On the status of the Michaelis-Menten equation and its implications for enzymology

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The Michaelis-Menten equation (MME) is considered to be the fundamental equation describing the rates of enzyme-catalysed reactions, and thus the 'physico-chemical key' to understanding all life processes.[1,2] It is the basis of the current view of enzymes as generally proteinaceous macromolecules that bind the substrate reversibly at the active site, and convert it to the product in a relatively slow overall sequence of bonding changes ('turnover'). The manifested 'saturation kinetics', by which the rate of the enzymic reaction (essentially) increases linearly with the substrate concentration ([*S*]) at low [*S*] but reaches a plateau at high [*S*], is apparently modelled by the MME. However, it is argued herein that the apparent success of the MME is misleading, and that it is fundamentally flawed by its equilibrium-based derivation (as can be shown mathematically). Thus, the MME cannot be classed as a formal kinetic equation *vis-à-vis* the law of mass action, as it does not involve the 'incipient concentrations' of enzyme and substrate; indeed, it is inapplicable to the reversible interconversion of substrate and product, not leading to the expected thermodynamic equilibrium constant. Furthermore, the principles of chemical reactivity do not necessarily lead from the above two-step model of enzyme catalysis to the observed 'saturation kinetics': other assumptions are needed, plausibly the inhibition of product release by the substrate itself. (Ironically, thus, the dramatic graphical representation of the

MME encrypts its own fundamental flaw!) Perhaps the simplest indictment of the MME, however, lies in its formulation that the rate of the enzymic reaction tends towards a maximum of $k_{cat}[E_o]$ in the saturation regime. This implies – implausibly – that the turnover rate constant k_{cat} can be known from the overall rate, but independently of the dissociation constant (K_M) of the binding step. (Many of these arguments have been presented previously in preliminary form.[3])

The original formulation of the MME, based on the reaction scheme in Fig. 1, is shown in equation (1). Its derivation is based on three distinct steps:[1-4] defining the overall rate, *v*, as the product of the turnover number and the concentration of the enzyme-substrate complex *ES* [equation (2)]; defining the initial 'pre-equilibrium' formation of *ES via* the Michaelis constant K_M [equation (3)]; expressing the equilibrium concentration [ES_{eq}] as a fraction of the total enzyme concentration [E_o], *via* K_M and the equilibrium substrate concentration [S_{eq}] [equation (4)]. The MME can also be formulated in terms of the free enzyme concentration, [E_{eq}], as in equation (5) [from equations (2) and (3)]. Note in particular that [E_{eq}] and [S_{eq}] refer to equilibrium values, arising upon reversible formation of *ES*, and that *v* is the initial rate.

However, the rate equation for an enzyme catalysed reaction may also be derived from the fundamental principles of chemical kinetics, essentially comprising the classical law of mass action and modern transition state theory.[5,6] Accordingly, the overall rate constant for the enzyme catalysed reaction would be (k_{cat}/K_M), as may be formally derived from the overall Gibbs free energy of activation and the Eyring equation (*cf.* Supplementary Information). If the 'incipient' concentrations of enzyme and substrate, *i.e.* at any given moment of time, are [*E*] and [*S*] respectively, the overall rate of the enzymic reaction *v* is given by equation (6). (*v* is defined as the rate of decrease of [*S*] with time 't', *i.e.* -d[*S*]/dt, noting that d[*E*]/dt = 0, as *E* is regenerated in

the reaction.) Thus, equation (6) represents the general and formally correct rate equation for an enzyme catalysed reaction, based on the scheme in Fig. 1.

$$v = k_{cat}[E_o][S_{eq}]/(K_M + [S_{eq}]) \quad (1)$$

$$v = k_{cat}[ES_{eq}] \quad (2)$$

$$[ES_{eq}] = [E_{eq}][S_{eq}]/K_M \quad (3)$$

$$[ES_{eq}] = \{[S_{eq}]/(K_M + [S_{eq}])\}[E_o] \quad (4)$$

$$v = (k_{cat}/K_M)[E_{eq}][S_{eq}] \quad (5)$$

$$v = -d[S]/dt = (k_{cat}/K_M)[E][S] \quad (6)$$

$$v = (k_{cat}/K_M)[E_o][S_o] \quad (7)$$

$$v_{ES} = -d[ES_{eq}]/dt = -(1/K_M)d([E_{eq}][S_{eq}])/dt =$$

$$-(1/K_M)\{([E_{eq}]d[S_{eq}]/dt) + ([S_{eq}]d[E_{eq}]/dt)\} \neq -d[S]/dt \quad (8)$$

$$K = [P]/[S] = (k_{cat}^{f}/K_M^{f})/(k_{cat}^{r}/K_M^{r}) \quad (9)$$

$$k_{cat}^{f} = k_{cat}^{r} \quad (10)$$

A comparison of equation (6) with the MME formulations [equations (1) and (5)] is instructive. Consider the rate of reaction at the very instant of mixing enzyme and substrate, *i.e.* before the formation of *ES*; since all enzyme and substrate are unbound and free, their concentrations may be represented as $[E_o]$ and $[S_o]$ respectively. The overall rate [*cf.* equation (6)] would then be given by equation (7).

Equation (7) is clearly at variance with equations (1) and (5). Thus, as $[E_o] > [E_{eq}]$ and $[S_o] > [S_{eq}]$, the MME rate is less than that predicted by equation (7). [(This is glaringly clear in the case of equation (5), but also apparent in the case of equation (1), as $[S_o] >> [S_{eq}]/(K_M + [S_{eq}])$]. The discrepancy between the formally correct relation equation (7), and the MME relations equations (1) and (5), is intriguing, but firmly invalidates the MME.

Note that $[E_o]$ and $[S_o]$ are the known and measured values to be related to the initial rate v. Also, although $[S_o] \sim [S_{eq}]$, $[E_o] >> [E_{eq}]$ (as the substrate is in considerable excess of the enzyme); thus, large errors are involved in employing $[E_{eq}]$ instead of $[E_o]$ [*cf.* equations (1) and (5)]. Furthermore, although the rate v may – in practice – be measured upon equilibration of the substrate and enzyme, $[E_{eq}]$ remains unknown, so the left and right hand sides of equations (1) and (5) would not correspond.

The above conundrum, apparently, may be traced to the equilibrium-based derivation of the MME, which suffers from the following flaws. The key assumption that the overall rate v is equal to the rate of decomposition of ES (v_{ES}), is seen to be invalid by differentiating the equilibrium expression for $[ES_{eq}]$ with respect to time [*cf.* equations (3) and (8), and Supplementary Information]:[7] thus, v is defined as the rate of disappearance of S, but this is not equal to v_{ES}. Clearly, equation (5) is highly misleading and the key source of the confusion: importantly, $[ES_{eq}]$ derives from 'initial' concentrations of E and S that do not correspond to $[E_{eq}]$ and $[S_{eq}]$.

Equation (2) also does not reflect the linked equilibrium between E, S and ES. Thus, ES is continuously replenished as it reacts (by E and S), a feature not captured by equation (2). In a hypothetical case in which ES is 'isolated' from E and S, the rate of turnover of ES would still be given by equation (2)! Also, in view of the above invalidation of equation (2), it is clear that the MME essentially reflects only the dependence of $[ES]$ on $[S]$ (k_{cat} being of no particular significance)!

The 'saturation kinetics', apparently modelled by the MME, is also to be viewed in this light (*cf.* Fig. 2). Thus, $[ES]$ would indeed tend towards a maximum of $[E_o]$ [*cf.* equation (4)], but in the absence of any further reaction: intriguingly, therefore, there is no causal relationship between the asymptotic behaviour of $[ES]$ and the overall rate,

with increasing [*S*]. (The possible origin of the observed 'saturation kinetics' is discussed below.)

In fact, equations (6) and (7) also imply that neither k_{cat} nor K_M can be derived independently of the other, from the overall rate *v*. This invalidates an important practical application claimed by the MME, *i.e.* the purported derivation of k_{cat} in the 'saturation regime'. Thus, the currently determined values of k_{cat} and K_M apparently possess no rigorous basis.

The MME is also inapplicable under conditions of overall equilibrium between substrate and product. Thus, equation (1) does not lead to the thermodynamic equilibrium constant (*K*), which can be reached from equation (6). This is shown in equation (9) (the superscripts *f* and *r* referring to the forward and reverse reactions respectively). Insofar as the rate expressions for the forward and reverse reactions must lead to the equilibrium constant, the MME is thus invalidated. [In fact, in the 'saturation regime' under conditions of reversibility, the MME leads to the absurd result shown in equation (10).]

Interestingly, equation (7) *per se* does not lead to the 'saturation' kinetics normally observed in enzyme catalysed reactions (Fig. 2). Indeed, a second order enzyme catalysed reaction (*cf.* Fig. 1) would become pseudo-first order in [*E*] at high [*S*]: this, however, does not imply that the rate becomes invariant with [*S*]! [It can be shown that the rate then ~ $(k_{cat}/K_M)[S_o][E_o]$, *cf.* Supplementary Information.[8] Note that the saturation idea is even less likely when one of the reactants is regenerated, as in the enzymic case!]

The saturation idea is also seen to be invalid qualitatively as follows. Increasing [*S*] would lead to a proportionate increase in [*ES*], the turnover number and the overall

rate *v*: as this would produce free enzyme, there would be no 'saturation'. Clearly, therefore, the experimentally observed invariance of rate at high [*S*] must have a basis other than 'saturation' of the enzyme active site.

The observed kinetics (*cf.* Fig. 2) implies that the enzyme catalysed reaction is inhibited at high [*S*]. A possible explanation could be that there exists a secondary site adjacent to the active site at which the substrate binds relatively weakly. At high [*S*] a second molecule of substrate could bind at this site, and sterically hinder the release of the product and the regeneration of the free enzyme.

Thus, the reaction sequence encounters a fork at *EP*, because of the presence of two kinetically competing pathways: formation of the final product *P* (along with free enzyme *E*), and weak binding of substrate at the secondary site to form the complex *S*--*EP* (Scheme 3 and Fig. 4). In *S*--*EP* release of product and free enzyme are sterically hindered, so it can only revert to *EP* and *S*.

It can be shown that, under these conditions, the rate of the enzyme catalysed reaction tends towards a maximum constant value of $(k_{cat}/K_M)(k_1/k_2)[E_o]$, where k_1 and k_2 are the rate constants for the conversion of *EP* to *P* and *S*--*EP* respectively. (The steady state approximation is employed for this derivation, *cf.* Supplementary Information; however, the problems involving the *ES* complex in the MME derivation do not apply here.) Although this is an unproven mechanism, it is in accord with fundamental principles of chemical reactivity. Thus, the invalidation of the MME has a far-reaching practical consequence, in suggesting a fundamental reappraisal of the general mechanism of enzyme catalysis.

It is noteworthy that the equilibrium-based approach in general, and the 'saturation' idea in particular, militate against the principles of transition state theory.[5,6]

Accordingly, the path taken by the reactants to reach the transition state – and the intermediates encountered along the way – are inconsequential to the overall rate of the enzyme catalysed reaction: this cannot be related to the existence of *ES* in any way. (The law of mass action requires the overall rate to be related to the starting concentrations of the substrate and enzyme, a stage at which *ES* has not formed at all.)

It is also noteworthy that the MME was formulated much before the currently accepted principles of chemical kinetics were developed.[4] All the same, it is particularly ironic that a flawed derivation – by apparently modelling the observed 'saturation' phenomenon – directed chemical biology along a fruitless course.

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Legends for Figures

Figure 1. The two-step sequence of an enzyme catalysed reaction. The relatively rapid formation of the enzyme-substrate complex (*ES*) from the substrate (*S*) and enzyme (*E*), is followed by the slow conversion of *ES* to the final product *P* (*E* being regenerated as shown).

Figure 2. The dependence of the overall rate of an enzyme catalysed reaction (*v*) on the substrate concentration ([*S*]), as experimentally observed ('saturation' kinetics).

Figure 3. The possible origin of the observed 'saturation' kinetics in enzyme catalysis. An additional molecule of substrate *S* binds adjacent to the active site in the initially formed enzyme-product complex *EP*, forming the weak complex *S--EP* in which the release of product and free enzyme is sterically inhibited. The formation of *S--EP* competes with the release of product and free enzyme, thus producing the observed 'saturation' kinetics.

Figure 4. Energy profile diagram for the reaction sequence in Fig. 3, representing the proposed inhibition of an enzyme catalysed reaction at high [*S*]. The effect originates in the formation of the weak complex *S--EP*, *via* the competitive binding of an additional molecule of substrate adjacent to the active site in the enzyme-product complex *EP*. In *S--EP* the release of product and free enzyme are sterically hindered. (*ES* is the enzyme-substrate complex, and TS_{EC} represents the rate determining transition state for the overall reaction.

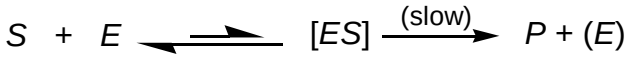

Figure 1.

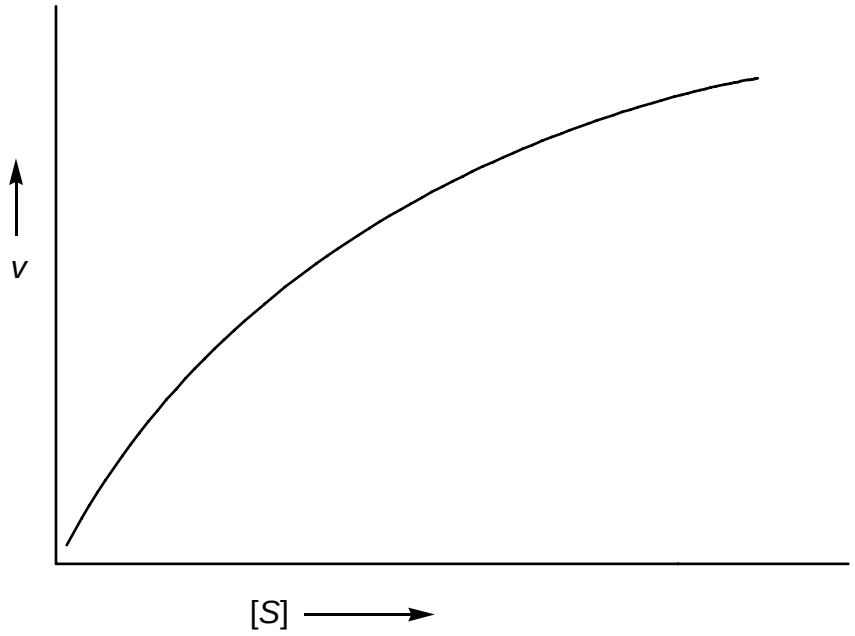

Figure 2.

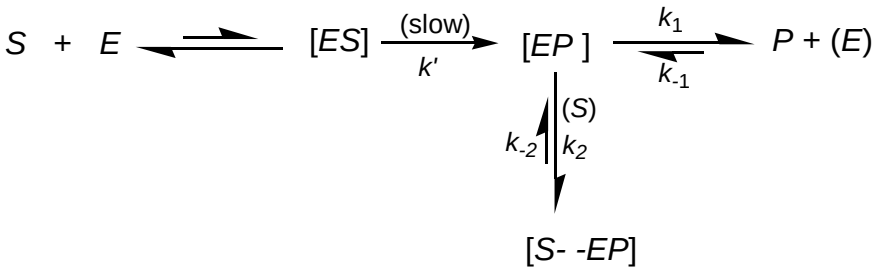

Figure 3

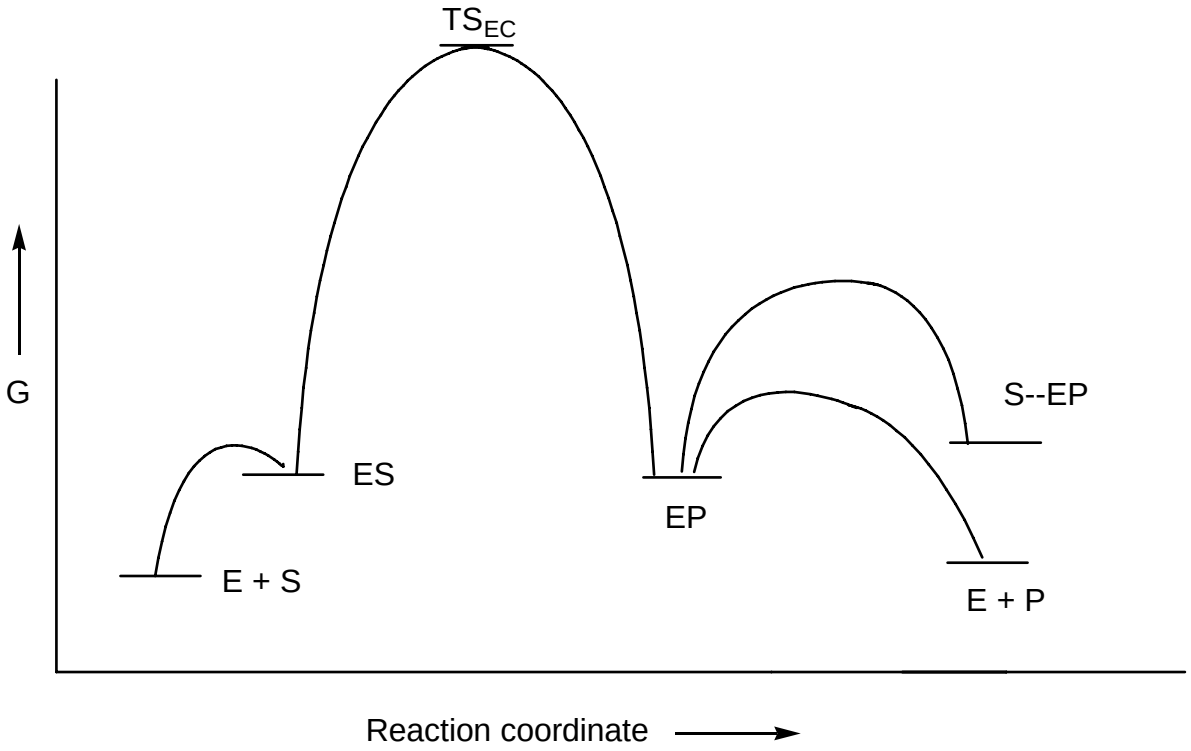

Figure 4