}[\mathrm{ES}]}{\mathrm{d}t} + \frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = 0 \,, \tag{2.30}$$

whose integral is:

$$[\mathrm{S}](t) + [\mathrm{ES}](t) + [\mathrm{P}](t) = C_2 \,. \tag{2.31}$$

The integration constant C_2 is fixed by the initial conditions from (2.23) as:

$$C_2 = [\mathrm{S}]_0 \,, \tag{2.32}$$

so that Eq. (2.31) becomes the substrate mass conservation law:

$$[\mathrm{S}](t) + [\mathrm{ES}](t) + [\mathrm{P}](t) = [\mathrm{S}]_0 \,. \tag{2.33}$$

This indicates that for any time t, the sum of the concentrations $[\mathrm{S}](t)$, $[\mathrm{ES}](t)$ and $[\mathrm{P}](t)$ for the free, bound and the inverted substrate, respectively is constant and equal to the substrate concentrations at the initial time $[\mathrm{S}](0) = [\mathrm{S}]_0$. Here, the substrate which is inverted by enzyme catalysis (2.1) to the product $[\mathrm{P}](t)$ is called the inverted substrate or invertase.

By inserting (2.28) into Eq. (2.19) and (2.33) into Eq. (2.21), we have:

$$\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t} = -k_1 \left([\mathrm{E}]_0 - [\mathrm{ES}]\right)[\mathrm{S}] + k_{-1}[\mathrm{ES}] \,, \tag{2.34}$$

$$\frac{\mathrm{d}[\mathrm{ES}]}{\mathrm{d}t} = k_1 \left([\mathrm{E}]_0 - [\mathrm{ES}]\right)[\mathrm{S}] - (k_{-1} + k_2)[\mathrm{ES}] \,. \tag{2.35}$$

This shows that the two mass conservation laws (2.28) and (2.33) effectively reduce the dimension of the original system with four Eqs. (2.19)–(2.22) to only two Eqs. (2.34) and (2.35). No approximation was made thus far in passing from the original to the reduced system of equations. Nevertheless, although very useful, this reduction alone cannot be of help in finding the exact analytical solution of the system of coupled non-linear differential Eqs. (2.34) and (2.35). An analytical approximate solution is possible by using the assumption (2.3) of the QSS model. Thus, under the condition (2.3) for which the substrate molecules are much more abundant than the available enzymes, [E] is converted to [ES] via $[\mathrm{E}] + [\mathrm{S}] \xrightarrow[k_1]{} [\mathrm{ES}]$ and reconverted to [E] through $[\mathrm{E}] + [\mathrm{S}] \xleftarrow[k_{-1}]{} [\mathrm{ES}]$ many times prior to reaching the quasi-equilibrium. This makes the rate $(\mathrm{d}/\mathrm{d}t)[\mathrm{ES}]$ negligibly small relative to the other competitive terms in the coupled kinetic Eqs. (2.19)–(2.22). Therefore, within the assumption (2.3), to a

high degree of accuracy, it is justified to view the rate $(\mathrm{d}/\mathrm{d}t)[\mathrm{ES}]$ as being close to zero:

$$\frac{\mathrm{d}[\mathrm{ES}]}{\mathrm{d}t} \approx 0 \qquad (\mathrm{Quasi-steady\ state,\ or\ QSS})\,. \tag{2.36}$$

In other words, for $[\mathrm{S}]_0 \gg [\mathrm{E}]_0$ the state of the investigated system in the intermediate complex in reaction (2.1) can be considered as being nearly stationary (quasi-stationary) or nearly steady. As usual, the condition for a stationary (i.e. time-invariant) state is expressed through equating the first derivative (with respect to time) of that state to zero. This is the content of the approximation (2.36). With (2.36) at hand, the rhs of Eq. (2.35) simplifies as:

$$\left.\begin{array}{l} k_1\,([\mathrm{E}]_0 - [\mathrm{ES}])\,[\mathrm{S}] - (k_{-1} + k_2)[\mathrm{ES}] \approx 0 \\ \therefore\ \left(\dfrac{k_{-1} + k_2}{k_1} + [\mathrm{S}]\right)[\mathrm{ES}] \approx [\mathrm{E}]_0[\mathrm{S}] \end{array}\right\}, \tag{2.37}$$

so that,

$$[\mathrm{ES}] \approx \frac{[\mathrm{E}]_0[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}\,, \tag{2.38}$$

where K_M is the MM rate constant from (2.11). Expression (2.38) is the same as formula (2.13) from the QE formalism. The result (2.38) expresses the unknown concentration [ES] of the intermediate complex in terms of the three other, experimentally measurable concentrations $[\mathrm{E}]_0$, [S] and K_M. It is the formula (2.38) for [ES], which was sought to complete the task of using (2.6) to find the initial velocity $v_0 = k_2[\mathrm{ES}]$ of enzyme catalysis (2.1):

$$\begin{aligned} v_0 &= k_2[\mathrm{ES}] \\ &= k_2 \left\{ \frac{[\mathrm{E}]_0[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]} \right\}. \end{aligned} \tag{2.39}$$

Substitution of the approximate concentration (2.38) for the intermediate complex [ES] into Eq. (2.34) yields:

$$\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t} \approx -k_2[\mathrm{E}]_0 \frac{[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}\,. \tag{2.40}$$

On the other hand, insertion of (2.38) into Eq. (2.35) gives the rate of the product formation:

$$\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} \approx k_2[\mathrm{E}]_0 \frac{[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}\,. \tag{2.41}$$

The lhs of this equation is the initial velocity v_0 for reaction (2.1) according to (2.24) and this yields the same result for v_0 as in (2.39), but written in an alternative form:

$$v_0 \approx \frac{v_{\max}[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}\,, \tag{2.42}$$

in agreement with (2.14) where $v_{\max}$ is given by (2.15).

2.2.2 An alternative derivation of enzyme velocity

Here, we shall give an alternative analysis in the QSS formalism. To this end, using the QSS hypothesis $\mathrm{d}[\mathrm{ES}]/\mathrm{d}t \approx 0$ from (2.36), we can simplify Eq. (2.30) as:

$$\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} \approx -\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t}, \qquad \frac{\mathrm{d}[\mathrm{ES}]}{\mathrm{d}t} \approx 0\,. \tag{2.43}$$

In other words, the consequence of the condition (2.36) is the twofold definition of the enzyme velocity:

$$\begin{aligned} v_0 &= \frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} \\ &\approx -\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t} \qquad \text{if} \qquad \frac{\mathrm{d}[\mathrm{ES}]}{\mathrm{d}t} \approx 0\,, \end{aligned} \tag{2.44}$$

where the first relation $v_0 = \mathrm{d}[\mathrm{P}]/\mathrm{d}t$ is taken from (2.6) and (2.22). Employing (2.44), the left hand sides of Eqs. (2.19) and (2.22) become approximately equal to each other. Thus, we can also equate the corresponding right hand sides of Eqs. (2.19) and (2.22) to obtain the relation $k_1[\mathrm{E}][\mathrm{S}] + k_{-1}[\mathrm{ES}] = k_2[\mathrm{ES}]$, which can be rewritten as:

$$[\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{M}} \qquad (\text{Consequence of the quasi} - \text{steady state hypothesis}). \tag{2.45}$$

This result coincides with (2.10) which was derived in the QE formalism as a direct consequence of assuming the existence of an equilibrium between formation and destruction of intermediate complex [ES]. As in the QE formalism, using the mass conservation law (2.12) for enzyme concentration, we replace [E] from (2.45) by $[\mathrm{E}]_0 - [\mathrm{ES}]$ to transform [ES] to $[\mathrm{ES}] = ([\mathrm{E}]_0 - [\mathrm{ES}])[\mathrm{S}]/K_\mathrm{M}$ which gives:

$$[\mathrm{ES}] = \frac{[\mathrm{E}]_0[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}\,. \tag{2.46}$$

This is Eq. (2.13) from the QE formalism. Combining Eqs. (2.45) and (2.46) yields the same MM equation from (2.14) according to:

$$v_0 = \frac{v_{\max}[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}\,, \tag{2.47}$$

where $v_{\max} = k_2[\mathrm{E}]_0$ as in (2.15). The analysis from this sub-subsection is more straightforward than the conventional derivation from 2.2.1. In particular, it is shown

here that there is no need at all to use the reduced system of two coupled rate equations (2.34) and (2.35) to obtain the MM equation. Moreover, during a short derivation, this alternative analysis makes a closer step-by-step link to the corresponding development from the QE formalism than what is done in the usual calculations from 2.2.1. Specifically, it is demonstrated that the identical key relation (2.45) between the concentrations of the intermediate complex [ES] and the product [E] [S] of enzyme and substrate concentrations stems equivalently from the QE and QSS hypotheses, as seen in (2.10) and (2.45).

Using the substrate mass conservation law (2.28) to replace $[\mathrm{E}]_0$ by $[\mathrm{E}] + [\mathrm{ES}]$ in (2.38), we can deduce the MM constant K_M in the following form of the quotient of concentrations [E][S] and [ES]:

$$K_\mathrm{M} \approx \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]} \qquad \text{at} \qquad t = t_{\max}\,, \tag{2.48}$$

in agreement with (2.10) and (2.45). Here, $t_{\max}$ is the extremal time, i.e. the root of the equation $(\mathrm{d}/\mathrm{d}t)[\mathrm{ES}] \approx 0$ as in (2.36). Relation (2.48) gives information about the abundance of enzymes [E] in the complex [ES] at the time $t_{\max}$ when the quasi steady-state is reached via (2.36), i.e. while enzymes are actively transforming (turning over) the substrates [S] to the products [P]. Care should be exercised with (2.48) in that the MM constant K_M is viewed only as a quasi steady-state approximation to the concentration quotient [E][S]/[ES] at a single point in time $t = t_{\max}$ via $K_\mathrm{M} \approx \{[\mathrm{E}][\mathrm{S}]/[\mathrm{ES}]\}_{t=t_{\max}}$. In other words, it is not strictly justified to write the *equality* in (2.48) at all times viz $K_\mathrm{M} = \{[\mathrm{E}][\mathrm{S}]/[\mathrm{ES}]\}_{\forall t} \equiv \{[\mathrm{E}](t)\}\{[\mathrm{S}](t)\}/\{[\mathrm{ES}](t)\}$ for arbitrary t because the quotient [E][S]/[ES] is not always constant. Rather, this latter quotient can best *approximate* the constant K_M at only one special instant, $t = t_{\max}$. Nevertheless, practice shows that K_M estimated from [E][S]/[ES] is nearly constant also at $t \neq t_\mathrm{R}$.

Overall, the same expression for reaction velocity v_0 from (2.14) or (2.41) is obtained for two different conditions and these are:

- (2.7) in the Michaelis–Menten model with a quasi-equilibrium for creation-destruction of the intermediate complex [ES], or
- (2.36) in the Briggs–Haldane model with the existence of the quasi-steady state of [ES].

The identical final results demonstrate the equivalence of the QE and QSS formalisms. Nevertheless, the theoretical framework of the latter is more general than that of the former formalism.

2.3 Time summation for two irreversible subreactions in enzyme catalysis (the van Sylke-Cullen theory)

In the TS formalism of van Slyke and Cullen [2], the irreversible version of enzyme catalysis (2.1) for $k_{-1} = 0$ is considered:

$$[\mathrm{E}] + [\mathrm{S}] \underset{k_1}{\longrightarrow} [\mathrm{ES}] \underset{k_2}{\longrightarrow} [\mathrm{E}] + [\mathrm{P}]\,. \tag{2.49}$$

Similarly to Michaelis and Menten [1], reaction (2.49) is also viewed by van Slyke and Cullen [2] as being comprised of two parts whose completion requires total time $t_{\rm tot}$. The first part $[{\rm E}]+[{\rm S}] \xrightarrow[k_1]{} [{\rm ES}]$, which necessitates time t_1, is an irreversible formation of the intermediate complex [ES] with the rate constant k_1. The second part $[{\rm ES}] \xrightarrow[k_2]{} [{\rm E}]+[{\rm P}]$ is an irreversible destruction of [ES] with the rate constant k_2 for which time t_2 is needed. Therefore, the total time $t_{\rm tot}$ for enzyme [E] to complete the cycle consisting of combining with the substrate [S] into the complex [ES] and subsequently liberating itself by throwing off the product [P] from [ES], as per reaction (2.49), is given by the sum of the time intervals consumed by the said two separate stages:

$$t_{\rm tot} = t_1 + t_2\,. \tag{2.50}$$

Time t_1 required for the first subreaction $[{\rm E}]+[{\rm S}] \xrightarrow[k_1]{} [{\rm ES}]$ is inversely proportional to the substrate concentration:

$$t_1 = \frac{1}{k_1[{\rm S}]}\,. \tag{2.51}$$

However, time t_2 for the second subreaction $[{\rm ES}] \xrightarrow[k_2]{} [{\rm E}]+[{\rm P}]$ is independent on [S] and it reads as:

$$t_2 = \frac{1}{k_2}\,, \tag{2.52}$$

so that,

$$t_{\rm tot} = \frac{1}{k_1[{\rm S}]} + \frac{1}{k_2}\,. \tag{2.53}$$

On the other hand, time $t_{\rm tot}$ is inversely proportional to velocity v_0 of the complete reaction (2.49) via $t_{\rm tot} \sim 1/v_0$. Here, the constant of proportionality depends of the amount of available enzyme $[{\rm E}]_0$, so that:

$$t_{\rm tot} = \frac{[{\rm E}]_0}{v_0}\,. \tag{2.54}$$

Hence, it follows from (2.53):

$$\begin{aligned} v_0 &= [{\rm E}]_0 \left(\frac{1}{k_1[{\rm S}]} + \frac{1}{k_2}\right)^{-1} \\ &= \frac{v_{\rm max}[{\rm S}]}{K_{\rm SC}+[{\rm S}]}, \qquad K_{\rm SC} = \frac{k_2}{k_1}\,. \end{aligned} \tag{2.55}$$

In this derivation, we have $v_{\rm max} = k_2[{\rm E}]_0$, as in (2.15). Here, $K_{\rm SC}$ is the van Slyke-Cullen constant, which can also be deduced from the more general Michaelis–Menten constant $K_{\rm M}$ in (2.11) for $k_{-1} = 0$ via:

$$K_{\mathrm{SC}} = \{K_{\mathrm{M}}\}_{k_{-1}=0}\,. \tag{2.56}$$

The result (2.55) of van Slyke and Cullen [2] coincides with the Michaelis–Menten equation (2.14) for the special case $k_{-1} = 0$.

2.4 Halved harmonic mean for velocity of enzyme catalysis (Present theory)

The HHM formalism for the general reversible version of enzyme catalysis (2.1) is based on the concept of the halved harmonic mean, which is known to be the truest average value in any process governed by rate equations, as is the case with (2.1). The harmonic mean (average) h of two quantities or functions f and g is defined by:

$$\frac{1}{h} = \frac{1}{2}\left(\frac{1}{f} + \frac{1}{g}\right) \quad \text{or} \quad h = 2\frac{fg}{f+g} \qquad \text{(Harmonic mean)}\,. \tag{2.57}$$

The so-called effective value denoted by h_{eff} is introduced as the halved harmonic mean:

$$h_{\mathrm{eff}} = \frac{1}{2}h \quad , \quad \frac{1}{h_{\mathrm{eff}}} = \frac{1}{f} + \frac{1}{g} \quad \text{or} \quad h_{\mathrm{eff}} = \frac{fg}{f+g} \qquad \text{(Effective value)}. \tag{2.58}$$

Similarly to the QE and QSS model, the HHM formalism views the whole reversible reaction (2.1) as being composed of three components. Here, the resultant velocities are calculated for the associative and dissociative parts of process (2.1). To proceed, it is convenient to introduce the branching probabilities p_1 and p_2 for formation of [ES] via $[\mathrm{E}] + [\mathrm{S}] \xrightarrow[k_1]{} [\mathrm{ES}]$ with rate k_1 and for creation of [P] through $[\mathrm{ES}] \xrightarrow[k_2]{} [\mathrm{E}] + [\mathrm{P}]$ with rate k_2 relative to the total dissociation rate $k_2 + k_{-1}$ as:

$$p_1 = \frac{k_1}{k_{\leftrightarrow}}, \qquad p_2 = \frac{k_2}{k_{\leftrightarrow}}, \qquad k_{\leftrightarrow} \equiv k_2 + k_{-1}\,. \tag{2.59}$$

Since there is only one associative subreaction $[\mathrm{E}] + [\mathrm{S}] \xrightarrow[k_1]{} [\mathrm{ES}]$, with velocity $v_1 \equiv v_{\rightarrow}$ which is directly proportional to substrate concentration [S], we can write:

$$\begin{aligned} v_{\rightarrow} &\equiv v_{\mathrm{assoc}} \\ &= k_1[\mathrm{E}]_0[\mathrm{S}]\,. \end{aligned} \tag{2.60}$$

By contrast, there are two dissociative subreactions $[\mathrm{ES}] \xrightarrow[k_2]{} [\mathrm{E}] + [\mathrm{P}]$ and $[\mathrm{E}] + [\mathrm{S}] \xleftarrow[k_{-1}]{} [\mathrm{ES}]$ that develop in time with their respective velocities v_2 and v_{-1}, neither of which is dependent upon substrate concentration [S]. We can define the compound or resultant velocity $v_{\leftrightarrow}$ for this twofold dissociation of [ES] as the sum of the corresponding components v_2 and v_{-1} :

$$
\begin{aligned}
v_{\leftrightarrow} &\equiv v_{\text{dissoc}} \\
&= v_2 + v_{-1}, \qquad v_2 = k_2[\mathrm{E}]_0, \qquad v_{-1} = k_{-1}[\mathrm{E}]_0 \\
&= k_{\leftrightarrow}[\mathrm{E}]_0 \,.
\end{aligned} \tag{2.61}
$$

Finally, given that we are here dealing with rate processes, the overall velocity of the whole reaction (2.1) can be introduced via the effective velocity v_{eff} as per (2.58) by means the halved harmonic mean of velocities $v_{\rightarrow}$ and $v_{\leftrightarrow}$, respectively:

$$
\begin{aligned}
\frac{1}{v_{\text{eff}}} &= \frac{1}{v_{\text{assoc}}} + \frac{1}{v_{\text{dissoc}}} \\
&= \frac{1}{v_{\rightarrow}} + \frac{1}{v_{\leftrightarrow}} \\
&= \frac{1}{k_1[\mathrm{E}]_0[\mathrm{S}]} + \frac{1}{k_{\leftrightarrow}[\mathrm{E}]_0} \,,
\end{aligned} \tag{2.62}
$$

or equivalently,

$$
\begin{aligned}
v_{\text{eff}} &= \frac{v_{\rightarrow} v_{\leftrightarrow}}{v_{\rightarrow} + v_{\leftrightarrow}} \\
&= \frac{k_1 k_{\leftrightarrow} ([\mathrm{E}]_0)^2 [\mathrm{S}]}{k_1[\mathrm{E}]_0[\mathrm{S}] + k_{\leftrightarrow}[\mathrm{E}]_0} \,,
\end{aligned} \tag{2.63}
$$

so that,

$$
v_{\text{eff}} = \frac{k_{\leftrightarrow}[\mathrm{E}]_0[\mathrm{S}]}{K_{\mathrm{M}} + [\mathrm{S}]}, \qquad K_{\mathrm{M}} = \frac{k_{\leftrightarrow}}{k_1} \,, \tag{2.64}
$$

where K_{M} is the same Michaelis–Menten constant (2.11) as in the QE and QSS formalisms, since $k_{\leftrightarrow} = k_{-1} + k_2$ according to (2.59). Finally, the enzyme velocity v_0 is introduced as $v_0 \equiv p_2 v_{\text{eff}}$ and this becomes:

$$
\begin{aligned}
v_0 &\equiv p_2 v_{\text{eff}} \\
&= \frac{k_2[\mathrm{E}]_0[\mathrm{S}]}{K_{\mathrm{M}} + [\mathrm{S}]} \,,
\end{aligned} \tag{2.65}
$$

or alternatively,

$$
v_0 = \frac{v_{\max}[\mathrm{S}]}{K_{\mathrm{M}} + [\mathrm{S}]}, \qquad v_{\max} = k_2[\mathrm{E}]_0 = v_2 \,. \tag{2.66}
$$

Here, $v_{\max}$ is the maximal value of enzyme velocity with the same definition (2.15) from the QE and QSS formalisms. Once the formula (2.66) for enzyme velocity v_0

becomes available, we can examine the two asymptotic cases for small and large substrate concentrations relative to K_M in the formal limits $[S] \ll K_M$ and $[S] \gg K_M$, respectively. This would yield another useful interpretation of the HHM formalism. Thus, at $[S] \ll K_M$, it follows that (2.66) simplifies to:

$$v_0 \underset{[S] \ll K_M}{\longrightarrow} v_{inf} \,, \tag{2.67}$$

where,

$$v_{inf} = k[S] \,, \tag{2.68}$$

with,

$$k = \frac{v_{max}}{K_M} \,. \tag{2.69}$$

In the opposite case $[S] \gg K_M$, the enzyme velocity (2.66) is reduced to the form:

$$v_0 \underset{[S] \gg K_M}{\longrightarrow} v_{sup} \,, \tag{2.70}$$

with,

$$v_{sup} = v_{max} \,, \tag{2.71}$$

where v_{max} is from (2.15). Note that the rate constant k from (2.69) can be determined directly by standard enzyme experiments. The effective reaction velocity v'_{eff} can be introduced as the halved harmonic mean of the two limiting velocities v_{inf} and v_{sup} from (2.68) and (2.71):

$$\frac{1}{v'_{eff}} = \frac{1}{v_{inf}} + \frac{1}{v_{sup}} \,. \tag{2.72}$$

This relationship can be cast into the following form by using (2.68) and (2.71):

$$\frac{1}{v'_{eff}} = \frac{1}{k[S]} + \frac{1}{v_{max}} \,, \tag{2.73}$$

so that,

$$v'_{eff} = \frac{v_{max}\{k[S]\}}{v_{max} + k[S]} = \frac{v_{max}[S]}{K_M + [S]} \,. \tag{2.74}$$

Comparing (2.74) with (2.65) and (2.66), it follows:

$$v'_{eff} = v_{eff} = v_0 \,. \tag{2.75}$$

Hence, the effective reaction velocity v'_{eff} from (2.74) derived differently from v_{eff} in the combined opposite limits of lesion concentration $[\text{S}] \ll K_{\text{M}}$ and $[\text{S}] \gg K_{\text{M}}$, coincides exactly with the enzyme velocity v_0 from (2.66) or (2.14) in the QE and HHM formalisms. Moreover, juxtaposing (2.60) to (2.68) and (2.61) to (2.71), we deduce at once:

$$v_{\leftrightarrow} = v_{\text{inf}}, \qquad v_2 = v_{\text{sup}}. \tag{2.76}$$

This shows that enzyme molecules are able to eject the repaired lesions [P] with maximal velocity $v_2 = v_{\text{sup}} = v_{\text{max}}$ from the complex [ES] and simultaneously set themselves free. This occurs in the exit channel via the reaction $[\text{ES}] \xrightarrow[k_2]{} [\text{E}] + [\text{P}]$, which develops with velocity v_2. In the rectangular hyperbola obtained by plotting v_0 as a function of lesion concentrations [S], enzyme velocity v_0 levels off as $v_0 \approx v_{\text{max}}$ for $[\text{S}] \gg K_{\text{M}}$. Here, velocity v_0 is said to have produced a saturation effect. This saturation for v_0 means that enzyme activity is maximized in the process of transforming radiation damages to repaired lesions.

2.5 Relative merits of four different formalisms for enzyme catalysis

Although the identical formula (2.14) or (2.55) for velocity v_0 is obtained in both the Michaelis–Menten and van Slyke-Cullen derivations, the two formalisms differ in the assumed modalities of the same mechanism. The Michaelis–Menten mechanism for enzyme catalysis (2.1) is based on an equilibrium established rapidly between formation of the intermediate compound via $[\text{E}] + [\text{S}] \xrightarrow[k_1]{} [\text{ES}]$ and its destruction through $[\text{ES}] \xrightarrow[k_2]{} [\text{E}] + [\text{P}]$, where $K_{\text{M}} = (k_{-1} + k_2)/k_1$ is the equilibrium constant. Stated equivalently, Michaelis and Menten assume that the reaction:

$$[\text{E}] + [\text{S}] \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [\text{ES}], \tag{2.77}$$

is practically always at the thermodynamic equilibrium. This effectively amounts to supposing that $k_2 \ll k_{-1}$ in which case $K_{\text{M}} = (k_{-1} + k_2)/k_1 \approx k_2/k_1 = K_{\text{SC}}$. On the other hand, the van Slyke-Cullen mechanism for enzyme catalysis via (2.49) assumes the existence of an irreversible intermediate step with formation of the complex [ES]. This implies $k_{-1} = 0$ from the outset.

Briggs and Haldane [3], while examining the theoretical basis of enzyme catalysis, concluded that both the Michaelis–Menten and the van Slyke-Cullen assumptions are unnecessarily restrictive. To overcome these restrictions, a more general assumption was introduced in Ref. [3] for enzyme catalysis yielding the Briggs-Haldane mechanism, which is based upon the concept of a quasi-steady state of the intermediate complex [ES]. According to this mechanism, concentration $[\text{ES}](t)$ is nearly stationary (time-independent) implying the condition $(\text{d}/\text{d}t)[\text{ES}](t) \approx 0$ for the existence of a quasi-steady state of the intermediate compound [ES]. The meaning of this extremal or stationary condition, which gives the Michaelis–Menten equation (2.14), is that in a steady state of [ES], enzymes [E] are seen as being at maximal activity in converting

[S] to [P]. The actual amount of enzyme molecules [E] in a steady state of [ES] is provided by the quotient [E][S]/[ES]. This latter ratio is recognized as the second of the two definitions (2.11) and (2.48) of the rate constant K_M. By comparison, in the Michaelis–Menten mechanism, the same constant K_M from (2.10) also tells us how much of enzyme [E] is contained in [ES], but at the thermodynamic equilibrium of the intermediate complex.

The significance of the Briggs–Haldane model is in establishing the Michaelis–Menten equation on a firmer theoretical basis with an enhanced flexibility for extensions to more complicated multicycle chemical reactions catalyzed by enzymes involving more than one substrate and intermediate complex molecules, as well as inhibitors and/or co-inhibitors. Indeed, it is the Briggs–Haldane quasi-steady state formalism, which conveys the contemporary and more general essence of the Michaelis–Menten equation for enzyme catalysis.

The Briggs-Haldane, the van Slyke-Cullen and the present models for enzyme catalysis were analyzed here not just to state these three alternative derivations of the MM equation. Rather, this was done primarily to highlight that the identical expression (2.14) could be rationalized by at least four different explanations of basically the same mechanism through which reaction (2.1) develops in time:

$$k_2 \ll k_{-1} \ : \ \text{Reversibility; Quasi} - \text{equilibrium, or QE (Michaelis} - \text{Menten [1])}, \tag{2.78}$$

$$k_{-1} = 0 \quad : \text{Irreversibility; Time summation, or TS (van Slyke} - \text{Cullen [2])}, \tag{2.79}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{ES}] \approx 0 \ : \text{Reversibility; Quasi} - \text{stationary state, or QSS (Briggs} - \text{Haldane [3])}, \tag{2.80}$$

$$v_{\mathrm{eff}} \ : \quad \text{Reversibility; Halved harmonic mean, or HHM (Present)}. \tag{2.81}$$

And even such a fourfold explanation is not the sole reason from comparing these four formalisms associated with (2.78)–(2.81). The real motivation for such a comparison is to emphasize the fact that the van Slyke-Cullen modality was pivotal for putting forward the QSS framework by Briggs and Haldane.

Briggs and Haldane [3] judiciously connected the two main limitations (2.78) and (2.79) in Refs. [1] and [2], respectively. Specifically, the van Slyke-Cullen starting premise of reducing a more involved reaction (2.1) to its simpler counterpart (2.49), as a model for enzyme catalysis, gave an opportunity to Briggs and Haldane to introduce a generalization of the QE and TS formalisms of Michaelis and Menten [1] and van Slyke and Cullen [2] by proposing the QSS model. This was achieved by realizing that the van Slyke and Cullen re-derivation of the same formula (2.14) for reaction (2.49) implies that the main working hypothesis (2.78) of Michaelis and Menten about the existence of a quasi-equilibrium via (2.7) is, in fact, unnecessary. This observation of Briggs and Haldane was further supported by the fact that the condition $k_2 \ll k_{-1}$ of Michaelis and Menten is impossible to verify through measurements. Namely, as pointed out by Briggs and Haldane, the experimental data on the time course of reaction (2.1) can give no information about a relationship between the rate constants k_2 and k_{-1}. As such, those in favor of the QE formalism could object to the TS platform for its restriction to an irreversible enzyme catalysis (2.49). Likewise, those supporting the TS formalism could criticize the QE framework for being applicable only to equilibrium-characterized enzymatic reactions via (2.77), especially given that

not all enzyme-catalyzed reactions proceed through the said equilibrium. The rescue by Briggs and Haldane is in preserving the good parts of both Refs. [1] and [2]. This is, first of all, the MM equation from Ref. [1] and the possibility to place doubt on the necessity of the existence of a quasi-equilibrium, as implicit in Ref. [2]. Finally, rather than using a weaker condition (2.79) to place doubt on a stronger limitation (2.78), Briggs and Haldane, resorted to a more general quasi-steady state formalism of Bodenstein [13] to simultaneously lift both restrictions inherent in Refs. [1] and [2]. This is the case since the quasi-stationary state condition (2.80) leads straight to the MM equation (2.14), but without invoking either (2.78) or (2.79). For this reason, the adaptation of the QSS formalism in the work of Briggs and Haldane [3] bypasses the assumption $k_2 \ll k_{-1}$ and $k_{-1} \equiv 0$ of the QE and TS formalisms, respectively. In other words, the QSS formalism can be applied to reversible enzyme catalysis reactions with or without the potentially existing equilibrium. Moreover, by simply setting $k_{-1} = 0$, the QSS formalism remains valid for irreversible reactions, as well.

Like the QSS model, the present HHM formalism also lifts the Michaelis–Menten ($k_2 \ll k_{-1}$) and the van Slyke and Cullen ($k_{-1} \equiv 0$) constraints. Advantageously, the HHM formalism considers the reversible reaction (2.1) in a much simpler way than in the work of Briggs and Haldane [3] by exploiting the fact that the harmonic mean is the truest average value among all the averages for any rate phenomena. This circumstance permits a direct identification of enzyme velocity v_0 as the branched halved harmonic mean of the limiting velocities for the two characteristic and sharply distinct pathways of enzyme catalysis. One pathway corresponds to the beginning of the development at the earliest time near the onset of catalysis when enzyme velocity is linearly dependent on substrate concentration [S]. The other pathway occurs significantly later at substrate concentrations that are much larger than the equilibrium concentration K_{M} and at which enzyme velocity attains a constant value. The attractiveness of the HMM formalism is in capturing these two dominant features of enzyme catalysis in a single formula for the enzyme effective velocity v_{eff} without the need to ever set and solve any kinetic rate equation, let alone a system of four coupled Eqs. (2.19)–(2.22) from the QSS model.

2.6 Time evolution as complete progress curves in integrated rate equations

Standard enzyme kinetic experiments do not directly measure the reaction rates. Instead, concentrations of substrate [S](t) or product [P](t) are measured as functions of time. Therefore, in order to directly compare the same type of data acquired by both experiment and theory, it is necessary to integrate the modeled rate equations. In this way, the theory too would give the complete progress curves that are the concentrations as functions of time. In the first sentence of their paper, Michaelis and Menten [1] stated that the ultimate goal of studying enzyme kinetics is to determine the complete time course of catalysis. To obtain the time evolution of e.g. the substrate concentration in reaction (2.1), they integrated Eq. (2.14) where the term $k_2[\mathrm{E}]_0$ is $v_{\max}$, according to (2.15). This important final step was also accomplished by van Slyke and Cullen [2], who through a different and independent derivation obtained the equivalent result (2.55) for reaction (2.49).

2.6.1 Exponential and linear asymptotic progress curves for low and high substrate concentrations

For a general, unspecified relationship between [S] and K_M, the MM equation (2.14) does not have a definite order which stems from the degree of the invoked polynomial in variable [S]. This is because the binomial $1/(K_M + [S])$ itself is a series with infinitely many powers $[S]^k$ ($k = 0, 1, 2, 3, \cdots$). However, the situation is simplified in the two asymptotic cases of low and high substrate concentrations. In Subsect. 2.4, these asymptotic cases have already been considered in terms of the reaction velocity v_0 at the idealized limits $[S] \to 0$ and $[S] \to \infty$. The results (2.67) and (2.70) for the two asymptotes of v_0 at very low and high substrate concentrations can be interpreted to also represent the equivalent limits $[S] \gg K_M$ and $[S] \ll K_M$ that are more realistically encountered in experimental measurements than $[S] \to 0$ and $[S] \to \infty$, respectively. Thus, for $[S] \ll K_M$, the MM Eq. (2.40) is seen to be of the first-order:

$$\frac{d[S]}{dt} \approx -k[S], \qquad [S] \ll K_M\,, \tag{2.82}$$

with the solution:

$$[S](t) \approx [S]_{inf}(t), \qquad [S]_{inf}(t) = [S]_0 e^{-kt}, \qquad [S] \ll K_M. \tag{2.83}$$

As such, at $[S] \ll K_M$, we have $[S](t) \approx [S]_{inf}(t)$, where $[S]_{inf}$ is a single exponential with the damping $k = v_{max}/K_M$ taken from (2.69).

On the other hand, when $[S] \gg K_M$, the MM Eq. (2.40) becomes of zero-order ([S] raised to zeroth order, i.e. $[S]^0 = 1$):

$$\frac{d[S]}{dt} \approx -v_{max}, \qquad [S](t) \gg K_M\,, \tag{2.84}$$

whose solution reads as:

$$[S](t) \approx [S]_{sup}(t), \qquad [S]_{sup}(t) = [S]_0 - v_{max}t, \qquad [S](t) \gg K_M. \tag{2.85}$$

Here, the asymptote $[S](t) \approx [S]_{sup}(t)$ for $[S] \gg K_M$ represents a linear progress curve versus t. The intercept of $[S]_{sup}$ from (2.84) with the ordinate at $t = 0$ is the initial substrate concentration $[S]_0$, whereas the slope of this progress curve is the saturation rate v_{max}. We also have the proper limit of $[S]_{inf}(t)$ and $[S]_{sup}(t)$ at $t = 0$:

$$[S]_{inf}(0) = [S]_{sup}(0) = [S](0) = [S]_0\,. \tag{2.86}$$

These asymptotic relations for $[S] \ll K_M$ and $[S] \gg K_M$ can also be written directly in terms of enzyme velocity v_0, since $v_0 = -d[S]/dt$ according to (2.44). Thus, Eqs. (2.82) and (2.84) can equivalently be stated as:

$$v_0 \approx k[S], \qquad [S] \ll K_M\,, \tag{2.87}$$

and

$$v_0 \approx v_{\max}, \qquad [\mathrm{S}] \gg K_\mathrm{M}\,, \tag{2.88}$$

respectively.

2.6.2 *The Lambert W_0 function for exact progress curves at arbitrary substrate concentrations*

- *The use of the definition of the Lambert W_0 function involving the logarithm*

To obtain the time evolution of e.g. the substrate concentration, without any further approximation, we integrate Eq. (2.40). Such a direct integration of Eq. (2.40) is possible because of separation of the time variable t from the substrate concentration [S] :

$$\int \mathrm{d}[\mathrm{S}]\frac{K_\mathrm{M}+[\mathrm{S}]}{[\mathrm{S}]} = -v_{\max}\int \mathrm{d}t\,,$$

so that,

$$K_\mathrm{M}\ln[\mathrm{S}] + [\mathrm{S}] = -v_{\max}t + C\,. \tag{2.89}$$

Here, the integration constant C is found by applying the initial condition (2.23) to (2.89) at $t = 0$ for the substrate concentration $[\mathrm{S}](0) = [\mathrm{S}]_0$, so that:

$$C = K_\mathrm{M}\ln[\mathrm{S}]_0 + [\mathrm{S}]_0\,. \tag{2.90}$$

This completes the derivation of the primitive function (2.89), which is usually called the integrated rate equation:

$$-v_{\max}t = K_\mathrm{M}\ln\frac{[\mathrm{S}]}{[\mathrm{S}]_0} + [\mathrm{S}] - [\mathrm{S}]_0\,. \tag{2.91}$$

Thus, the exact solution (2.91) is given in a nonlinear implicit form relative to the dependent variable [S]. This means that the independent variable t is given as a function of the dependent variable [S]. Such a reverse role of the dependent and independent variables is precisely opposite to what is encountered in explicit functions that express a dependent variable in terms of an independent variable. To pass from (2.91) onto the field of explicit functions, it would be necessary to write [S] by means of a function $\mathcal{F}(t)$ which, as a function of the independent variable t, could contain some constants, but *not* the dependent variable [S]. With this aim, we first rewrite (2.91) as:

$$\ln(\sigma_\mathrm{M}[\mathrm{S}]) + \sigma_\mathrm{M}[\mathrm{S}] = \ln h(t) \tag{2.92}$$

with,

$$\ln h(t) \equiv \ln\left(\sigma_{\mathrm{M}}[\mathrm{S}]_0\right) + \sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}\,, \tag{2.93}$$

where $[\mathrm{S}]_{\mathrm{sup}}$ is taken from (2.85) and σ_{M} is the reciprocal of the MM rate constant:

$$\sigma_{\mathrm{M}} = \frac{1}{K_{\mathrm{M}}} = \frac{k_1}{k_{-1}+k_2}\,. \tag{2.94}$$

With the help of the identities:

$$\ln h(t) = \ln\left(\sigma_{\mathrm{M}}[\mathrm{S}]_0\right) + \sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}} = \ln\left(\mathrm{e}^{\ln\left(\sigma_{\mathrm{M}}[\mathrm{S}]_0\right)+\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}}\right) = \ln\left(\sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}}\right),$$

we can extract $h(t)$ as:

$$h(t) = \sigma_{\mathrm{M}}[\mathrm{S}]_0\, \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}} = \sigma_{\mathrm{M}}[\mathrm{S}]_0\, \mathrm{e}^{\sigma_{\mathrm{M}}\left([\mathrm{S}]_0 - v_{\max} t\right)}\,. \tag{2.95}$$

The exponential in (2.95) is always non-negative for any time t and, moreover, the physical concentrations $[\mathrm{S}]_0$ and K_{M} are also positive or zero, so that:

$$h(t) \geq 0, \qquad \forall\, t\,. \tag{2.96}$$

Using (2.85) and (2.95), the expression (2.91) can be rewritten via:

$$\ln\left(\sigma_{\mathrm{M}}[\mathrm{S}]\right) + \sigma_{\mathrm{M}}[\mathrm{S}] = \ln\left(\sigma_{\mathrm{M}}[\mathrm{S}]_0\, \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}}\right). \tag{2.97}$$

This transcendental equation can be solved exactly in the explicit form of the Lambert W function defined by [18,19]:

$$W(x)\mathrm{e}^{W(x)} = y\,. \tag{2.98}$$

An equivalent definition for $W(x)$ also exists in terms of the natural logarithms via:

$$\ln W(x) + W(x) = \ln y\,. \tag{2.99}$$

In the case of real x, there are only two real-valued Lambert functions, one of which is the principal branch $W_0(x)$ and the other is denoted by $W_{-1}(x)$. All the remaining branches $W_k(k = 1, \pm 2, \pm 3, \cdots)$, as the roots of Eq. (2.98), are complex-valued. The Lambert function $W(x)$ will be the principal branch $W_0(x)$ if $W(x) \geq -1$, for $x \in [-1/\mathrm{e}, +\infty]$:

$$W(x) = W_0(x) \qquad \text{if} \qquad W(x) \geq -1 \qquad \text{and} \qquad x \in [-1/\mathrm{e}, +\infty]\,. \tag{2.100}$$

Moreover, if $x \geq -1$ and the independent variable of W is $x\mathrm{e}^x$, we have the following useful relation:

$$W_0(x\mathrm{e}^x) = x \qquad \text{if} \qquad x \geq -1\,. \tag{2.101}$$

The asymptotic behaviors of the Lambert W_0 function at small and large values of real and non-negative x are given by:

$$W_0(x) \underset{x \to 0}{\approx} x - x^2 \underset{x \to 0}{\approx} \frac{x}{1+x}\,, \tag{2.102}$$

and,

$$W_0(x) \underset{x \to \infty}{\approx} \ln x - \ln(\ln x)\,, \tag{2.103}$$

respectively. We shall also need the differentiation rule of the Lambert W function as prescribed the two equivalent expressions:

$$\frac{\mathrm{d}W(x)}{\mathrm{d}x} = \frac{\mathrm{e}^{-W(x)}}{1+W(x)}, \quad x \neq -\frac{1}{\mathrm{e}}\,, \tag{2.104}$$

and,

$$\frac{\mathrm{d}W(x)}{\mathrm{d}x} = \frac{W(x)}{x\,[1+W(x)]}, \quad x \neq 0, \quad x \neq -\frac{1}{\mathrm{e}}. \tag{2.105}$$

Comparing (2.97) with definition (2.99) of the Lambert W function, we can deduce:

$$[\mathrm{S}] = [\mathrm{S}](t) = \frac{1}{\sigma_\mathrm{M}} W_0\left(\sigma_\mathrm{M}[\mathrm{S}]_0\,\mathrm{e}^{\sigma_\mathrm{M}[\mathrm{S}]_\mathrm{sup}}\right)\,, \tag{2.106}$$

$$= \frac{1}{\sigma_\mathrm{M}} W_0\left(\sigma_\mathrm{M}[\mathrm{S}]_0\,\mathrm{e}^{\sigma_\mathrm{M}([\mathrm{S}]_0 - v_\mathrm{max} t)}\right)\,, \tag{2.107}$$

or equivalently, by means of (2.93):

$$[\mathrm{S}] = [\mathrm{S}](t) = \frac{1}{\sigma_\mathrm{M}} W_0(h(t))\,. \tag{2.108}$$

This shows that the mentioned unique explicit function $\mathcal{F}(t)$ indeed exists and is proportional to the Lambert function, $\mathcal{F}(t) \propto W_0(h(t))$. The solution (2.107) is unique, as indicated by the specification $W(h(t)) = W_0(h(t))$. In (2.107), the principal branch W_0 is chosen for W for the reason which runs as follows. First of all, the independent variable $h(t)$ of W is always non-negative for every t, as per (2.96). Further, all physical concentrations must be positive and finite, so that $[\mathrm{S}](t) > 0$ and $K_\mathrm{M} > 0$, as well as $\sigma_\mathrm{M} > 0$. Therefore, the equality in (2.107) implies $W_0(h(t)) \geq 0$. Non-negativity

of the Lambert function $W(x)$ for real non-negative x implies $W(x) = W_0(x)$, where W_0 is the principal branch. Hence, since $h(t) \geq 0$, we have $W(h(t)) = W_0(h(t))$:

$$W\left(\sigma_M[S]_0\, e^{\sigma_M([S]_0 - v_{max}t)}\right) = W_0\left(\sigma_M[S]_0\, e^{\sigma_M([S]_0 - v_{max}t)}\right) \geq 0. \quad (2.109)$$

The obtained result $[S](t) = \sigma_M^{-1} W_0(h(t)) = \sigma_M^{-1} W_0(\sigma_M[S]_0\, e^{\sigma_M[S]_{sup}})$ for the time evolution of the substrate concentration can be checked by taking the first derivative with respect to time of both sides of Eq. (2.107) as:

$$\frac{d[S]}{dt} = \frac{1}{\sigma_M}\frac{d}{dt}W_0(h(t)) = \frac{1}{\sigma_M}\left\{\frac{d}{dh(t)}W_0(h(t))\right\}\left\{\frac{dh(t)}{dt}\right\}. \quad (2.110)$$

By using the differentiation rule (2.105) for the Lambert function and the first derivative of the auxiliary function $h(t)$, it follows:

$$\frac{d}{dh(t)}W_0(h(t)) = \frac{1}{h(t)}\frac{W_0(h(t))}{1 + W_0(h(t))} \quad , \quad \frac{dh(t)}{dt} = -\sigma_M v_{max} h(t)\,. \quad (2.111)$$

This yields:

$$\begin{aligned}\frac{d[S]}{dt} &= \frac{1}{\sigma_M}\left\{\frac{d}{dh(t)}W_0(h(t))\right\}\left\{\frac{dh(t)}{dt}\right\} \\ &= K_M\left\{\frac{1}{h(t)}\frac{W_0(h(t))}{1 + W_0(h(t))}\right\}\left\{-\frac{v_{max}}{K_M}h(t)\right\} \\ &= -v_{max}\frac{K_M W_0(h(t))}{K_M + K_M W_0(h(t))} \\ &= -v_{max}\frac{[S]}{K_M + [S]}\end{aligned}$$

so that,

$$\frac{d[S]}{dt} = -v_{max}\frac{[S]}{K_M + [S]}\,, \qquad \text{(QED)} \quad (2.112)$$

which is the MM Eq. (2.40) where $k_2[E]_0$ is equal to v_{max} in accord with (2.15). Note that in a previous application [20,21] of the MM formalism to dose-effect curves, the implicit Eq. (2.91) was used with $k_{-1} = 0$ ($K_M = K_{SC}$) and solved numerically for the cell surviving fraction.

- *The use of the definition of the Lambert W_0 function involving the exponential*

In an alternative derivation, we employ (2.85) to express (2.91) as:

$$\ln\frac{[S]}{[S]_0} = -\frac{[S] - [S]_{sup}}{K_M}\,. \quad (2.113)$$

With the help of (2.113), we can obtain the following transcendental equation involving exponentials instead of logarithms from (2.92):

$$\sigma_{\mathrm{M}}[\mathrm{S}]\,\mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]} = \sigma_{\mathrm{M}}[\mathrm{S}]_0\,\mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}}\,. \tag{2.114}$$

Making use of the definition (2.98) of the Lambert function, we can immediately identify the following result for the root [S] of the transcendental Eq. (2.114):

$$[\mathrm{S}] = [\mathrm{S}](t) = \frac{1}{\sigma_{\mathrm{M}}} W_0\left(\sigma_{\mathrm{M}}[\mathrm{S}]_0\,\mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}}\right)\,, \tag{2.115}$$

in agreement with (2.106). The importance of the relation (2.113) is in showing that the measure or degree of the departure of the complete solution $[\mathrm{S}](t)$ from its high-substrate asymptote $[\mathrm{S}]_{\mathrm{sup}}$, i.e. the difference $[\mathrm{S}](t) - [\mathrm{S}]_{\mathrm{sup}}$ is proportional to the difference between the logarithm of the initial concentration $\ln [\mathrm{S}]_0$ and $\ln [\mathrm{S}](t)$, i.e. $[\mathrm{S}](t) - [\mathrm{S}]_{\mathrm{sup}} = \sigma_{\mathrm{M}}\{\ln [\mathrm{S}]_0 - \ln [\mathrm{S}](t)\}$. On the other hand, the logarithmic function is the slowest decreasing function with augmentation of its independent variable. This fact, alongside Eq. (2.113), implies that $[\mathrm{S}](t)$ will tend very slowly to its asymptote $[\mathrm{S}]_{\mathrm{sup}}$ at high substrate concentrations. Such a feature is common to both the reaction velocity v_0 through its rectangular hyperbola (2.14) and the integrated rate Eq. (2.113).

2.7 Logarithm of the complete progress curve as the Lambert W_0 function with the factored asymptote at high substrate concentrations

Within the MM formalism, it is important to establish a direct link between the complete progress curve in its exact and asymptotic forms $[\mathrm{S}](t)$ and $[\mathrm{S}]_{\mathrm{sup}}(t)$. It is clear that (2.91) already exhibits a link between $[\mathrm{S}](t)$ and $[\mathrm{S}]_{\mathrm{sup}}(t)$, albeit in the form of an iterated exponential condensed in the independent variable of the Lambert function, $W_0(\sigma_{\mathrm{M}}[\mathrm{S}]_0\,\mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}})$. However, it would be instructive if at least a part of this link could be factored out. A hint towards this goal is provided by (2.91), which shows that the difference $[\mathrm{S}](t) - [\mathrm{S}]_{\mathrm{sup}}(t)$ is proportional to the logarithm of the quotient $[\mathrm{S}](t)/[\mathrm{S}]_0$, i.e. $[\mathrm{S}]-[\mathrm{S}]_{\mathrm{sup}} = -K_{\mathrm{M}} \ln ([\mathrm{S}]/[\mathrm{S}]_0)$. Let this latter difference be denoted by $[B_{\mathrm{M}}]$:

$$[B_{\mathrm{M}}] \equiv [\mathrm{S}] - [\mathrm{S}]_{\mathrm{sup}}\,, \tag{2.116}$$

$$= -K_{\mathrm{M}} \ln \frac{[\mathrm{S}]}{[\mathrm{S}]_0}\,. \tag{2.117}$$

From here, the substrate concentration $[\mathrm{S}](t)$ can be written in terms of the quantity $B_{\mathrm{M}}(t)$ as:

$$[\mathrm{S}](t) = [\mathrm{S}]_0 \mathrm{e}^{-\sigma_{\mathrm{M}}[B_{\mathrm{M}}]}\,. \tag{2.118}$$

In this notation, the integrated MM rate Eq. (2.91) becomes:

$$\begin{aligned} -v_{\max} t &= K_{\mathrm{M}} \ln \frac{[\mathrm{S}]}{[\mathrm{S}]_0} + [\mathrm{S}] - [\mathrm{S}]_0 \\ &= -[B_{\mathrm{M}}] + [\mathrm{S}]_0 \mathrm{e}^{-\sigma_{\mathrm{M}}[B_{\mathrm{M}}]} - [\mathrm{S}]_0 \\ &= -[B_{\mathrm{M}}] - [\mathrm{S}]_0 \left\{ 1 - \mathrm{e}^{-\sigma_{\mathrm{M}}[B_{\mathrm{M}}]} \right\} , \end{aligned}$$

so that,

$$v_{\max} t = [B_{\mathrm{M}}] + [\mathrm{S}]_0 \left\{ 1 - \mathrm{e}^{-\sigma_{\mathrm{M}}[B_{\mathrm{M}}]} \right\} . \tag{2.119}$$

This equation can equivalently be rewritten as:

$$[B_{\mathrm{M}}] + [\mathrm{S}]_{\mathrm{sup}} - [\mathrm{S}]_0 \mathrm{e}^{-\sigma_{\mathrm{M}}[B_{\mathrm{M}}]} = 0 , \tag{2.120}$$

which also follows from the definition (2.116) via $B_{\mathrm{M}}(t) = [\mathrm{S}] - [\mathrm{S}]_{\mathrm{sup}}$, when (2.116) is used for [S]. The unknown quantity in this implicit transcendental equation is the function $B_{\mathrm{M}}(t)$. The specific form (2.119) is of the type of the following transcendental equation:

$$z - q_1 - q_2 \mathrm{e}^{-q_3 z} = 0 , \tag{2.121}$$

which has the exact explicit solution:

$$z = q_1 + \frac{1}{q_3} W \left(q_2 q_3 \mathrm{e}^{-q_1 q_3} \right) , \tag{2.122}$$

where W is the Lambert function from (2.98) or (2.99). When comparing (2.120) with (2.130) and identifying:

$$\left. \begin{aligned} z &= [B_{\mathrm{M}}] \\ q_1 = -[\mathrm{S}]_{\mathrm{sup}} &= v_{\max} t - [\mathrm{S}]_0 \\ q_2 &= [\mathrm{S}]_0 \\ q_3 &= \sigma_{\mathrm{M}} \end{aligned} \right\} , \tag{2.123}$$

it follows,

$$[B_{\mathrm{M}}] = -[\mathrm{S}]_{\mathrm{sup}} + \frac{1}{\sigma_{\mathrm{M}}} W_0 \left(\sigma_{\mathrm{M}} [\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}} [\mathrm{S}]_{\mathrm{sup}}} \right) , \tag{2.124}$$

or more explicitly,

$$[B_{\mathrm{M}}] = v_{\max} t - [\mathrm{S}]_0 + \frac{1}{\sigma_{\mathrm{M}}} W_0 \left(\sigma_{\mathrm{M}} [\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}} ([\mathrm{S}]_0 - v_{\max} t)} \right) . \tag{2.125}$$

The reason for having the principal branch W_0 for the Lambert W is given earlier in connection with (2.108). Solution (2.124) can be verified by inserting the definition $[B_{\mathrm{M}}] = [\mathrm{S}](t) - [\mathrm{S}]_{\mathrm{sup}}(t)$ from (2.116) into the lhs of (2.124) to write:

$$[\mathrm{S}](t) - [\mathrm{S}]_{\mathrm{sup}} = -[\mathrm{S}]_{\mathrm{sup}} + \frac{1}{\sigma_{\mathrm{M}}} W_0\left(\sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}}\right), \tag{2.126}$$

which gives,

$$[\mathrm{S}](t) = \frac{1}{\sigma_{\mathrm{M}}} W_0\left(\sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}}\right), \tag{2.127}$$

in agreement with (2.91). The explicit result (2.124) for the function $[B_{\mathrm{M}}]$ exhibits an additive separation of the first-order kinetic term $-[\mathrm{S}]_{\mathrm{sup}}$ for high-substrate concentration and the rest $W_0(\sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}})$ which is itself dependent upon $[\mathrm{S}]_{\mathrm{sup}}$.

When the alternative and equivalent definition (2.117) is used for $[B_{\mathrm{M}}]$ in the lhs of Eq. (2.124), we have:

$$[\mathrm{S}](t) = [\mathrm{S}]_{\mathrm{sup}}(t)\mathcal{M}(t), \tag{2.128}$$

where,

$$\mathcal{M}(t) = \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_0} - W_0(\sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}}). \tag{2.129}$$

At $t = 0$, we have $[\mathrm{S}]_{\mathrm{sup}}(0) = [\mathrm{S}]_0$, as in (2.86), so that:

$$\mathcal{M}(0) = \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_0} - W_0(\sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_0}) = \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_0} - \sigma_{\mathrm{M}}[\mathrm{S}]_0 = 1.$$

Here, relation $W_0(x\mathrm{e}^x) = x$ from (2.101) is used and, therefore:

$$\mathcal{M}(0) = 1. \tag{2.130}$$

This reduces Eq. (2.129) to $[\mathrm{S}](0) = [\mathrm{S}]_0$, as the correct limit to the initial condition (2.23).

The compact result (2.128) expresses the complete progress curve $[\mathrm{S}](t)$ in a form which factors out the high-dose asymptote $[\mathrm{S}]_{\mathrm{sup}}(t)$, as a multiplicative term. In such a factorization, the remainder $\mathcal{M}(t)$ is the exponential function containing the Lambert function $W_0(\sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}})$, which is also given in terms of $[\mathrm{S}]_{\mathrm{sup}}(t)$. Therefore, neither (2.124) nor (2.128) can fully isolate a clear-cut contribution of the first-order kinetics through a single term $[\mathrm{S}]_{\mathrm{sup}}(t)$. Rather, the factored term $[\mathrm{S}]_{\mathrm{sup}}(t)$ in (2.124) and (2.128) is accompanied by the $[\mathrm{S}]_{\mathrm{sup}}-$dependent Lambert function $W_0(\sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}})$. The impossibility to single out the contribution of a pure first-order kinetics, with the rest being totally independent of it can be traced back to the fact that the rate equations of the MM kinetics (2.19)–(2.22) are of mixed order (zero, first and second) and, hence, inseparable, i.e. coupled.

Overall, the asymptote $[\mathrm{S}]_{\mathrm{sup}}(t)$ at high substrate concentrations is seen to be the main constituent of the exact progress curve $[\mathrm{S}](t)$. This dependence of $[\mathrm{S}](t)$ on $[\mathrm{S}]_{\mathrm{sup}}(t)$ is succinctly contained in the Lambert function $W_0(\sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}}})$. However, if the Lambert function were unknown, the first thing which would come to mind while solving the transcendental Eqs. (2.91) and (2.120) is an iterative self-substitution. Thus, we could insert $[\mathrm{S}]_{\mathrm{sup}} + K_{\mathrm{M}} \ln([\mathrm{S}]_0/[\mathrm{S}])$ in place of $[\mathrm{S}]$ in the rhs of Eq. (2.91) to arrive at the first iteration. Subsequently repeating the same procedure with the outcome from the first iteration, we would obtain the expression for the second iteration:

$$\left.\begin{aligned}
[\mathrm{S}] &= [\mathrm{S}]_{\mathrm{sup}} + K_{\mathrm{M}} \ln \frac{[\mathrm{S}]_0}{[\mathrm{S}]} \\
[\mathrm{S}] &= [\mathrm{S}]_{\mathrm{sup}} + K_{\mathrm{M}} \ln \frac{[\mathrm{S}]_0}{[\mathrm{S}]_{\mathrm{sup}} + K_{\mathrm{M}} \ln \dfrac{[\mathrm{S}]_0}{[\mathrm{S}]}} \\
[\mathrm{S}] &= [\mathrm{S}]_{\mathrm{sup}} + K_{\mathrm{M}} \ln \frac{[\mathrm{S}]_0}{[\mathrm{S}]_{\mathrm{sup}} + K_{\mathrm{M}} \ln \dfrac{[\mathrm{S}]_0}{[\mathrm{S}]_{\mathrm{sup}} + K_{\mathrm{M}} \ln \dfrac{[\mathrm{S}]_0}{[\mathrm{S}]}}}
\end{aligned}\right\}. \tag{2.131}$$

An entirely similar iterative self-substitution could also be applied to the transcendental Eq. (2.120), which would give:

$$\left.\begin{aligned}
[B_{\mathrm{M}}] &= -[\mathrm{S}]_{\mathrm{sup}} + [\mathrm{S}]_0 \mathrm{e}^{-\sigma_{\mathrm{M}}[B_{\mathrm{M}}]} \\
[B_{\mathrm{M}}] &= -[\mathrm{S}]_{\mathrm{sup}} + [\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}} - \sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{-\sigma_{\mathrm{M}}[B_{\mathrm{M}}]}} \\
[B_{\mathrm{M}}] &= -[\mathrm{S}]_{\mathrm{sup}} + [\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}} - \sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{\sigma_{\mathrm{M}}[\mathrm{S}]_{\mathrm{sup}} - \sigma_{\mathrm{M}}[\mathrm{S}]_0 \mathrm{e}^{-\sigma_{\mathrm{M}}[B_{\mathrm{M}}]}}}
\end{aligned}\right\}. \tag{2.132}$$

These formulae for $[\mathrm{S}](t)$ or $[B_{\mathrm{M}}]$ still represent implicit, transcendental equations because the sought solution $[\mathrm{S}](t)$ or $[B_{\mathrm{M}}]$ appear on the rhs of Eqs. (2.131) and (2.132). However, this is only an apparent obstacle, since the iterative approximations, that are accurate to any preassigned degree of precision, could be obtained by truncating the self-substitution at a given step, as customarily done with continued fractions [22]. Moreover, there are many continued fractions that can be summed up explicitly to yield a number of the existing elementary and/or special functions. The outlined continued fractions generated from (2.91) and (2.120) is an example of exactly summable iterative self-substitutions in the form of the Lambert W_0 function. The advantage of giving the final result for $[\mathrm{S}](t)$ and $[B_{\mathrm{M}}]$ in terms of the Lambert function is in the fact that this function possesses a number of very useful properties, such as the asymptotic behaviors at small as well as large values of its independent variable, power series expansions with the known convergence radius, etc. Furthermore, recognizing the Lambert function in the outlined continued fractions is also of great practical usefulness due to the existence of a number of efficient and accurate algorithms for numerical computations of $[\mathrm{S}](t)$ or $[B_{\mathrm{M}}]$ to any fixed accuracy [22,23].

3 Repair by the Michaelis and Menten enzyme catalysis

An application of the formalism of Sect. 2 to the problem of repair in cell survival is possible with a few straightforward specifications. To this end, substrate [S] will be relabeled by [L], which is the concentration of lesions that are the DNA substrate molecules. Likewise, product [P] shall be relabeled by [R], which is the concentration of repaired lesions. The notation [E] for the concentration of enzyme molecules will remain unaltered. Moreover, the irreversible version ($k_{-1} = 0$) of enzyme catalysis (2.1) will be considered, i.e. reaction (2.49). As such, lesion repair mediated by enzyme catalysis of the type of Michaelis–Menten, or equivalently, van Slyke-Cullen, can schematically be written as:

$$[\mathrm{E}] + [\mathrm{L}] \underset{k_1}{\longrightarrow} [\mathrm{EL}] \underset{k_2}{\longrightarrow} [\mathrm{E}] + [\mathrm{R}]\,. \tag{3.1}$$

For this reaction, the system of Eqs. (2.19)–(2.22) takes the form:

$$\frac{\mathrm{d}[\mathrm{L}]}{\mathrm{d}t} = -k_1[\mathrm{E}]\,[\mathrm{L}] \tag{3.2}$$

$$\frac{\mathrm{d}[\mathrm{E}]}{\mathrm{d}t} = -k_1[\mathrm{E}]\,[\mathrm{L}] + k_2[\mathrm{EL}] \tag{3.3}$$

$$\frac{\mathrm{d}[\mathrm{EL}]}{\mathrm{d}t} = k_1[\mathrm{E}]\,[\mathrm{L}] - k_2[\mathrm{EL}] \tag{3.4}$$

$$\frac{\mathrm{d}[R]}{\mathrm{d}t} = k_2[\mathrm{EL}]\,, \tag{3.5}$$

and the initial conditions at $t = 0$ are,

$$[\mathrm{L}](0) = [\mathrm{L}]_0,\quad [\mathrm{E}](0) = [\mathrm{E}]_0,\quad [\mathrm{EL}](0) = [\mathrm{EL}]_0,\quad [\mathrm{R}](0) = [\mathrm{R}]_0. \tag{3.6}$$

Then under the QSS approximation, the velocity (2.14) for reaction (3.1) is:

$$v_0 \equiv \frac{\mathrm{dR}}{\mathrm{d}t} = -\frac{\mathrm{dL}}{\mathrm{d}t} = \frac{v_{\max}[\mathrm{L}]}{\widetilde{K}_{\mathrm{M}} + [\mathrm{L}]}\,, \tag{3.7}$$

where $v_{\max}$ is the maximal enzyme velocity given by (2.15) and $\widetilde{K}_{\mathrm{M}}$ is the Michaelis–Menten constant, which is for $k_{-1} = 0$ identical to the van Slyke-Cullen constant K_{SC}, as per (2.55) and (2.56):

$$\widetilde{K}_{\mathrm{M}} = \frac{k_2}{k_1}, \qquad \widetilde{K}_{\mathrm{M}} = K_{\mathrm{SC}}. \tag{3.8}$$

The integral of the differential equation $\mathrm{dL}/\mathrm{d}t = v_{\max}[\mathrm{L}]/(\widetilde{K}_{\mathrm{M}} + [\mathrm{L}])$ in (3.7) is deduced from (2.127) as:

$$[\mathrm{L}](t) = \frac{1}{\tilde{\sigma}_{\mathrm{M}}} W_0\left(\tilde{\sigma}_{\mathrm{M}}[\mathrm{L}]_0 \mathrm{e}^{\tilde{\sigma}_{\mathrm{M}}[\mathrm{L}]_{\sup}}\right) = \frac{1}{\tilde{\sigma}_{\mathrm{M}}} W_0\left(\tilde{\sigma}_{\mathrm{M}}[\mathrm{L}]_0 \mathrm{e}^{\tilde{\sigma}_{\mathrm{M}}[\mathrm{L}]_0 - kt}\right)\,, \tag{3.9}$$

where,

$$[\mathrm{L}]_{\mathrm{sup}}(t) = [\mathrm{L}]_0 - v_{\max} t\,, \tag{3.10}$$

and,

$$\tilde{\sigma}_{\mathrm{M}} = \frac{1}{\widetilde{K}_{\mathrm{M}}}, \qquad k = \tilde{\sigma}_{\mathrm{M}} v_{\max} = \frac{v_{\max}}{\widetilde{K}_{\mathrm{M}}}. \tag{3.11}$$

Assuming, as usual, that the initial number of lesions $[\mathrm{L}]_0$ is proportional to the absorbed dose, we write:

$$[\mathrm{L}]_0 = k_0 D\,, \tag{3.12}$$

where,

$$k_0 = \frac{1}{D_0}\,. \tag{3.13}$$

Here, D_0 or D_{37} is the dose defined as the dose at which the survival fraction $S_{\mathrm{F}}(D)$ is reduced by a factor of $1/\mathrm{e} \approx 0.37$, or by $\sim$37 %. This definition stems from a purely exponential decay law for the cell survival probability, $S_{\mathrm{F}}(D) = \mathrm{e}^{-D/D_0}$, where at $D = D_0$ we have $S_{\mathrm{F}}(D_0) = 1/\mathrm{e}$. Using (3.12) and choosing t to be equal to the repair time t_{R}, we set:

$$\{[\mathrm{L}](t)\}_{[\mathrm{L}]_0 = k_0 D, t = t_{\mathrm{R}}} \equiv [\mathrm{L}(D)]\,. \tag{3.14}$$

With this convention, Eq. (3.10) can be written as the following expression:

$$[\mathrm{L}(D)] = \widetilde{K}_{\mathrm{M}} W_0(y_D)\,, \tag{3.15}$$

where,

$$y_D = \lambda_{\mathrm{M}} D \mathrm{e}^{\lambda_{\mathrm{M}} D - \omega_{\mathrm{R}}}\,, \tag{3.16}$$

and the three-parameters $\{\widetilde{K}_{\mathrm{M}}, \lambda, \omega\}$ of the lesions $[\mathrm{L}(D)]$ are:

$$\widetilde{K}_{\mathrm{M}} = \frac{k_2}{k_1}, \qquad \lambda_{\mathrm{M}} = \frac{k_0}{\widetilde{K}_{\mathrm{M}}}, \qquad \omega_{\mathrm{R}} = k t_{\mathrm{R}}\,. \tag{3.17}$$

The three parameters $\{\widetilde{K}_{\mathrm{M}}, k, t_{\mathrm{R}}\}$ could, in principle, be directly accessible to in vitro experimental measurements. Therefore, ω_{R} as the product of two observables k and t_{R} can also be deduced from experimental data. Alternatively, it might also be convenient to introduce the equivalent set of three parameters $\{\alpha, \beta, \gamma\}$ that can directly be connected to $\{\widetilde{K}_{\mathrm{M}}, \lambda_{\mathrm{M}}, \omega_{\mathrm{R}}\}$ via:

$$\alpha = k_0 \mathrm{e}^{-\omega_{\mathrm{R}}}, \quad \beta = k_0 \gamma, \quad \gamma = \frac{\alpha}{\widetilde{K}_{\mathrm{M}}}, \quad \frac{\beta}{\gamma} = k_0 = \frac{1}{D_0}, \tag{3.18}$$

to cast (3.15) to the form:

$$[\mathrm{L}(D)] = \frac{\alpha}{\gamma} W_0\left(\gamma D \mathrm{e}^{\beta D/\alpha}\right) . \tag{3.19}$$

3.1 The integrated Michaelis–Menten model for cell survival

Quantity $[\mathrm{L}(D)]$ represents the biological effect in the integrated version (3.15) of the MM formalism and, therefore, the underlying theory will hereafter be called the "Integrated Michaelis–Menten" (IMM) model:

$$[\mathrm{L}(D)] \equiv \mathrm{E}_{\mathrm{B}}^{(\mathrm{IMM})}(D) , \tag{3.20}$$

where,

$$\mathrm{E}_{\mathrm{B}}^{(\mathrm{IMM})}(D) = \frac{\alpha}{\gamma} W_0\left(\gamma D \mathrm{e}^{\beta D/\alpha}\right) . \tag{3.21}$$

Supposing that the Poisson distribution is applicable to a random generation of lesions, the surviving fraction in the IMM model can be written in terms of the three-set parameters $\{\alpha, \beta, \gamma\}$ as:

$$S_{\mathrm{F}}^{(\mathrm{IMM})}(D) = \mathrm{e}^{-\mathrm{E}_{\mathrm{B}}^{(\mathrm{IMM})}} = \mathrm{e}^{-(\alpha/\gamma) W_0(\gamma D \mathrm{e}^{\beta D/\alpha})} . \tag{3.22}$$

The equivalent expressions for the biological effect and cell surviving fraction, given by way of the parameters $\{\widetilde{K}_{\mathrm{M}}, \lambda_{\mathrm{M}}, \omega_{\mathrm{R}}\}$ take their respective forms:

$$\mathrm{E}_{\mathrm{B}}^{(\mathrm{IMM})}(D) = \widetilde{K}_{\mathrm{M}} W_0\left(\lambda_{\mathrm{M}} D \mathrm{e}^{\lambda_{\mathrm{M}} D - \omega_{\mathrm{R}}}\right) , \tag{3.23}$$

$$S_{\mathrm{F}}^{(\mathrm{IMM})}(D) = \mathrm{e}^{-\widetilde{K}_{\mathrm{M}} W_0(\lambda_{\mathrm{M}} D \mathrm{e}^{\lambda_{\mathrm{M}} D - \omega_{\mathrm{R}}})} . \tag{3.24}$$

In the IMM model, the repair time t_{R} plays a very important role, as it is used for differentiating between repairable and irreparable lesions. Thus, if at least one unrepaired lesion is still present at the time t such that $t \geq t_{\mathrm{R}}$, the cell is considered as being dead. This means that enzymes could not repair (i.e. remove) the last remaining lesion at the critical time $t = t_{\mathrm{R}}$. In other words, the IMM model assumes that there is a finite time $t_{\mathrm{R}} < \infty$ for which all the lesions formed at $t < t_{\mathrm{R}}$ must be repaired in order for the irradiated cell (or an organism) to survive. Such a survival is the proof that the repair process by enzyme catalysis was not saturated. Here, the term "saturated" refers to the ability of enzymes to bind to lesions. A situation in which enzymes could not bind any longer to lesions signifies saturation of enzymes with lesions. Hence, if the enzymatic repair is saturated, any remaining lesion would become irreparable (i.e. lethal) and, as such, would yield the cell death with certainty.

For easy and extremely fast computations of the Lambert W_0 function, several freely available and powerful numerical algorithms exist in the literature [24–27]. They have recently been complemented by very accurate analytical expressions for the Lambert

function [28,29]. Such closed formulae are useful in practice not only for the IMM model, but also for the recently proposed "Pool Repair Lambert" (PRL) model [30].

3.2 Low-dose approximation

At small values of the absorbed dose D, variable y_D from (3.16) in (3.15) is also small, in which case we can use the asymptotic formula (2.102) of the Lambert function as $W_0(y_D) \approx y_D(1 - y_D) \approx y_D/(1 + y_D)$. This, together with the accompanying approximation $e^{\lambda_M D} \approx 1 + \lambda_M D$ at small D, will cast the biological effect (3.21) and the surviving fraction (3.22) from the IMM model into the forms:

$$E_B^{(IMM)}(D) \underset{D\to 0}{\approx} E_B^{(PLQ)}(D)\,, \tag{3.25}$$

and,

$$S_F^{(IMM)}(D) \underset{D\to 0}{\approx} S_F^{(PLQ)}(D)\,. \tag{3.26}$$

Here, $E_B^{(PLQ)}(D)$ and $S_F^{(PLQ)}(D)$ are the biological dose and cell surviving fraction in the Padé linear-quadratic, or alternatively, the differential Michaelis–Menten model as abbreviated by PLQ and DMM, respectively:

$$E_B^{(PLQ)}(D) = \frac{\alpha D + \beta D^2}{1 + \gamma D} = E_B^{(DMM)}(D)\,, \tag{3.27}$$

and,

$$S_F^{(PLQ)}(D) = e^{-\frac{\alpha D + \beta D^2}{1+\gamma D}} = S_F^{(DMM)}(D)\,. \tag{3.28}$$

Hence, at low doses, the biological effect $E_B^{(IMM)}(D)$ and the surviving fraction $S_F^{(IMM)}(D)$ in the IMM model coincide with the corresponding quantities $E_B^{(PLQ)}(D)$ or $E_B^{(DMM)}(D)$ and $S_F^{(PLQ)}(D)$ or $S_F^{(DMM)}(D)$ from the PLQ or DMM model.

3.3 High-dose approximation

At high doses D, variable y_D from (3.16) in (3.15) likewise becomes large, so that it is appropriate to employ the asymptote (2.103) for the Lambert function as $W_0(y_D) \approx \ln y_D - \ln(\ln y_D)$ from (2.103). This gives:

$$\begin{aligned} S_F^{(IMM)}(D) &\underset{D\to\infty}{\approx} \left(\frac{\ln y_D}{y_D}\right)^{\widetilde{K}_M} \\ &= \left(\frac{\ln \lambda_M D e^{\lambda_M D - k t_R}}{\lambda_M D e^{\lambda_M D - k t_R}}\right)^{\widetilde{K}_M} \end{aligned}$$

$$= \left(1 - \frac{kt_R}{D} + \frac{\ln \lambda_M D}{\lambda_M D}\right)^{\widetilde{K}_M} e^{v_{max} t_R - k_0 D}$$
$$\approx \left(1 + \frac{\ln \lambda_M D}{\lambda_M D}\right)^{\widetilde{K}_M} e^{v_{max} t_R - k_0 D}$$

so that the high-dose asymptote of the surviving fraction becomes:

$$S_F^{(IMM)}(D) \underset{D\to\infty}{\approx} n \left(1 + \frac{\ln \lambda_M D}{\lambda_M D}\right)^{k_0/\lambda_M} e^{-k_0 D}, \tag{3.29}$$

where,

$$\ln n = v_{max} t_R = \omega_R . \tag{3.30}$$

The corresponding asymptotic form of the biological effect $E_B^{(IMM)}(D) = -\ln S_F^{(IMM)}$ reads as:

$$E_B^{(IMM)}(D) \underset{D\to\infty}{\approx} (k_0 D - \ln n) - \frac{k_0}{\lambda_M} \ln\left(1 + \frac{\ln \lambda_M D}{\lambda_M D}\right). \tag{3.31}$$

The term $ne^{-k_0 D}$ in (3.29) represents the usual proper high-dose asymptote of surviving fractions. There is also the extra multiplicative binomial term $\{1 + (\lambda_M D)^{-1} \ln \lambda_M D\}^{k_0/\lambda_M}$ in (3.29). However, the limiting value of this latter contribution is equal to 1, on account of $\lim_{y\to 1} y^{-1} \ln y = 0$, so that:

$$\lim_{D\to\infty} \left(1 + \frac{\ln \lambda_M D}{\lambda_M D}\right)^{k_0/\lambda_M} = 1, \qquad \lim_{D\to\infty} \frac{\ln \lambda_M D}{\lambda_M D} = 0 . \tag{3.32}$$

As a consequence, the surviving fraction $S_F^{(IMM)}(D)$ exhibits the required correct high-dose asymptotic behavior:

$$S_F^{(IMM)}(D) \underset{D\to\infty}{\approx} ne^{-D/D_0}, \tag{3.33}$$

where $k_0 = 1/D_0$ from (3.13) is used.

3.4 Initial and final slopes

Using (2.106), we can extract the initial (s_i) and final (s_t) slopes $s_i = \lim_{D\to 0} E_B^{(IMM)}(D)$ and $s_f = \lim_{D\to\infty} E_B^{(IMM)}(D)$ in the IMM model as:

$$\text{Initial slope} \equiv s_i = \frac{k_0}{n}, \tag{3.34}$$

and,

$$\text{Final slope} \equiv s_{\mathrm{f}} = k_0 = \frac{1}{D_0}\,, \tag{3.35}$$

where, on account of the relation $n > 1$, we have:

$$\text{Initial slope } (s_{\mathrm{i}}) < \text{Final slope } (s_{\mathrm{f}})\,, \tag{3.36}$$

as it should be.

4 Results and discussion

The performance of the IMM models is presently tested by comparisons with experimental data for cell surviving fractions. The full-effect plots, or the Fe-plots, are also used in these testings. As to surviving fractions, a relatively satisfactory agreement can be obtained at lower doses by using a number of quite different radiobiological models. Simultaneously, however, the same theoretical results when displayed by way of the Fe-plots could exhibit strikingly different behavior. This is most remarkably evidenced within the LQ model which is reasonably accurate for dose-effect curves at small doses, but often flagrantly fails for the Fe-plots. Moreover, even regarding surviving fractions, the LQ model breaks down at larger doses. These features are illustrated in Figs. 1 and 2 where the overall performance of the LQ and IMM models is assessed. It is seen from these figures that the IMM model provides the most favorable agreements with the measurement, as opposed to the LQ model. These illustrations deal explicitly with acute doses that are instantaneously delivered. However, equally remarkable superiority of the IMM model over the LQ model is also encountered when doses are administered through fractions.

5 Conclusions

The ultimate success of radiotherapy will be determined by the way in which cell repair is understood and accordingly incorporated into the modern dose-planning systems, particularly for fractionation schedules with high-dose-per fraction and high-dose rates. This key role of repair originates from the fact that the dose required to inactivate a cell is determined not only by the extent of the primary chemical damage and imparted lesions, but also by the overall capacity of the cell and its microenvironment to recover from the radiation insult and restore the proliferation function.

The presently introduced biophysical theory of cell repair after radiation damage has several notable advantages over the other available radiobiological models. The most important advantage of the proposed Integrated Michaelis–Menten model, or IMM, is its foundation in the well-established second-order Michaelis–Menten chemical kinetics for reactions involving interactions of the cell with radiation. In these

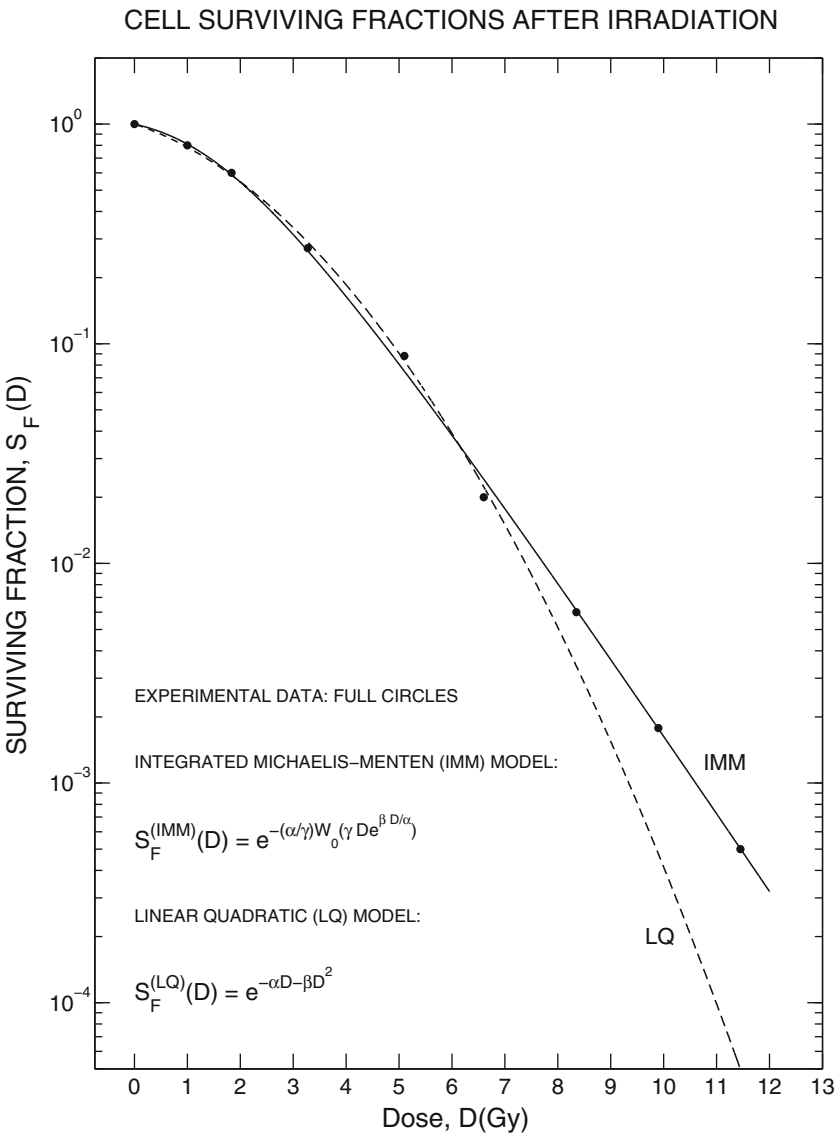

Fig. 1 Cell surviving fractions $S_F(D)$ as a function of radiation dose D in Gy. Experimental data (*symbols*) [31]: the mean clonogenic surviving fractions $S_F(D)$ for Chinese hamster V79 cells irradiated by 50 kVp X-rays. Theories: *full line*: IMM (Integral Michaelis–Menten) model and dashed line: LQ (Linear Quadratic) model

reactions, alongside the readily understood pathway of direct cell inactivation by single radiation events, the channel of damage repair, which plays the most critical role, represents the greatest challenge for radiobiological models. The major goal of the present study is to explore Michaelis–Menten enzyme catalysis as the most efficient mechanism of damage repair. This approach is enhanced by recourse to the recent

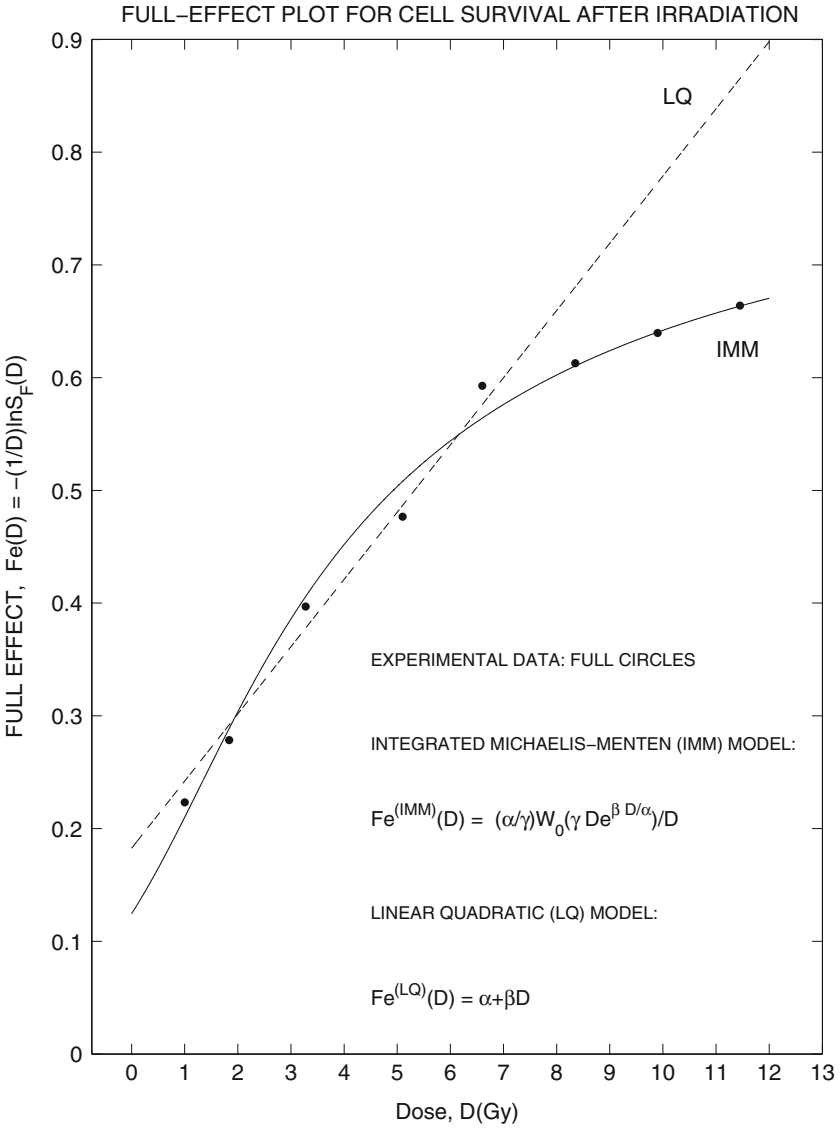

Fig. 2 The Full-effect (Fe) plot from the cell surviving fractions as given by the product of the reciprocal dose $1/D$ and the negative natural logarithm of $S_F(D)$ on the ordinate versus D as the abscissa: $\mathrm{Fe}(D) \equiv -(1/D)\ln(S_F) = R(D)$. Experimental data (*symbols*) [31]: the corresponding values for Chinese hamster V79 cells irradiated by 50 kVp X-rays. Theories: *solid curve*: IMM model and dashed curve: LQ model

advances in applied mathematics through exact, analytical solutions of transcendental equations that represent the integrated rate equations of the underlying chemical kinetics.

The IMM model is valid at any absorbed dose, from low through intermediate to high irradiation exposures. Such a property is particularly important for dose-planning

systems in hypofractionated radiotherapy, such as stereotactic radiotherapy, stereotactic body radiotherapy and high dose-rate brachytherapy. The current dose-planning systems for these newer radiotherapies are still dominated by the linear quadratic model, despite its repeatedly demonstrated inadequacy at high doses. It appears necessary to amend this practice and adopt much more adequate theoretical descriptions for cell survival, such as the IMM model stemming from a clear and proper mechanistic formalism of chemical kinetics for Michalelis-Menten enzyme catalysis of repair of different kinds of radiation damage.

Acknowledgments This work is supported by research grants from Radiumhemmet at the Karolinska University Hospital and the City Council of Stockholm (FoUU) to which the author is grateful.

References

1. L. Michaelis, M.L. Menten, Die kinetik der invertinwirkung. Biochem. Z. **49**, 333–369 (1913) [English translation by R.S. Goody and K.A. Johnson, The kinetics of invertase action. Biochem. **50**, 8264–8269 (2011); Supporting Information: The full text (34 pp) of the German to English translation of the original paper by Michaelis and Menten (1913, op. cit.) available at: http://pubs.acs.org]
2. D.D. van Slyke, G.E. Cullen, The mode of action of urease and of enzymes in general. J. Biol. Chem. **19**, 141–180 (1914)
3. G.E. Briggs, J.B.S. Haldane, A note on the kinetics of enzyme action. Biochem. J. **19**, 338–339 (1925)
4. Dž Belkić, Parametric analysis of time signals and spectra from perspective of quantum physics and chemistry. Adv. Quantum Chem. **61**, 145–260 (2011)
5. Dž Belkić, K. Belkić, Padé-Froissart exact signal-noise separation in nuclear magnetic resonance spectroscopy. J. Phys. B: At. Mol. Opt. Phys. **44**, 125003.1–125003.18 (2011)
6. Dž Belkić, K. Belkić, High-resolution signal processing in magnetic resonance spectroscopy for early cancer diagnostics. Adv. Quantum Chem. **62**, 243–347 (2011)
7. Dž Belkić, K. Belkić, Mechanistic repair-based Padé linear-quadratic model for cell response to radiation damage. Adv. Quantum Chem. **65**, 407–449 (2013)
8. Dž Belkić, K. Belkić, Mechanistic description of survival of irradiated cells: repair kinetics in Padé linear-quadratic or differential Michaelis-Menten model. J. Math. Chem. **51**, 2572–2607 (2013)
9. B. Andisheh, M. Edgren, Dž Belkić, P. Mavroidis, A. Brahme, B.K. Lind, A comparative analysis of radiobiological models for cell surviving fractions at high doses. Technol. Cancer Res. Treat. **12**, 183–192 (2013)
10. A. Sols, R. Marco, Concentrations of metabolites and binding sites: Implications in metabolic regulation. in *Cellular Regulation*, eds. by B. Horecker, E. Stadtman (Academic Press, New York, 1972), **2**, 227–273
11. S. Schnell, P.K. Maini, Enzyme kinetics at high enzyme concentrations. Bull. Math. Biol. **62**, 483–499 (2000)
12. A.R. Tzafriri, Michaelis-Menten kinetics at high enzyme concentration. Bull. Math. Biol. **65**, 1111–1129 (2003)
13. M. Bodenstein, Theorie der photoschemischen reaktionsgeschwindigkeiten. Z. Phys. Chem. **85**, 329–397 (1913)
14. T. Alper, P. Howard-Flanders, Role of oxygen in modifying the radiosensitivity of E. Colli B. Nature **178**, 978–979 (1956)
15. P. Howard-Flanders, T. Alper, The sensitivity of microorganisms to irradiation under controlled glass conditions. Radiat. Res. **7**, 518–543 (1970)
16. T. Alper, *Cellular Radiobiology* (Cambridge University Press, Cambridge, 1979)
17. D.L. Dewey, Effect of glycerine on the X-ray sensitivity of serratia marcescens. Nature **187**, 1008–1010 (1960)
18. J.H. Lambert, Observationes varie in mathesin puram. Acta Helvetica, Physico-mathematico-anatomico-botanico-medica, Basel **3**, 128–168 (1758)
19. R.M. Corless, G.H. Gonnet, D.E.G. Hare, D.J. Jeffrey, D.E. Knuth, On the Lambert *W* function. Adv. Comput. Math. **5**, 329–359 (1996)

20. J. Calkins, A method of analysis of radiation response based on enzyme kinetics. Radiat. Res. **45**, 50–52 (1971)
21. J. Calkins, Alternative repair models capable of generating shouldered dose-response curves. Int. J. Radiat. Biol. **59**, 997–999 (1991)
22. Dž Belkić, *Quantum Theory of Spectral Analysis and Signal Processing* (Taylor & Francis, London, 2005)
23. W.H. Press, S.A. Teukolsky, W.T. Vetterling, B.P. Flannery, *Numerical Recipes*, 2nd edn. (Cambridge University Press, Cambridge, 1992)
24. F.N. Fritsch, R.E. Shafer, W.P. Crowley, Solution of transcendental equation $we^w = x$ algorithm 443: function $WEW(x)$.. Commun. Assoc. Comput. Mach. **16**, 123–124 (1973)
25. D.A. Barry, P.J. Culligan-Hensley, S.J. Barry, Real values of the W-function. Assoc. Comput. Mach. Trans. Math. Softw. **21**, 161–171 (1995)
26. D.A. Barry, S.J. Barry, P.J. Culligan-Hensley. Algorithm 743: WAPR: A FORTRAN routine for calculating real values of the W-function. Assoc. Comput. Mach. Trans. Math. Softw. **21**, 172–181 (1995) [Web reference for free downloading both fortran (wapr.f) and matlab (wapr.m) programs: http://www.netlib.org/toms/743]
27. D. Veberič, Having fun with Lambert $W(x)$ function, arXiv:1003.1628v1 [cs.MS], 15pp, Last updated: 8 March 2010
28. S. Winitzki, Uniform approximations for transcendental functions, in *Lecture Notes in Computer Science*, Nr. 2667, eds. V. Kumar, M.L. Gavrilova, C.J.K. Tan, P. L'Ecuyer (Springer, Berlin, 2003), pp. 780–789
29. Dž. Belkić, Theory and practice with the transcendental Lambert W function in interdisciplinary research: introduction of a highly accurate single analytical formula. J. Math. Chem. (2013, submitted)
30. Dž. Belkić, Survival of radiation-damaged cells via mechanism of repair by pool molecules: the Lambert function as the exact analytical solution of coupled kinetic equations. J. Math. Chem. doi:10.1007/s10910-014-0307-3
31. M.M. Elkind, H. Sutton, Radiation response to mammalian cell growth in culture: I. Repair of X-ray damage in surviving Chinese Hamster cells. Rad. Res. **13**, 556–593 (1960)