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(RESEARCH ARTICLE)

Association-dissociation equations, distinct from Michaelis-Menten equation for the quantification of the net flux of reactants with or without immobiliser.

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Abstract

The formation of enzyme-substrate complex, often in connection with the adsorption of the enzyme leading to either partial immobilisation in which the enzymes are adsorbed on a colloid or total immobilisation in which the enzyme is adsorbed on a rigid immobile phase is the concern of some researchers. The interest in immobilised substrate common in biological system is not very common. The objectives of this theoretical research are the rederivation of the equations of association and dissociation of reactants in the presence of adsorbents, insoluble larger macro-or supra-molecule and elucidation of why such equations are important and generalisable. The derivations produced two different equations that describe mathematically the net flux of either the substrate where the enzyme is adsorbed or the net flux of the enzyme where the substrate is adsorbed. The derivation also produced equations of translational velocities, given the probabilities that reactions occur following complex formation or that an escape of bullet molecules or dissociation reactions occur. In conclusion two different equations need separate derivation for association and dissociation of reactants. The needs for the flux of reactants have both biological and industrial relevance, respectively due to importance of time-dependent digestive processes and for the optimisation of the production of desired products of enzymatic action. The equations describing net flux seem generalisable in that information about the physicochemical properties of both crowding agent and immobilisers may not be needed for calculations.

Keywords: Dissociation-association interaction equations; Approach and escape translational velocities; Probabilities of reaction and an escape; Net flux of reactants; Importance and generalisability of equations.

1. Introduction

Many years ago (with due respect to the researcher whose work is in 1970 is to my surprise still in active research.), Shurr [1] published a paper describing the effect of the adsorption of enzyme on larger spherical particles via mathematical modeling. However, the mathematical equations arising from such modeling need to be adapted to biochemical and abiotic situations in quantitative manner. Hence an association-dissociation equations distinct from the usual Michaelis-Menten equation need to be derived for the quantification of the net flux of reactants in the presence and absence of an immobiliser or adsorbent. The paper by Shurr [1] could be of biological and industrial relevance. The biological perspective, one of the interests in this research, stems from the fact that in the gastrointestinal tract (GIT), the stomach 1st, and the small intestine 2nd, there are complex mixtures of biomolecules of different degrees of complexity. The dieticians (or nutritionist) appreciate the fact that a meal described as balanced diet contains sources

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of carbohydrate, protein, fat *etc*. Bypassing the oral cavity does not preclude the observation that the first-pass digestion of the carbohydrate component of the diet is in the mouth.

The interest in the stability of enzymes for industrial application has increased in recent time [2]. Thus, the major challenge in an enzymatic process is the instability of the enzyme under repetitive or prolonged use and inhibition by high substrate and product concentration [3]. An "immobilisation is a very effective alternative in overcoming problems of instability and repetitive uses of enzymes" [3]. Similar to this issue is the phenomenon of biological sequestration [4-6]. Sequestration simply "arrests" a protein and removes it from circulation within the medium of biological activity. There is a view that enzyme sequestration in a given motif enables it to generate both ultrasensitive and adaptive dynamics under biologically relevant parameter regimes. While acknowledging that the dynamics of such a motif can be tuned between adaptive and ultrasensitive responses through modulation of the concentration or kinetic parameters of the sequestrating protein [4], it needs to be made clear that it is not only proteins as scaffoldings that can sequestrate, but other macromolecules that has strong binding affinity with the enzyme or protein in general. There could be transient self-sequestration due to favourable orientational alignment. An enzyme can be sequestrated by its own much larger molecular weight substrate [7, 8]. The observed signaling networks resulting from combinatorial interactions among many enzymes and scaffolding proteins [4] may be applicable to the interactions between masticated food components, large molecular weight carbohydrate molecules, masticated fibres, and existing enzyme (pepsin) molecules in the stomach. This suggestion is anchored on the fact that if existing pepsin molecules are sequestrated by ingested masticated food, a "feeling" of deficit or at least a concentration gradient may be created which may activate the protein synthetic machinery for the synthesis of the needed enzyme (there is no doubt a single protein diet alone can trigger the synthesis of the needed enzyme in the stomach.). This line of thought seems to be opposite of the view that the substrate can also be a sequestrated by the enzyme [5] but in line with the claim elsewhere of the possibility of substrate enzyme-sequestration analysed from deterministic and a more difficult and complex stochastic perspective (being in the domain of chemical master equation) [6]. The objectives of this research are 1) to rederive the equations of association and dissociation of reactants in the presence of adsorbents, insoluble larger macro-or supra-molecule and 2) elucidate why such equations are important and generalisable.

2. Theory

In this section preliminary issues regarding ingestion of masticated food, distribution of secreted enzyme in the stomach, binding to the masticated food seen as "colloid" and the development of alternative mathematical model are embarked upon.

2.1. Preliminary facts

The digestion of the proteins begins in the stomach even in the presence of non-protein components, the carbohydrate component for instance. Thus the presence of masticated food, seen as a form of pseudo-colloid, in the stomach, constitutes a medium in which, in the presence of ingested water, the secreted enzyme, pepsin, can diffuse to the surface of the carbohydrate components, interact and bind. It is therefore, likely that a fraction (γ) of the total enzyme molecules bind on a fraction (α) of the total surface area of the food colloid, leaving $1-\gamma$ as the fraction forming enzyme substrate complex. There is a need to add too, that, the protein component of food could also bind strongly to other food components, while the incoming enzyme, the pepsin molecules are mobile. Thus, there could be two models, one for which some of the enzyme molecules are bound, a part of which is free, while the remaining formed enzyme substrate complex, and the other, for which the substrate (e.g. protein) is bound on other diet components, while the enzyme, pepsin in the stomach are in free motion. By seeing the colloid (which for the purpose of this research is dispersed masticated food in the stomach) as a single multivalent reacting species A at a concentration C_{A}^{0} (spheres/m^3), one, in the same vein, can see the concentration of substrate molecules near the surface of presumed colloidal sphere in number density given symbolically as $C_S(r_0)$. A modified (alternative) approach is hereby presented as follows.

2.2. Alternative Approach

Before proceeding, it must be made clear that the fixation of the enzyme molecules on a stationary material (perhaps a rigid adsorbent) constitutes immobilisation of the enzyme. This is unlike the fixation of an enzyme on a mobile colloid. In other words the enzyme molecule is "vehicularly mobile". Given that the substrate number density, the number of molecules concentration per litre is $C_S(r_0)$ near the surface of the sphere (colloid), rate of association between the substrate and the adhered enzyme is given by the total inward flux ($\mathcal{F}_{\mathrm{in}}$) of substrate molecules across the reaction surface provided by the food colloid. Thus, similar to information in the literature in which the enzyme molecules are regarded as being bound on the colloidal particles while the substrate molecules are in a direct free motion [1], is the following equation

$$\mathcal{F}_{in} = 4\,\pi\, r_0^2\, P\, \bar{\nabla}_{T(+)}\, C_A^0\, C_S(r_0)\, \alpha\, \gamma\, /\, 2 \quad (1)$$

Equation (1), where r_0, P, and $\bar{\nabla}_{T(+)}$ are the hydrodynamic radius of the colloid, the probability of reaction per collision and mean thermal approach translational velocity, seems very plausible because the number of molecules of the substrate which flux could be very large even if the molar concentration is in Pico scale such that its product with Avogadro's number is always expectedly » 1. However, the 2nd order rate (k_1) constant equation for such a system presented here unlike in the literature [1] includes a constant. Thus,

$$k_1 = 4\,\pi\, R^2\, k_{1(0)}\, P\, \bar{\nabla}_{T(+)}/2 \quad (2)$$

Where, $k_{1(0)}$ and R are the introduced constant whose unit is suggested to be 1/mol considering the fact that the unit of the intrinsic 2nd order rate constant, k_1 is (L / mol) /minute and the sum of the particle radius of the protein (R_E) and substrate (R_S). There is a need to point out the fact that both enzyme and substrate either as gelatinised starch molecules or as starch granules are in a state of direct flux unlike a situation where either the enzyme or substrate is adsorbed on the surface of a colloid which acts as a vehicle for the random motion of the macromolecules. The constant, $k_{1(0)}$, is taken to be » k_1 in magnitude albeit, speculatively, considering the fact that in most, if not all, enzyme catalysed reaction, with Michaelian characteristics, the 2nd order rate constant is usually very high, approaching, and in most cases several folds larger than a mega scale.

Research elsewhere [9] has shown that the translational velocity of a solute in solution is far lower than the mean root mean square velocity ($u_{(rms)}$) ascribable to matter in gas phase; to assume otherwise would imply that a cell can dissolve and dissociates into micro- and macro-molecular components and reassemble into a supramolecular structure in what may be described as an unguided "reverse evolution". Indeed dissolution produces an instantaneous translational velocity followed by a tendency to lower terminal velocity resulting from solvent resistance [9]. The translational terminal velocity ($u_{(ter)}$) is « translational instantaneous velocity ($u_{(int)}$). Both are much lower than $u_{(rms)}$. Therefore, considering that R is several folds less than the micro scale, its product with $\bar{\nabla}_{T(+)}$ (the equivalent of $u_{(int)}$) and p (this being <1 but » milli scale) gives the impression that k_1 is « femto scale in magnitude. This is contrary to recent results which showed that k_1 is higher than the effective or apparent 2nd order constant [10]. Thus, this may justify the presence of $k_{1(0)}$ in Eq. (2) and the issue raised about it. From Eq. (2), $P\, \bar{\nabla}_{T(+)} = 2\, k_1/4\,\pi\, R^2\, k_{1(0)}$, and substituting same into Eq. (1) gives:

$$\mathcal{F}_{in} = r_0^2 C_A^0\, C_S(r_0)\, \alpha\, \gamma\, k_1\, /k_{1(0)}\, R^2 \quad (3)$$

For the outward flux ($\mathcal{F}_{out}$), the equation is given as

$$\mathcal{F}_{out} = \frac{P'\, \bar{\nabla}_{T(-)}}{2} \frac{3}{4\,\pi\, R^3} (\alpha(1-\gamma)\, 4\,\pi\, r_0^2 C_A^0) \quad (4)$$

Here, unlike in the literature [1], a constant is introduced to give intrinsic 1st order rate constant k_2, as follows:

$$k_2 = 3\, k_{2(0)}\, P'\, \bar{\nabla}_{T(-)}\, /2R \quad (5)$$

Where, $k_{2(0)}$ is the dimensionless constant. Thus,

$$P'\, \bar{\nabla}_{T(-)} = 2\, k_2\, R\, /\, 3\, k_{2(0)} \quad (6)$$

Substituting Eq. (6) into Eq. (4) gives

$$\mathcal{F}_{out} = \frac{r_0^2\, k_2\, \alpha\, (1-\gamma)\, C_A^0}{R^2\, k_{2(0)}} \quad (7)$$

2.3. Mathematical equations of flux for the two cases, bound *i.e.* Adsorbed enzyme and substrate.

Although the equations that may be stated or derived may assume the same form, they are likely to give different result because different molecular species are involved in either being mobile or immobilised. Therefore, there is a need to derive separate equations subsequently.

2.3.1. The case in which the enzyme is bound.

The expression for the net reaction rate per unit concentration of spheres is thus, given as:

$$\frac{\mathcal{F}_{\text{in}} - \mathcal{F}_{\text{out}}}{C_{\text{A}}^{0}} = \frac{[S_0]\,(1 - \exp\,(-k\,t))\,N_{\text{A}}}{M_3\,C_{\text{A}}^{0}\,t} = \frac{r_0^2 C_{\text{A}}^{0}\,C_{\text{S}}(r_0)\,\alpha\,\gamma\,k_1/k_{1(0)}\,R^2 - \frac{r_0^2\,k_2\,\alpha\,(1-\gamma)\,C_{\text{A}}^{0}}{R^2\,k_{2(0)}}}{C_{\text{A}}^{0}} \tag{8a}$$

Where, M_3 is the molar mass of the substrate. The time, t (duration of assay) is introduced based on the assumption that the rate of utilisation of the substrate should be equal to the net reaction rate per unit concentration of sphere. Equation (8a) simplifies to give

$$\frac{[S_0]\,(1 - \exp\,(-k\,t))\,N_{\text{A}}}{M_3\;t} = r_0^2 C_{\text{A}}^{0}\;\;C_{\text{S}}(r_0)\,\alpha\,\gamma\,k_1/k_{1(0)}\,R^2\;-\;\frac{r_0^2\,k_2\,\alpha\,(1-\gamma)\,C_{\text{A}}^{0}}{R^2 k_{2(0)}} \tag{8b}$$

Equation (8b) results from the assumption that at distance r_0 (the value of $r_0 \gg R$) from the surface of the colloid the number density of the substrate may be the same as in the bulk (or at least $C_{\text{S}}(bulk) \approx C_{\text{S}}(r_0)$); t and k are the duration of assay and the pseudo-first order rate constant for the hydrolysis of the substrate.

Meanwhile,

$$\alpha = \frac{N_{\text{A}} n_{\text{E}(0)}\,R_{\text{E}}^2}{C_{\text{A}}^{0}\,r_0^2} \tag{9}$$

Where, $n_{\text{E}(0)}$ and N_{A} are the number of moles of the enzyme per cubic litre and Avogadro number respectively. Since, from Eq. (9), $\alpha\,C_{\text{A}}^{0}\,r_0^2 = N_{\text{A}} n_{\text{E}(0)}\,R_{\text{E}}^2$, then,

$$\gamma = \frac{N_{\text{A}} n_{\text{f}}\,R_{\text{E}}^2}{\alpha\;C_{\text{A}}^{0}\,r_0^2}\;\text{Or}\;\frac{n_{\text{f}}}{n_{E(0)}} \tag{10}$$

Where n_{f} is the number of moles of free enzyme per unit volume (volume in litre is to be converted to cubic metre in this research where elucidation is needed.)

Therefore,

$$\alpha\,\gamma = \frac{N_{\text{A}} n_{\text{f}}\,R_{\text{E}}^2}{C_{\text{A}}^{0}\,r_0^2} \tag{11}$$

Also,

$$1 - \gamma = 1 - \frac{N_{\text{A}} n_{\text{f}}\,R_{\text{E}}^2}{\alpha\;C_{\text{A}}^{0} r_0^2} \tag{12}$$

Another possibility is that

$$\alpha\,(1 - \gamma) = \alpha - \frac{N_{\text{A}} n_{\text{f}}\,R_{\text{E}}^2}{C_{\text{A}}^{0}\,r_0^2} \tag{13a}$$

Substitution of Eq. (9) into Eq. (13a) and simplification give

$$\alpha\,(1 - \gamma) = \frac{N_{\text{A}}\,R_{\text{E}}^2}{C_{\text{A}}^{0}\,r_0^2}\left(n_{\text{E}(0)} - n_{\text{f}}\right) \tag{13b}$$

From Eq. (10),

$$C_{\text{A}}^{0} = \frac{N_{\text{A}} n_{\text{f}}\,R_{\text{E}}^2}{\alpha\,\gamma\,r_0^2}. \tag{14}$$

Substitution of Eqs (11) and (13b) into Eq. (8b) and simplification gives

$$\frac{[S_0](1-\exp(-k\,t))\,N_A}{M_3\,n_f\,t} = \frac{N_A\,R_E^2\,k_1\,C_S(r_0)}{k_{1(0)}\,R^2} - \frac{N_A\,R_E^2\,k_2}{k_{2(0)}\,R^2}\frac{(n_{E(0)}-n_f)}{n_f} \tag{15a}$$

Where, as usual, n_f = ([E(0)] − [ES]) where [E(0)] and [ES]) are the concentration of the enzyme at time equal to zero, and the concentration of enzyme-substrate complex, all in moles per litre. Equation (15a) seems to be a general equation applicable to reaction mixture containing very large insoluble gelatinised substrate (starch) and much smaller enzymes. One needs to understand that given a mixture of the colloidal particles, the substrate, and the enzyme, there is a possibility that some enzyme molecules may be in the bulk, if the number of enzyme molecules is overwhelmingly larger than the number of colloidal particles even if each colloid particle can speculatively hold up to 2–3 enzyme molecules. One needs to note that where either the substrate or enzyme is adsorbed on the colloid, the macromolecules can be said to be in an indirect Brownian motion since they are being transported "vehicularly" by the colloid particles.

"A simple step-by-step approach is intended to reach out to all and sundry being the essence of communication". Thus, for the purpose of computational convenience, $C_S(r_0)$, which can be rewritten as [S_0] N_A/M_3 is substituted into Eq. (15a) and simplified further, to give

$$\frac{(1-\exp(-k\,t))}{n_f\,t} = \frac{N_A\,R_E^2\,k_1}{k_{1(0)}\,R^2} - \frac{R_E^2\,M_3\,k_2}{k_{2(0)}\,R^2[S_0]}\frac{(n_{E(0)}-n_f)}{n_f} \tag{15b}$$

A plot of $(1-\exp(-k\,t))/n_f\,t$ versus $(n_{E(0)}-n_f)/[S_0]\,n_f$ should give slope (S_{lope}) and intercept I_{nt} from where, $k_{2(0)}$ and $k_{1(0)}$ respectively can be calculated.

The following are derivable from the plot of $(1-\exp(-k\,t))/n_f t$ versus $(n_{E(0)}-n_f)/[S_0]\,n_f$ in Eq. (15b).

$$k_{2(0)} = \frac{R_E^2\,M_3\,k_2}{R^2\,S_{lope}} \tag{16}$$

Substitution of Eq. (16) into Eq. (6) eliminates the intrinsic 1st order rate constant, k_2 to give

$$P' = \frac{2\,R^3\,S_{lope}}{3\,R_E^2\,M_3\,\bar{v}_{T(-)}} \tag{17}$$

If other parameters are known, R can be determined using the following equation.

$$R = \sqrt[3]{\frac{3\,R_E^2\,M_3P'\,\bar{v}_{T(-)}}{2\,S_{lope}}} \tag{18}$$

The equation for $k_{1(0)}$ can be derived from the intercepts as follows.

$$k_{1(0)} = \frac{N_A\,R_E^2\,k_1}{R^2\,I_{int}} \tag{19}$$

Where, I_{nt} is the intercept. Substitution of Eq. (18) into Eq. (19) gives another equation for $k_{1(0)}$.

$$k_{1(0)} = \frac{N_A\,R_E^2\,k_1}{I_{nt}}\left(\frac{2\,S_{lope}}{3\,R_E^2\,M_3P'\,\bar{v}_{T(-)}}\right)^{2/3} \tag{20a}$$

Equation (20a) simplifies to

$$k_{1(0)} = \frac{0.763143\,N_A k_1\sqrt[3]{R_E^2\left(\frac{S_{lope}}{P'\bar{v}_{T(-)}M_3}\right)^2}}{I_{nt}} \tag{20b}$$

Meanwhile, substituting Eq. (20 b) into Eq. (2) eliminates the 2nd order intrinsic rate constant, such that P can be given as

$$P = \frac{0.655185\, I_{\mathrm{nt}}}{\pi\, R^2\, \bar{\nabla}_{\mathrm{T}(+)}\, N_{\mathrm{A}} \sqrt[3]{R_{\mathrm{E}}^2 \left(\frac{S_{\mathrm{lope}}}{P' \bar{\nabla}_{\mathrm{T}(-)} M_3}\right)^2}} \tag{21}$$

If $P = 1 - P'$, then substitution into Eq. (21) and rearrangement gives

$$P' + \frac{0.655185\, I_{\mathrm{nt}}}{\pi\, R^2\, \bar{\nabla}_{\mathrm{T}(+)}\, N_{\mathrm{A}} \sqrt[3]{R_{\mathrm{E}}^2 \left(\frac{S_{\mathrm{lope}}}{P' \bar{\nabla}_{\mathrm{T}(-)} M_3}\right)^2}} = 1 \tag{22}$$

To simplify Eq. (22), $P' \bar{\nabla}_{\mathrm{T}(-)}$ needs to be made subject of the formula in Eq. (17) to give

$$P' \bar{\nabla}_{\mathrm{T}(-)} = \frac{2\, R^3\, S_{\mathrm{lope}}}{3\, R_{\mathrm{E}}^2\, M_3} \tag{23}$$

Then, substitution of Eq. (23) into Eq. (22) and rearrangement give

$$P' \cong 1 - \frac{0.5\, I_{\mathrm{int}}}{\pi\, R_{\mathrm{E}}^2\, \bar{\nabla}_{\mathrm{T}(+)}\, N_{\mathrm{A}}} \tag{24}$$

Equation (24) implies that P can also be given as

$$P = \frac{0.5\, I_{\mathrm{int}}}{\pi\, R_{\mathrm{E}}^2\, \bar{\nabla}_{\mathrm{T}(+)}\, N_{\mathrm{A}}} \tag{25}$$

Therefore, two algebraic equations namely Eqs (21) and (25) may be available for the determination of P, while Eqs (17) and (24) are for the determination of P'.

In order to quantify the inward flux of the macromolecule, the substrate in this case, Eq. (1) have to be re-derived as follows. Substitution of Eq. (11) into Eq. (1) gives

$$\mathcal{F}_{\mathrm{in}} = 2P\, \bar{\nabla}_{\mathrm{T}(+)}\, C_{\mathrm{S}}(r_0)\, N_{\mathrm{A}}\, n_{\mathrm{f}}\, R_{\mathrm{E}}^2\, \pi \tag{26}$$

Rearranging Eq. (25) to give $P\bar{\nabla}_{\mathrm{T}(+)}$ and substitute same into Eq. (26) gives after simplification the following

$$\mathcal{F}_{\mathrm{in}} = I_{\mathrm{nt}} C_{\mathrm{S}}(r_0)\, n_f \tag{27a}$$

Equation (27a) is with the understanding that $C_{\mathrm{S}}(r_0)$ is the number density of the substrate $[S_0]N_{\mathrm{A}}/M_3$, a number per litre and n_{f} is also in moles per litre.

$$\mathcal{F}_{\mathrm{in}} = \frac{I_{\mathrm{nt}}\, N_{\mathrm{A}}\, [S_0]\, n_{\mathrm{f}}}{M_3} \tag{27b}$$

The quantification of outward flux requires the rederivation of Eq. (4). To this end, Eq. (13b) is substituted into Eq. (4) to give after rearrangement and simplification the following.

$$\mathcal{F}_{\mathrm{out}} = 3P' \bar{\nabla}_{\mathrm{T}(-)}\, N_{\mathrm{A}}\, R_{\mathrm{E}}^2 \left(n_{\mathrm{E}(0)} - n_{\mathrm{f}}\right)/2R^3 \tag{28a}$$

Substituting Eq. (23) into Eq. (28a) gives after simplification the following.

$$\mathcal{F}_{\mathrm{out}} = \frac{S_{\mathrm{lope}}\, N_{\mathrm{A}}\, \left(n_{\mathrm{E}(0)} - n_{\mathrm{f}}\right)}{M_3} \tag{28b}$$

The net flux is then given as,

$$\mathcal{F}_{\mathrm{in}} - \mathcal{F}_{\mathrm{out}} = 2P\, \bar{\nabla}_{\mathrm{T}(+)}\, C_{\mathrm{S}}(r_0)\, N_{\mathrm{A}}\, n_{\mathrm{f}}\, R_{\mathrm{E}}^2\, \pi - 3P' \bar{\nabla}_{\mathrm{T}(-)}\, N_{\mathrm{A}}\, R_{\mathrm{E}}^2 \left(n_{\mathrm{E}(0)} - n_{\mathrm{f}}\right)/2R^3 \tag{29a}$$

$$= \frac{I_{\text{int}}\, N_{\text{A}}\, [S_0]\, n_{\text{f}}}{M_3} - \frac{S_{\text{lope}}\, N_{\text{A}}\, (n_{\text{E}(0)} - n_{\text{f}})}{M_3} \tag{29b}$$

Further simplifications give

$$\mathcal{F}_{\text{in}} - \mathcal{F}_{\text{out}} = N_{\text{A}} \left([S_0] I_{\text{int}}\, n_{\text{f}} - S_{\text{lope}} \left(n_{\text{E}(0)} - n_{\text{f}} \right) \right) / M_3 \tag{30}$$

The corollaries that cannot be ignored from Eqs (23) and (25) and are given as follows

$$\overline{\nabla}_{\text{T}(-)} = \frac{2\, R^3\, S_{\text{lope}}}{3\, R_{\text{E}}^2\, M_3\, P'} \tag{31}$$

$$\overline{\nabla}_{\text{T}(+)} = \frac{I_{\text{int}}}{2\, N_{\text{A}}\, R_{\text{E}}^2\, P\, \pi} \tag{32}$$

2.3.2. The case in which the substrate is bound to a much larger particle.

This section is important because biochemical reactions *in vivo* occur in a much crowded environment, very much different from a guided *in vitro* situation. Carbohydrate (gelatinised starch) may be bound on fibres, granular shell, tissue or cellular debris and the protein component of food may bind to the much larger, carbohydrate components and other luminal components of the gastrointestinal tract (GIT). Included in this scenario is also the binding of disaccharides and monosaccharides on larger diet polymers.

The expression for the net reaction rate per unit concentration of spheres is thus given as:

$$\frac{\mathcal{F}_{\text{in}} - \mathcal{F}_{\text{out}}}{C_{\text{A}}^0} = \frac{[S_0]\, (1 - \exp\, (-k\, t))\, N_{\text{A}}}{M_{\text{P}}\, C_{\text{A}}^0\, t\, k_3} = \frac{r_0^2 C_{\text{A}}^0\, C_{\text{E}}(r_0)\, \alpha\, \gamma\, k_1 / k_{1(0)}\, R^2 - \frac{r_0^2\, k_2\, \alpha\, (1-\gamma)\, C_{\text{A}}^0}{R^2\, k_{2(0)}}}{C_{\text{A}}^0} \tag{32a}$$

Where, M_{P} is the molar mass of the product and $C_{\text{E}}(r_0)$ is the number density of the enzyme assumed to be approximately uniform in concentration around the larger surface element and k_3 is the first order rate constant for the formation of the product, otherwise known as turnover number. For the sake of convenience, other symbols are retained, and, other arguments remain essentially the same.

Equation (32a) simplifies to give

$$\frac{[S_0]\, (1 - \exp\, (-k\, t))\, N_{\text{A}}}{M_{\text{P}}\, t\, k_3} = r_0^2 C_{\text{A}}^0\; C_{\text{E}}(r_0)\, \alpha\, \gamma\, k_1 / k_{1(0)}\, R^2 \; - \; \frac{r_0^2\, k_2\, \alpha\, (1-\gamma)\, C_{\text{A}}^0}{R^2 k_{2(0)}} \tag{32b}$$

Meanwhile,

$$\alpha = \frac{N_{\text{A}} n_{\text{S}(0)}\, R_{\text{S}}^2}{C_{\text{A}}^0\, r_0^2} \tag{33}$$

Where, $n_{\text{S}(0)}$ and N_{A} are the number of moles of the substrate per cubic litre and Avogadro number respectively. Since, from Eq. (33), $\alpha\, C_{\text{A}}^0\, r_0^2 = N_{\text{A}} n_{\text{S}(0)} R_{\text{S}}^2$, then,

$$\gamma = \frac{N_{\text{A}} n_{\text{f}}\, R_{\text{S}}^2}{\alpha\; C_{\text{A}}^0\, r_0^2} \quad \text{Or} \frac{n_{\text{f}}}{n_{\text{S}(0)}} \tag{34}$$

Where n_{f} (This is = $[\text{S}_0]/M_3 \exp(-k\, t)$, where M_3 is the molar mass of the substrate) is the number of moles of free substrate per unit volume.

Therefore,

$$\alpha\, \gamma = \frac{N_{\text{A}} n_{\text{f}}\, R_{\text{S}}^2}{C_{\text{A}}^0\, r_0^2} \tag{35}$$

Also,

$$1-\gamma = 1-\frac{N_A n_f R_S^2}{\alpha\ C_A^0 r_0^2} \tag{36}$$

Another possibility is that

$$\alpha\,(1-\gamma) = \alpha - \frac{N_A n_f R_S^2}{C_A^0\, r_0^2} \tag{37}$$

Substitution of Eq. (33) into Eq. (37) and simplification give

$$\alpha\,(1-\gamma) = \frac{N_A\, R_S^2}{C_A^0\, r_0^2}\left(n_{S(0)} - n_f\right) \tag{38}$$

From Eq. (34),

$$C_A^0 = \frac{N_A n_f R_S^2}{\alpha\,\gamma\, r_0^2}. \tag{39}$$

Substitution of Eqs (35) and (38) into Eq. (32b) and simplification gives

$$\frac{[S_0]\left(1-\exp\left(-k\,t\right)\right) N_A}{M_P\, n_f\, t\, k_3} = \frac{N_A\, R_S^2\, k_1\, C_E(r_0) t_+}{k_{1(0)}\, R^2} - \frac{N_A\, R_S^2\, k_2}{k_{2(0)}\, R^2}\frac{\left(n_{S(0)} - n_f\right) t_-}{n_f} \tag{40}$$

$C_E(r_0)$, which can be rewritten as $[E_0^g]\, N_A / M_2$, (where $[E_0^g]$ is the mass concentration of the enzyme) is substituted into Eq. (40) to give

$$\frac{[S_0]\left(1-\exp\left(-k\,t\right)\right) N_A}{M_P\, n_f\, t k_3} = \frac{N_A^2\ R_S^2\ k_1\, [E_0^g] t_+}{M_2\, k_{1(0)}\, R^2} - \frac{N_A\, R_S^2\ k_2}{k_{2(0)}\, R^2}\frac{\left(n_{S(0)} - n_f\right) t_-}{n_f} \tag{41a}$$

Where, M_2 is the molar mass of the enzyme. Besides,

$$[ES] \approx \frac{[S_0]\left(1-\exp\left(-k\,t\right)\right)}{M_P\, t\, k_3} \tag{41b}$$

Therefore, Eq. (41a) can be restated in a simplified form to give

$$\frac{[ES]}{n_f} = \frac{N_A\, R_S^2\, k_1\, [E_0^g] t_+}{M_2\, k_{1(0)}\, R^2} - \frac{R_S^2\, k_2}{k_{2(0)}\, R^2}\frac{\left(n_{S(0)} - n_f\right) t_-}{n_f} \tag{41c}$$

A plot of $[ES]/n_f$ versus $(n_{S(0)} - n_f)/n_f$ should give slope (S_{lope}) and intercept I_{nt} from where, $k_{2(0)}$ and $k_{1(0)}$ respectively can be calculated.

The following are derivable from the plot of $[ES]/n_f$ versus $(n_{E(0)} - n_f)/n_f$ in Eq. (41c).

$$k_{2(0)} = \frac{R_S^2 k_2\, t_-}{R^2\, S_{lope}} \tag{42}$$

Substitution of Eq. (42) into Eq. (6) eliminates the intrinsic 1st order rate constant, k_2 to give

$$P' = \frac{2\, R^3\, S_{lope}}{3\, R_S^2\ \bar{v}_{T(-)}\, t_-} \tag{43}$$

$$R = \sqrt[3]{\frac{3\, R_S^2\, P'\, \bar{v}_{T(-)}\, t_-}{2\ S_{lope}}} \tag{44}$$

The equation for $k_{1(0)}$ can be derived from the intercepts in Eq. (41c) as follows.

$$k_{1(0)} = \frac{N_A R_S^2\, k_1\, [E_0^g] t_+}{M_2\, I_{nt}\, R^2} \tag{45}$$

Substitution of Eq. (44) into Eq. (45) gives another equation for $k_{1(0)}$.

$$k_{1(0)} = \frac{N_A R_S^2 \, k_1 \, [E_0^g] t_+}{M_2 \, I_{nt}} \left(\frac{2 \, S_{lope}}{3 \, R_S^2 \, P' \, \bar{\nabla}_{T(-)} \, t_-} \right)^{2/3} \tag{46a}$$

Equation (46a) simplifies to

$$k_{1(0)} = \frac{0.763143 k_1 [E_0] N_A \, t_+ \sqrt[3]{R_S^2 \left(\frac{S_{lope}}{P' \, \bar{\nabla}_{T(-)} t_-} \right)^2}}{I_{nt}} \tag{46b}$$

Where, $[E_0^g]/M_2$ is rewritten as $[E_0]$.

Meanwhile, substituting Eq. (46b) into Eq. (2) eliminates the 2nd order intrinsic rate constant, such that P can be given as

$$P = \frac{0.655185 \, I_{nt}}{\pi \, R^2 \, \bar{\nabla}_{T(+)} [E_0] \, N_A \, t_+ \sqrt[3]{R_S^2 \left(\frac{S_{lope}}{P' \, \bar{\nabla}_{T(-)} t_-} \right)^2}} \tag{47}$$

If $P = 1 - P'$, then substitution into Eq. (47) and rearrangement give

$$P' + \frac{0.655185 \, I_{nt}}{\pi \, R^2 \, \bar{\nabla}_{T(+)} [E_0] \, N_A \, t_+ \sqrt[3]{R_S^2 \left(\frac{S_{lope}}{P' \, \bar{\nabla}_{T(-)} t_-} \right)^2}} = 1 \tag{48}$$

To simplify Eq. (48), $P' \, \bar{\nabla}_{T(-)}$ needs to be made subject of the formula in Eq. (43) to give

$$P' \, \bar{\nabla}_{T(-)} = \frac{2 \, R^3 \, S_{lope}}{3 \, R_S^2 \, t_-} \tag{49}$$

Then, substitution of Eq. (49) into Eq. (48) and rearrangement give

$$P' \cong 1 - \frac{0.5 \, I_{nt}}{\pi \, R_S^2 \, \bar{\nabla}_{T(+)} [E_0] \, N_A \, t_+} \tag{50}$$

Equation (50) implies that P can also be given as

$$P = \frac{0.5 \, I_{nt}}{\pi \, R_S^2 \, \bar{\nabla}_{T(+)} [E_0] \, N_A \, t_+} \tag{51}$$

Therefore, two algebraic equations namely Eqs (47) and (51) may be available for the determination of P, while Eqs (43) and (50) are for the determination of P'.

In order to quantify the inward flux of the macromolecule, the enzyme in this case, Eq. (1) have to be re-derived as follows. Substitution of Eq. (35) into Eq. (1) gives

$$\mathcal{F}_{in} = 2P \bar{\nabla}_{T(+)} \, C_E \, (r_0) \, N_A \, n_f \, R_S^2 \pi \tag{52}$$

Rearranging Eq. (51) to give $P \bar{\nabla}_{T(+)}$ and substitute same into Eq. (52) where $C_E \, (r_0)$ is equal to $[E_0] N_A$, gives after simplification the following

$$\mathcal{F}_{in} = I_{nt} N_A n_f / t_+ \tag{53a}$$

With the understanding that n_f is equal to $[S_0]/M_3 . \exp(kt)$ Eq. (53a) can be rewritten as

$$\mathcal{F}_{in} = \frac{I_{nt} \, N_A \, [S_0]}{M_3 \exp(kt) t_+} \tag{53b}$$

The quantification of outward flux requires the rederivation of Eq. (4). To this end, Eq. (38) is substituted into Eq. (4) to give after rearrangement and simplification the following.

$$\mathcal{F}_{\text{out}} = 3P'\,\bar{\nabla}_{\text{T}(-)}\,N_{\text{A}}\,R_{\text{S}}^2\left(n_{\text{S}(0)}-n_{\text{f}}\right)/2R^3 \tag{54a}$$

Substituting Eq. (49) into Eq. (54a) gives after simplification the following.

$$\mathcal{F}_{\text{out}} = \frac{S_{\text{lope}}\;N_{\text{A}}\left(n_{\text{S}(0)}-n_{\text{f}}\right)}{t_{-}} \tag{54b}$$

Therefore, the net flux of the enzyme is given as

$$\mathcal{F}_{\text{in}} - \mathcal{F}_{\text{out}} = 2P\bar{\nabla}_{\text{T}(+)}\,C_{\text{E}}(r_0)\,N_{\text{A}}\,n_{\text{f}}\,R_{\text{S}}^2\pi - 3P'\,\bar{\nabla}_{\text{T}(-)}\,N_{\text{A}}\,R_{\text{S}}^2\left(n_{\text{S}(0)}-n_{\text{f}}\right)/2R^3 \tag{55a}$$

$$= \frac{I_{\text{nt}}\,N_{\text{A}}\,[S_0]}{M_3\exp(kt)t_{+}} - \frac{S_{\text{lope}}\;N_{\text{A}}\left(n_{\text{S}(0)}-n_{\text{f}}\right)}{t_{-}} \tag{55b}$$

Further simplifications ($[S_0]/M_3 = n_{\text{S}(0)}$) give

$$\mathcal{F}_{\text{in}} - \mathcal{F}_{\text{out}} = N_{\text{A}}\left(\frac{I_{\text{nt}}n_{\text{S}(0)}}{\exp(kt)t_{+}} - S_{\text{lope}}\frac{\left(n_{\text{S}(0)}-n_{\text{f}}\right)}{t_{-}}\right) \tag{56}$$

The corollaries that cannot be ignored from Eqs (49) and (51) are given as follows

$$\bar{\nabla}_{\text{T}(-)} = \frac{2\,R^3\,S_{\text{lope}}}{3\,R_{\text{S}}^2\,t_{-}\,P'} \tag{57}$$

$$\bar{\nabla}_{\text{T}(+)} = \frac{0.5\,I_{\text{nt}}}{\pi\;R_{\text{S}}^2\;[E_0]\,N_{\text{A}}\,P\,t_{+}} \tag{58}$$

3. Results and discussion

This research which is purely theoretical needs to end in part with a brief analysis of derived equations and elucidation of the importance or applicability to both biological and non-biological (abiotic) situations. There are three main if not the only situations. The situations in which the enzyme is totally removed from bulk solution by being bound to a much larger particles such as colloid particles which however, are in constant random motion, the Brownian type due to solvent bombardment and in which the enzyme is bound to an immobile rigid surface thereby becoming immobilised; the colloid may be a gelatinised insoluble polysaccharide. This aspect, total immobilisation is of interest to food processing and pharmaceutical and automobile industries that are in search of alternative cleaner fuel, often called bio-fuels. The 3rd situation is one in which the substrate (*eg* short polypeptides, peptides *etc* are bound on polysaccharide components of partially digested food in the small intestine) and in particular the brush border enzymes (sucrase, isomaltase, maltase *etc*) is sequestrated by being bound to the luminal part of the biological compartment, GIT, cytosol *etc*.

The first situation, with respect to net flux of biomolecular reactants is given in Eq. (30). In this case, it is the net flux of the substrate molecules that is described within specified duration of assay. It addresses mostly the case in which the enzyme is immobilised on a rigid surface. This is notwithstanding the case in which the enzyme is adsorbed on much larger neutral colloid as discussed in the literature [1] but being generalisable to much larger molecules either as substrate or enzyme. This claim is anchored on the fact that there is nothing to show in Eq. Eq. (30) that the net flux is dependent on the adsorbent, the colloid on which all the enzyme are bound. Yet an inert adsorbent-the colloid- that is sufficiently larger than the enzyme and substrate cause molecular crowding (MC) otherwise called volume exclusion phenomenon [11]. The issue of MC has regularly been of intense research interest for years [12-18] considering its effect on enzyme kinetics and kinetic and thermodynamic stability. This is relevant to digestion or metabolism in general in an *in vivo* compartment in particular and *in vitro* compartment as may be the case in an industrial application.

Although the situation described above can be ascribed to abiotic or industrial cum laboratory application mainly, it is nevertheless applicable to a limited extent in a biological situation. There are brush border membrane proteins most of which are enzymes such as maltase, isomaltase, sucrase *etc* in the intestinal membranes of mammals, human beings being exclusively very important example. Hence, there is the view that translational diffusion of free substrate in a

crowded environment (metabolically active space such as cytoplasm or mitochondrial matrix) is interrupted by thermally controlled frequent collision and nonspecific interactions with soluble/immobile macromolecules/macrostructures in a variety of shapes/sizes [19]. This is due to molecular crowding. This seems to suggest a case whereby there are obstacles to free motion. But there is a need to redefine obstacle that is all encompassing. Whenever a substrate undergoing thermal motion cum weak or strong "electrostatic steering"[20] approaches the active site of the enzyme with functional groups that are not vulnerable to catalytic action, the purpose of catalytic function becomes unrealisable. Therefore, this constitutes an obstacle. In this situation, what has been described as non-equilibrium binding energy [21] may apply; but it may be weak and likely to be overcome by thermal energy unlike the situation where the catalytically vulnerable group binds correctly at the active site.

Nonetheless, diffusion enables substrates find their target enzyme's active site within the limited dimensions of the animal cell, taken to be ≈ 15 μm [19]. Some substrate molecules are however, insoluble, being colloidal or suspension with likelihood of the latter settling at the bottom of available 3-dimnesional space (3-D] space. Therefore, the process, production, and chemical engineers need to make special design that may involve constant agitation or steering. Biological relevance of Eq. (30) can be appreciated if consideration is given to the fact that, what constitutes a crowding agent to peptidases may be substrate for other enzymes, glucanohydrolase, triglyceridase and lipase in general, for instance, and *vice versa*. Thus, apart from volume exclusion, the crowding agent (seen to be colloidal in nature) as food material could bind the enzyme whose substrate is different from the crowding agent. The substrate in question in the presence of aqueous medium within the intestinal lumen can undergo a net flux around its enzyme.

The preceding discussion is very much amenable to Eq. (56), if in particular, for the biological situation, the stomach is the case. A balanced diet which has been masticated contains, fat/oil, carbohydrate, protein etc. While in the stomach the protein components may be strongly associated with the other components of the food. Thus, the enzyme, pepsin, secreted into the stomach can also undergo net flux around the protein component of food bound to other food components. This scenario may not be different from an *in vitro* situation whereby an aliquot of the enzyme is introduced into a highly heterogeneous mixture of macromolecules. This may take place in research, pharmaceutical, and institutional laboratories.

Immobilisation of the enzyme is definitely different from the immobilisation of the substrate. They may likely give different result because while Eqs (30) and (56) look alike, they are nevertheless different in one major aspect. The unit of the intercept and slope for the situation where the enzyme is removed from direct contact with the bulk solution or entirely immobilised is the same as the unit of a 2nd order rate constant. Whereas, where the substrate is removed from the bulk or entirely immobilised the slope and the intercept are dimensionless. What is important is that though the reactants, enzyme and substrate may be exposed to a third component, the insoluble colloidal particles or rigid immobilisers, the concentrations, areas, radii *etc* of the latter do not appear in the final equations as to imply that initial information about such may not be necessary for computational purpose. This is not withstanding the observation that the performance of some enzymes always depended on the amount of excluded volume; only large oligomeric proteins as enzymes, display an obstacle size-dependent behavior and crowding has the potential of hindering diffusion to the extent of shifting reaction control from activation (in which the enzyme-mediated transformation of the substrate to product is the rate limiting step) to diffusion (in which the reactive step is fast and complex formation step is diffusion-dependent) [11]. The presence of crowders can promote the probability of incorrect enzyme-substrate contact leading to higher probability of dissociation. Thus, it appears that the presence of crowding agents, immobilisers *etc* are indirectly observed via the kinetic parameters without the need for the appearance of their physicochemical parameters in both equations. This may therefore, justify the generalisability of the equations with or without the 3rd component of the reaction mixture. In other words, an insoluble large substrate (*e.g.* raw and gelatinised insoluble starch) can be a crowding agent and a substrate. This is very much applicable to every large enzyme (*e.g.* sucrase) and its substrate, sucrose.

What matters most is motion either mechanically or thermally and electrostatically driven. There is a need to examine the working equations. Many molecules of the enzyme advance towards the substrate molecules, but not all the enzyme molecules possess sufficient kinetic energy to overcome the solvent resistance to a substantial extent. Apart from the solvent resistance, the collision frequency and the impact must be sufficient to enable the displacement of molecular surface water of hydration that is loosely bound. As in the literature [10] thermal energy is not enough to enhance collision, but electrostatic attraction is needed for directionality that culminates into effective collision and complex formation. Therefore, there is a need to understand that the association between the bullet molecule and the target may occur with high probability. However, not all associations in the course of complex formation are catalytically oriented.

Although the statement to the effect that that P, P', and $\bar{v}_T$ may be assumed to have the same values which characterise the free enzyme [1], is not very clear, the approach in this research sees it differently. Thus, the probabilities P and P'

may not be equal (this is contrary if the initial position is that they are the same) otherwise it may imply that the rate of association is equal to the rate of dissociation into free enzyme and substrate. This is also in the light of the approach adopted by Szabo and Zhou [22] which showed that $P = k_2/(k_{-1} + k_2)$ and $P' = k_{-1}/(k_{-1} + k_2)$ where k_{-1} and k_2 are the 1st order rate constants for the dissociation of enzyme-substrate complex, *ES* to free enzyme, *E* and substrate, *S* and for the formation of the product respectively.

The corollary arising from Eq. (17) is that $\bar{v}_T$ can be calculated if the value of r_0 is known. Let it be known that $\bar{v}_T$ is « 1 m/s as explained elsewhere [9], otherwise biological fluid will completely dry up. Also, if values of $\bar{v}_T$ can be calculated and given any value of r_0 and R_S, the substrate particle radius, either in the literature or calculated, *P* could be calculated as long as other parameters can be experimentally determined. The most important corollary is that the probability of dissociation could be low if k_3 is very high. In other words *P* is inversely proportional to k_3.

4. Conclusion

In conclusion two different equations need separate derivation for association and dissociation of reactants. The needs for the flux of reactants have both biological and industrial relevance, respectively due to importance of time-dependent digestive processes and for the optimisation of the production of desired products of enzymatic action. The equations describing net flux seem generalisable in that information about the physicochemical properties of both crowding agent and immobilisers may not be needed for calculations despite the fact that their presence influences the rate of enzymatic action. Research in the feature may focus on the quantitative determination of the "approach and departing velocities" as well as the determination of the probabilities of escape and association of either the enzyme or substrate and formation of effective enzyme-substrate complex respectively.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares that there is no conflict of interest of any kind.

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Author's Short Biography

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My interest covers mainly subjects such as biochemistry, biophysics (and related field) and very limited extra-curricular subject such as atomic and nuclear physics. My general interest includes field of science amenable to basic mathematics. My recent interest in respiratory physiology is due to the challenge imposed by the global pandemic which compromised respiratory system to an extremely dangerous proportion. My highest degree is a Ph.D in Biochemistry.