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Determination of Translational Velocity of Reaction Mixture Components: Effect on the Rate of Reaction

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Abstract: The objectives of the research were to: 1) formulate a simple mathematical model for the determination of initial velocity and terminal velocity of dissolved solute in aqueous solvent, 2) to determine the minimum interparticle distance (l_{EM}) for which the periods of coverage determined according two methods namely Einstein model and Newtonian model are equal and 3) elucidate the importance of translational velocity and the minimum interparticle distance in the optimization of the purpose of enzyme catalyzed reaction. The values of l_{EM} for which Einstein and Newtonian approach for the determination of time for the coverage of such distance gave the same result were 3.15 exp (-8) m and 4.04 exp (-8) m when the concentrations of enzyme were ~ 2.4 exp (-8) mol/l and ~3.21 exp (-8) mo/l respectively. The terminal velocity was ~ 8.43 nm/s at 293.15K; the real/effective kinetic energy in solution was ~7.36 exp (-27) J at 293.15K. The initial velocity of solute was ~ 9.25 exp (-3) m/s. In conclusion, a model for the determination of terminal velocity and initial velocity was derived. The initial velocity is » the terminal velocity at a given temperature. Effective collision between the bullet molecule and the much larger target molecule at l_{EM} or less should be directional so as to achieve enzyme-substrate and drug-pathogen/poison complex formation.

Keywords: *Aspergillus Oryzea* Alpha Amylase, Model, Translational Velocity, Ballistic and Brownian Time, Minimum Interparticle Distance

1. Introduction

It is a well known fact that most elementary reactions are diffusion controlled while some enzyme catalyzed reactions may be diffusion controlled. In line with this standpoint is the claim that, "The interplay of intermolecular interactions at multiple time and length scales governs a fine-tuned system of reaction and transport processes, including particularly protein diffusion as a limiting or driving factor" [1, 2]. It has also been observed that a strong decrease of the translational diffusion coefficient occurs due to macromolecular crowding on nanosecond time scales; concentration seems to affect the rate of translational diffusion as it has been evidenced in the observation that at volume fractions $\varphi \approx 25\%$ as present in living cells, the translational diffusion is decreased to 20% of the dilute-limit value, implying a slowing down of diffusion-driven transport and diffusion-limited reactions [1]. Although, proteins not only show global motions like translational and rotational diffusion but also internal and inter-domain motions [1], it is the translational aspect that can easily be related to linear motion. All attention seemed to be focused on translational diffusion or self-diffusion of proteins with little concern for translational velocity of dissolved solute. Another issue that need to be considered is the usual calculation of time of diffusion according to Einstein's approach, $t = d^2/2D$. In this approach, d is average mean root square distance and so, the time, t should be average time; D is the diffusion coefficient. Advancing population of bullet molecules towards the target arrive at different time at the "finish line" at different times. Therefore, without necessarily questioning the use of Einstein's model, one out of the numerous molecules collides with the target at a time that may be different from the average given that all bullet molecules are similar for instance and they are located at

different locations before the target molecule. To elucidate this further, one can focus on known diffusion coefficient of oxygen molecule in air and water given as 1.8 exp (-5) m^2/s in water and 1.8 exp (-5) m^2/s in air [3]. If for the purpose of illustration, root mean square distance is 1um in air, for instance, then, $d^2/2D$~2.78 exp (-8) s. On the other hand, from the perspective of Newtonian mechanics and kinetic theory, t~2.09 exp (-9) s (from $d/(3k_BT/m)^{½}$ where k_B, T, and m are the Boltzmann constant, thermodynamic temperature, and mass of a particle.). In this case, d is used in line with Newtonian and kinetic theory as displacement without prejudice to earlier description as average quantity in the light of Brownian phenomenon as opposed to directionality implied in Newtonian and kinetic theory. One should know what may be the case if d = 1 m. Nonetheless, it is important to realize that collision between biomolecules in particular in the light of this subject may be greatly inhibited if directional forces that can bring about transition from random diffusional motion to "ballistic" or bullet-like motion are not imposed on reactants. This force may be hydrophobic, electrostatic *etc* in nature. There is also claim that "the solvent time scale τ_S is in general much shorter than the diffusive time scale of the dissolved particles, $\tau_B \approx m/6\pi\eta R$ on which the motion changes from ballistic to diffusive motion" [1]. If this implied "missile/bullet-like" motion (i.e. ballistic), then it can be seen once again that diffusion is a major concern to most investigators; but it can be seen also that, translational velocity as implied in ballistic motion, is relevant to biological function. After diffusion which depends on concentration gradient, each molecule has its individual motion with speed. There is no doubt that diffusion is vital to general biological function as may be attested to by subsequent write-up, but translational velocity being highly vectorial/directional is very useful for the ultimate delivery of biological substances to their target. Diffusion is multi-dimensional process enhancing the distribution of matter in a medium. Diffusion is very useful biologically in photosynthesis and cellular respiration and in the separation of different macromolecules via chromatography and centrifugation techniques. But direct delivery of a molecule to any biological target, haemoglobin, chlorophyll *etc* must be vectorial or "ballistic".

"To bind at an enzyme's active site, a ligand must diffuse or be transported to the enzyme's surface, and, if the binding site is buried, the ligand must diffuse through the protein to reach it"[2]. For instance alpha amylase hydrolysis is carried out by a side-by-side digestion mechanism but only after the enzyme diffuses and binds to the substrate [4]. Thus, despite the importance of diffusion, it is the velocity of reactants that lead to effective collision. Incidentally, there is a claim that it is impossible to individualize the motion of solute in solution except at infinite dilution [5]. Therefore, the objectives of this research are, 1) to derive a mathematical model for the determination of terminal velocity of solution components 2) to determine the minimum interparticle distance for which the periods of coverage determined according two models namely Einstein model and Newtonian model are equal and 3) elucidate the importance of translational velocity and the minimum interparticle distance in the optimization of the purpose of enzyme catalyzed reaction.

1.1. Theoretical Background

It is important to begin this section with what ought to be a scientific question: if the velocity of $H_3O^+_{(aq)}$ in aqueous solution is about 36 exp (-8) m/s due to accelerative force of electric field gradient, should the velocity of a macromolecule like protein be an exception and so, cannot be less, let alone under purely thermal environment only? When an aliquot of a solution of enzyme is placed into a solution of the substrate, there are forces driving the movement of molecules. These are gravitational force implicit in dropping the aliquot and in the concentration gradient or chemical potential gradient (CPG). Swirling may accelerate the mixing of the reaction component mixture. This according to Ahmedi et al. [6] leads to negligible effect of external diffusion. Industrial establishment has electrically powered mechanical way of mixing reaction mixture components in solution as may be applicable to, but not limited to, food, pharmaceutical, and beverage industries. Ultimately, the reaction mixture components become uniformly distributed in solution. In the absence of substantial CPG, magnitude of displacement by each molecule with time becomes a function of the translational velocity of the reactants. This is the case during every catalytic cycle. The implication is that it takes some time before every molecule of the enzyme gets involved in catalytic action. Hence the amount of product formed in one minute cannot be > the amount in 2 minutes. Therefore, the initial translational velocity of the molecules, not just enzymes, substrate, medicinal drug *etc* is vital to the optimization of the effect/benefit of reaction.

Using the mobility of electrolyte in an electric field as a "guiding principle" one sees that the velocity (u_e) of the solute or electrolyte is inversely proportional to the radius of the solute–$u_e = eqV/6\pi\eta rL$ where e, q, and V/L are the charge of an electron, oxidation number of the solute, and the potential gradient respectively. The parameters η and r are the viscousity constant and radius of the enzyme. The situation under purely thermal condition may not be an exception since the velocity may be inversely proportional to the radius of the dissolved solutes. However, the radius of proteins in particular is often determined using the solution of the enzyme by investigators [7]. A popular method is Stokes-Einstein model-$6\pi\eta r = k_BT/D$. One question that needs to be answered is: does the radius of the protein in particular remain the same at all temperatures, including temperature as low as 5 ^oC? The dry molar mass of most protein may be known such that given the general density of the protein one can calculate the radius of the protein, including human salivary alpha amylase for instance. Quillin and Matthews [8] showed that, the general accurate density of proteins is 1.43 ± 0.03 g/l. Other investigators have shown that the density of proteins with high molecular weight (say M > 30 kDa) is ρ = 1.41 g/ml (instead of 1.43 g/ml) which according to Fisher et al [9] represents well the average densities determined by other workers [8].

One can assume that the radius of the enzyme remains relatively stable/constant under the temperature at which the enzyme is assayed. Dissolution of substance in the dry state in water at the ambient temperature can increase the radius because of hydration of the polar and charged groups in particular.

Meanwhile, it must be born in mind that, globular proteins in general are subject to the effect of their thermal environment on account of which such proteins either possess conformational flexible or rigidity as an adaptive measure needed for function [10] without loss of 3-dimensional structure. Thus with increase in temperature, there may be higher conformational flexibility coupled with increase in radius of the molecule. The contrary is the case with decrease in temperature. This is a universal phenomenon which is partially opposed by the anomalous behaviour of water molecule.

1.2. Formulation of Translational Velocity Equation

It is important to realize that particles suspended in air or liquid or homogenous with the aqueous solvent or air are subject to Brownian motion. If a solution of a solute is introduced into an aqueous solvent gently to minimize gravity induced motion as the drops fall into the solvent, the solute particles separate but their motion is not unidirectional. Randomness implied that each solute particle covers distance several times longer than the distance between the initial position and subsequent position. This seems to be reflected in Einstein's equation which takes into account mean square displacement. The implication is that Einstein's model or equation may not be applicable to non-Brownian motion. Thus the results of any physico-biochemical investigation should be analyzed in the context of Brownian phenomenon and non-Brown phenomenon. This leads to the following inequalities. They are:

$$l/(\xi/m)^{½} > l^2/2D \quad (1)$$

where *m, l,* and ξ are the mass of the particle, interparticle distance that can be covered, and driving energy respectively. The parameter, ξ which is a general one, may not be the same for all molecules even though it is generally posited that the kinetic energy of matter including protein in solution is $3k_BT/2$ [11] when viewed in the context of the velocity of the particles as in gas phase. If the liquid phase velocity of water is ≠ its vapour phase velocity ($3k_BT/m_w$), where m_w is mass of a molecule of water, the velocity of a molecule of protein cannot be an exception.

$$l/(\xi/m)^{½} < l^2/2D \quad (2)$$

If Eq. (1) holds, then there is less Brownian motion. This means that the average interparticle distance is too short (≤ radius of much smaller bullet molecule). If Eq. (2) holds, then there is greater Brownian motion. However, there may be a magnitude of displacement at which the result from classical model become equal to that obtained from Einstein model, hence the following:

$$l/(\xi/m)^{½} = l^2/2D \quad (3)$$

Rearrangement of Eq. (3) gives:

$$l = 2D/(\xi/m)^{½} \quad (4)$$

Equation (3) is illustrated in figure (1). Alternative, is the value of *l*, which can be predetermined or chosen based on the concentration of reaction mixture components, such that the value of ξ can then be determined. If the value of *l* is taken as intermolecular distance between substrate and enzyme or between a therapeutic drug and its target pathogen then rapid distribution of the solutes is important and can be made possible via Brownian motion which is better illustrated by Eq. (2). With specific target in mind, there is a minimum distance between the bullets (drug for instance) and the target (pathogen for instance), at which non-Brownian motion assumes preeminence. This ensures encounter complex formation and consequently enzyme-substrate complex or drug-pathogen (or toxin) complex formation. Equation (3) may justify this situation.

In order to determine the terminal velocity of the solute, the energy or the force per unit time compelling motion need to be determined. With Einstein model which appears to preclude cohesive force that may retard motion particularly in solution or liquid state, the time, *t* expended in covering the displacement, equal to the distance, *l* by a solute is:

$$t = l^2/2\ D = l/(\xi_p/m_2)^{½} \quad (5)$$

where ξ_p and m_2 are the energy driving motion and mass of protein molecule respectively. Rearrangement of Eq. (5) and squaring gives:

$$\xi_p = 4m_2D^2/l^2 \quad (6)$$

Equations (5) and (6) are intended to address the fact there is a distance at which the same time is expended in the coverage of such distance by both Brownian and non-Brownian motion; it becomes necessary to quantitatively define the energy at which the same time can be expended by both Brownian and non-Brownian motion. The next step is to relate ξ_p with driving force, F_t, as follows:

$$F_t l = 4m_2D^2/l^2 \quad (7)$$

Rearrangement of Eq. (7) gives:

$$F_t = 4m_2D^2/l^3 \quad (8)$$

This force is related to Stokes-Einstein equation as follows:

$$4m_2D^2/l^3 = 6\pi\eta ru \quad (9)$$

where η and *r* are coefficient of viscousity and radius of the solute respectively; *t* and *u* are the time taken to cover the distance, *l* and the translational velocity of the protein in solution respectively. The "thermal field" is intrinsic to the system accounting for whether or not the system, solvent in particular and the dissolved solute(s) remain in liquid or

gaseous state. This issue is made clearer if one considers the fact that, molecules much higher in molar mass than water is gaseous suggesting therefore, that there is a strong cohesive force keeping water in liquid state at ordinary temperatures. The thermal energy opposes the cohesive force that enable both the solvent and solute to be in motion at speeds that are much lower than what it could have been if in the gaseous state. Equation (9) is justified because every diffusive motion of a solute in solution is subject to solvent resistance and not only during migration under the influence of an electric field. As usual in the light of this investigation, $D = k_B T/6\pi\eta r$ and substituting it into Eq. (9) gives:

$$4m_2 (k_B T)^2/(6\pi\eta r)^2 l^3 = 6\pi\eta r u \quad (10)$$

After rearrangement and simplification Eq. (10) becomes:

$$u = 4\, m_2 (k_B T)^2/(6\pi\eta r)^3 l^3 \quad (11)$$

Equation (11) can be transformed to:

$$u = 4\, m_2 D^2/6\pi\eta r\, l^3 \quad (12)$$

The magnitude of l must, subject to verification, be $> 2r$. The next task is to determine l.

When a liquid (which may be pure liquid or a solution) in test tube is placed in a constant temperature water bath, the liquid or solution undergoes changes in kinetic energy until temperature becomes constant at every location and equilibrates with the water bath. At thermal equilibrium, the molecules are subject to the same cohesive force and driving force in 3-dimension as applicable to molecules in the water bath. It is hereby postulated that every molecule in liquid state at any temperature, is subject to a "thermal field force" (TFF) = $3k_B T/L$ where $L = (V_{mL})^{1/3}$ where V_{mL} = molar volume of the liquid which has definite volume at temperatures below boiling point. Thus, TFF can relate to Eq. (12) as follows. When $(k_B T)^2/(6\pi\eta r)^2$ is substituted for D^2 in Eq (12) and the outcome re-substituted into $6\pi\eta r u$ the resulting equation is similar to TFF.

$$4\, m_2 \times 6\pi\eta r (k_B T)^2/(6\pi\eta r)^3 l^3 = 3k_B T/L \quad (13)$$

Rearrangement of Eq. (13) leads to:

$$l^3 = 4\, m_2 k_B T\, L/3(6\pi\eta r)^2 \quad (14)$$

$$l^3 = 4\, m_2 D^2 L/3\, k_B T \quad (15)$$

$$l = (4\, m_2 k_B T\, L/3)^{1/3}/(6\pi\eta r)^{2/3} \quad (16)$$

$$l = (4\, m_2 L/3\, k_B T)^{1/3} D^{2/3} \quad (17)$$

Equation (17) can be re-expressed as:

$$l = (4\, m_2 LD/18\pi\eta r)^{1/3} \quad (18)$$

Also, Eq. (18) can be expressed as:

$$l = (4\, m_2 L k_B T/108(\pi\eta r)^2)^{1/3} \quad (19)$$

$$l = (m_2 L k_B T/27(\pi\eta r)^2)^{1/3} \quad (20)$$

Equations (18), (19) and (20) are not unreasonable because $D = k_B T/6\pi\eta r$. The advantage of Eq. (19) lies in the fact that the viscousity constant (η) and L ($(V_{mL})^{1/3}$) values for water at different temperatures can be found in literature given that r can be taken to be constant though this may not be so, an assertion to be examined in the feature. This is against the backdrop of the fact that protein diffusing in aqueous medium is hydrated such that the radius is longer than that of the bare protein. There is also the contribution of anisotropic effect which increases the effective radius of the protein which according to review report by Roosen-Runge [1] is not totally spherical. Yet protein radius is calculated in various ways which includes: effective radius of the protein as the radius of a sphere whose volume is equal to the volume of the bare protein under investigation [1]. Another approach is to define the mean effective hydrodynamic radius as the radius of a sphere with the same diffusion coefficient as the species being studied [12].

Equation (15) can be re-substituted into Eq. (12) to give:

$$u = 3k_B T/6\pi\eta r L \quad (21)$$

Equation (21) can be re-written as:

$$u = 3D/L \quad (22)$$

For easy reference and usage, Eq. (6**)** can be re-written first, after substituting Eq. (18) into Eq. (6) as:

$$\xi_p = (4\, m_2)^{1/3} D^{4/3} (18\pi\eta r/L)^{2/3} \quad (23)$$

Equation (23) can also be restated as:

$$\xi_p = (m_2)^{1/3} (k_B T)^{4/3} (1/\pi\eta r\, L)^{2/3} \quad (24)$$

The advantage in this form (Eq. (24)) is as stated earlier.

"In the light of Eq. (22), one may need to know what makes one feel that if $H_3O_{(aq)}^+$ has a velocity of about 36 exp (-8) m/s, propelled by extra energy, the velocity of a macromolecule like enzyme should not possess lower velocity let alone under thermal environment (referred to as TFF) only". Then what should be the most probable value of L? Thus any solute in aqueous solution is subject to solvent resistance and cohesive forces that reduce velocity of displacement accounting for the fact that the velocity of water molecule in liquid state $\neq (3k_B T/m_w)^{½}$ let alone what the real velocity of macromolecules like proteins and soluble starch may be. What may be strange about Eq. (11) is that u is directly proportional to m contrary to the usual; but Eq. (9) explains the situation coupled with the fact that dimensionality with respect to the unit of mass is not lost. The interactive forces in liquid state and in multi-component solution differ to some extent from what may be obtainable in free gas phase. As interparticle cohesive force → zero ($3k_B T/L_{\to\infty} = 0$), the molecules occupy any space, taking up, in the process indefinite volume and shape at a definite absolute temperature and pressure. In liquid state at definite temperature, the cohesive force is very large acting in opposition to the thermal kinetic energy, thereby imposing fixed volume to the liquid or solution.

2. Materials and Method

The chemicals were *Aspergillus oryzea* alpha amylase (EC 3.2.1.1) whose molar mass M_2 is = 52 kDa [13] and diffusion coefficient, D, value at 293.15K is 7.37 exp(-11) m^2/s [14] and potato starch were purchased from Sigma–Aldrich, USA. Hydrochloric acid, sodium hydroxide, sodium chloride, and Tris 3, 5–dinitrosalicylic acid, maltose, and sodium potassium tartrate tetrahydrate were purchased from Kem light laboratories Mumbia, India; Distilled water was purchased from local market.

The equipment such as electronic weighing machine was purchased from Wenser Weighing Scale Limited and 721/722 visible spectrophotometer was purchased from Spectrum Instruments China. PH meter was purchased from Hanna Instruments, Italy.

Assay of the enzyme was according to Bernfeld method [15]. Duplicate assays were carried out with each concentration of substrate with blank readings subtracted from test readings at a wavelength = 540 nm with extinction coefficient = 181/M.cm.

2.1. Determination of Physical Parameters

Various values of the time t taken to cover interparticle distance, l were determined using Einstein equation $l^2/2D$, $2l/u$ where $u = (\xi_p/m_2)^{½}$, $(3k_BT/m_2)^{½}$, and Eq. (22) as the case may be. All concentrations, different concentration of the enzyme (2.4 exp (-8) mol/l and 3.21 exp (-8) mol/l) and different concentration of the substrate ranging from 4-10 g/l were converted to mol/ml. The values of l at different concentration of substrate, [S], is determined as follows: $(V_{ol}/N_A\Sigma n)^{1/3}$ where V_{ol} is the total reaction volume = 1 ml (1× exp (-6) m^3). Then the logarithm of t determined using Einstein approach and any other method was plotted versus log l to give two equations of straight line expected to cross each other with sufficient data points but not necessarily indispensible. From combined equations of straight line the slope, x, a logarithmic value, is converted to its antilog to give minimum interparticle distance, l_{EM}(taken as such to reflect a practical situation in terms of measurement made), for which the time, t, determined using Einstein approach and any other approach is equal. Substitution of l_{EM} into the equation of straight line obtained from the plot of Σn versus l yields after calculation, the value of Σn when $l \rightarrow l_{EM}$. The number of moles of the substrate is then given as $\Sigma n–n_2$ where n_2 is the number of moles of the enzyme per ml. The value of [S] in g/l is then obtained by multiplying $\Sigma n–n_2$ by exp (+3) and molar mass.

2.2. Statistics

The mean of velocities of hydrolysis of starch from duplicate assays was determined using Microsoft Excel.

3. Results and Discussion

Besides mathematical relationship which verifies the postulation that there must be interparticle distance for which the calculated time of its coverage by oxygen molecule based on Einstein model or Brownian diffusional model (BD) ($l^2/2D$) and Newtonian kinetic model (NK) ($l/(\xi_p/m_2)^{½}$) is equal, graphical approach is used to determine such interparticle distance as shown in Figure. (1). Hypothetical values of l ranging from exp (-9)–exp (-2) m were used to determine t values. Then log t is plotted versus log l. Diffusion coefficient of oxygen molecule is = 1.8 exp (-5) m^2/s [3] at 293.15 K. By combining the two equations of straight line ($2x + 4.443 = x$-1.074), the antilog of x, the value of l (l_{EN}) for which "BD and NK time" are equal is 3.041 exp (-6) m. This approach is applied to real reaction mixture solution in which different concentration of the enzyme is assayed at room temperature and pH = 6. Figure 2 is the case where the diffusion coefficient of the enzyme in solution and $(\xi_p/m_2)^{½}$ (where $\xi_p \ll 3k_BT$) are used to determine t in the usual way namely $l^2/2\ D$ and $2l/(\xi_p/m_2)^{½}$ where [E] = 2.40 exp (-8) mol/l. A plot of log t versus log of interparticle distance, l obtained from $(V_{ol}/N_A\Sigma n/\text{ml})^{1/3}$ (where V_{ol} and N_A are the volume of reaction mixture and Avogadro's number respectively.) is carried out. The l_{EN} value (~3.15exp (-8) m) obtained from the antilog of x from 2 combined equation of straight lines ($0.999x + 2.328 = 1.996x + 9.807$) is about 1.743-fold less than the interparticle distance at the highest [S] value. This expresses the possibility that the concentration of the substrate at which randomness → minimum is practically approached. Similar processes (figure 3) were carried out using $l^2/2D$ and $l/(3k_BT/m_2)^{½}$ for the purpose of holistic examination of what may or may not be permissible scientifically. Although l_{EN} (~8.97 Å) value obtained when antilog of x from $1.999\ x + 9.824 = 0.999\ x$–0.777 is taken is very short, it may not be permissible because the real kinetic energy of the solute is less than $3k_BT$ due to cohesive forces in solution. "This can be explained on the basis of a simple analogy such as the fact that, while the total charge of the nuclear space remains the same, the actual charge, the effective nuclear charge affecting the outer orbital electron (s) is < than total nuclear charge due to shielding effect of inner orbital electrons". Figure 4 represents a plot in which the time t from BD approach is determined with $l^2/2D$ while terminal velocity, $3D/L$ is used to determine t with respect to NK approach for [E] = 2.40 exp (-8) mol/l. From the combined equations of straight line, x (*i.e.* log l_{EN}) is a small negative value (-1.439) suggesting that l_{EN} (0.036 m) is very long as expected of a state of infinite dilution. This is not possible and cannot be possible even at higher [E]~3.20exp (-8) mol/l. The reason is simply based on Newtonian classical mechanics. In the first place it must be appreciated that $3D/L$ cannot be used directly because there is initial velocity,$(\xi_p/m_2)^{½}$; this imply that $(\xi_p/m_2)^{½} \gg 3D/L$ such that $\{(3D/L)+(\xi_p/m_2)^{½}\}t/2 = l$; this translates to $l \cong (\xi_p/m_2)^{½}t/2$ where it should be understood that$(\xi_p/m_2)^{½} \rightarrow 3D/L$ is evidence of deceleration due to solvent resistance. Figure 5 shows similar plot in which $l^2/2D$ and $l/(\xi_p/m_2)^{½}$ were used to determine the different t values where [E] ~3.20 exp (-8) mol/l. The l_{EN} value is ~ 4.04exp (-8) m. This is intended to examine the effect of higher concentration of the enzyme in particular apart from the use of different lower mass

concentration of the substrate. *Ab initio* it is understood that $\xi_p \ll 3k_BT$ in solution which can be explained in terms of another analogy such as the case of an athlete moving at steady velocity until confronted by opposing strong wind. Suddenly the athlete runs at a reduced velocity so that his/her kinetic energy decreases even if the rest mass remains the same. In the same vein, there are forces opposed to the motion of solute with the result that $\xi_p \ll 3k_BT$.

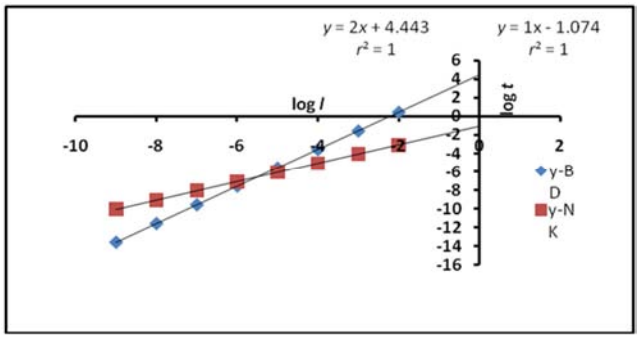

Figure 1. *Theoretical (hypothetical) plot illustrating the proposition that at some point in space the calculated time, by two different methods (BD and NK), taken to cover a given distance would be the same for both methods. BD and NK are Brownian diffusional approach ($l^2/2D$) and Newtonian-kinetic approach ($l/(3k_BT/m_2)^{½}$) respectively. D is however, the diffusion coefficient for oxygen molecule at 293.15K.*

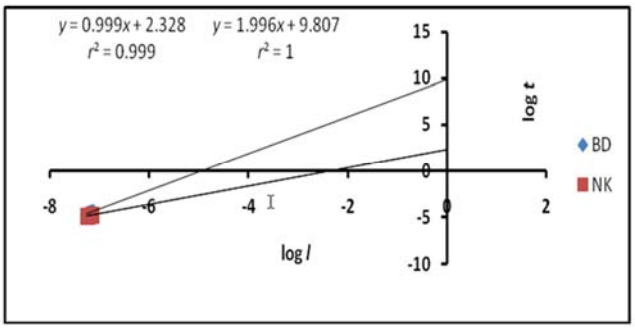

Figure 2. *Determination of interparticle distance where "Brownian time" and "ballistic time" are equal using $l^2/2D$ and kinetic energy (ξ_p) « $3k_BT/2$ as defined in Eq. (24) and Eq.(6) to determine u. [E] = [(0.1g/l)/80]/M_2~2.40exp(-8) mol/l.*

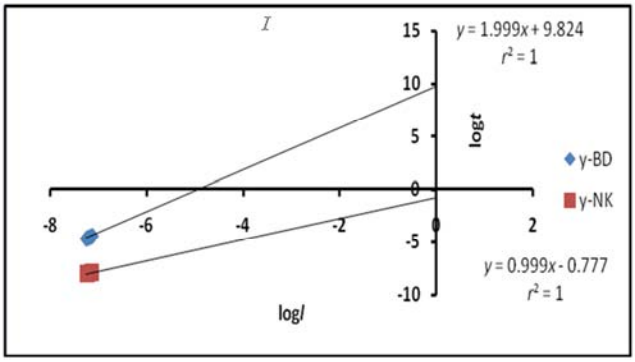

Figure 3. *Determination of interparticle distance in solution using $(3k_BT/m_2)^{½}$ for NK approach, and $l^2/2D$ for Brownian approach.*

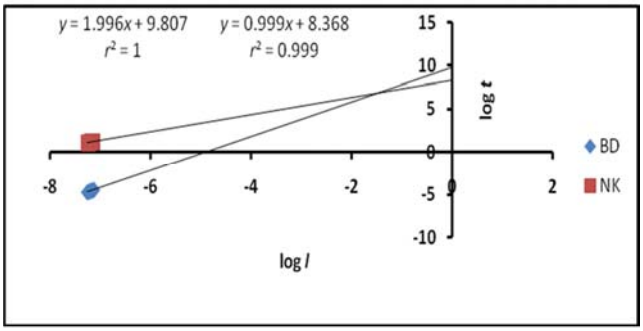

Figure 4. *Determination of interparticle distance where "Brownian time" and "ballistic time" are equal using intra-solvent velocity = 3D/L for "Ballistic time" determination and $l^2/2D$ for "Brownian time" determination. [E]~2.40 exp (-8) mol/l*

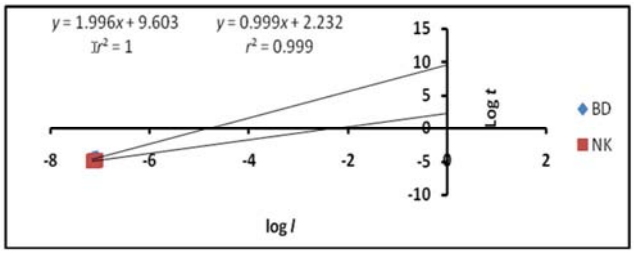

Figure 5. *Determination of interparticle distance where "Brownian time" and "ballistic time" are equal using intra-solvent velocity = $(\xi_p/m_2)^{½}$ for "Ballistic time" determination and $l^2/2D$ for "Brownian time" determination. [E] = (0.1/60) g/l~3.21exp(-8) mol/l.*

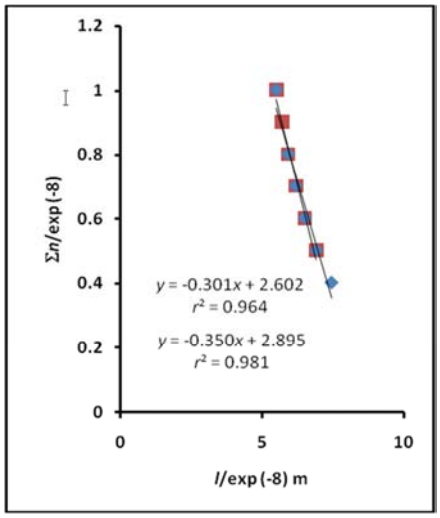

Figure 6. *Plot of Σn versus intermolecular distance for the determination of n_s/ml when the time of displacement determined by two different methods, Einstein (Brownian-Diffusion method) and Newtonian kinetic method, is equal. Σn is the sum of the number of moles of the substrate and the enzyme.*

Figure 6 is a plot of Σ*n*/ml versus *l* intended for the determination of what the value of Σ*n* should be when the interparticle distance, l_{EN}, for which "BD and NK time" are equal is reached. The number of mole per ml (n_s/ml) of substrate is: Σ*n*/ml-n_2/ml. Wide conduit pipe allows mass diffusional motion but does not enhance directional delivery of bullet molecules to specific targets: This is the advantage of terminal bronchiole/stomata in minimizing randomness for effective directional delivery of oxygen to target tissues and

cells. A model describing this issue was formulated but yet to see the light of the day. This graphical approach is applied to different concentration of the substrate subjected to *Aspergillus oryzea* alpha amylase action.

If the root mean square displacement of a solute is on a nano-scale quantity or less, before contact or collision is made then the concentration must be exceedingly high. Random motion or Brownian motion is most likely when intermolecular distance is long as expected in highly diluted solution of solutes or reactants. At much lower concentration the solutes are caged such that the interparticle distance between any two particles may be very narrow and close to average. But this is not without a limit. In other words cage effect may become of no effect if the reaction mixture solution is highly diluted. This is reflected in increasing velocity of hydrolysis of starch with increasing concentration of the substrate. This is not new; but what is new is the fact that the enzyme taken as bullet molecule must reach the substrate at a well defined translational velocity. Einstein model is predicated on randomness, hence the mean square displacement. If the time spent in covering the mean distance which may be close to average interparticle distance at high concentration is to be calculated using Einstein model, such can no longer be closer to the value expected as "Brownian time". Such time tend to "ballistic time" that is, time calculated on the basis of vectorial motion using translational velocity of a single "missile" or bullet molecule. The time taken to reach a target over long distance by some molecules may be smaller than average, while it may be longer than the average with other molecule in line with their differences in the initial position relative to the target.

One out of several bullet molecules caging the target collides with the target. This should be very probable at such interparticle distance where the "Brownian time" on the basis of Einstein model and "ballistic time" on the basis of Newtonian model is equal. This is very important for enzyme-substrate and drug target interaction leading to complex formation. This is buttressed with the observation that, the distance covered at which the time spent via Newtonian kinetic motion and Brownian diffusional motion is equal is exceptionally < interparticle distance at all concentrations of the substrate. Thus as long as terminal velocity is reached under the prevailing thermal energy (or temperature) the collision rate must be vectorially directed as opposed to random diffusional motion which only serve to disperse dissolved solutes without swirling or shaking. Within the range of mutual electrostatic influence of the bullet and target at microscopic level, the initial velocity under thermal influence changes, increasing to higher magnitude due to electrostatic force of attraction. The final velocity → zero as soon as effective collision occurs. Effective collision is one in which enzyme-substrate, drug-pathogen, and drug-poison complex is formed. Random diffusional motion expresses state of entropy such that collision between reactants becomes too probabilistic as against directionality enhanced by, first the terminal velocity and consequently electrostatically induced acceleration that determines the rate of collision. What may be uncertain is the interparticle distance at which mutual electrostatic attraction commences. Thus the value of $u \cong 8.432 \exp(-9)$ m/s at 293.15K may be justified. A simple question is: should the velocity of $Ca^{2+}_{(aq)}$ be < the velocity of a protein molecule under both TFF and electric field force (EFF)?

If interparticle distance → zero, it could be as result of infinite concentration of reaction mixture component. Consequently, random motion of advancing particle as bullet towards a target molecule cannot be the case because there should be much less solvent molecules bombarding the larger solution components. Therefore, it is inconceivable that Einstein model involving the input of mean square displacement should be applicable. Thus when the interparticle distance (l) → nano-scale or less, the time, $t = 2l/u$. The significance of electrostatic attraction can be understood from the perspective of different degrees of temperature dependences of some enzyme, notably the much studied alpha amylase. Psychrophiles are known to show not just much higher activity than their moderate thermophiles or mesophiles and the thermophiles [16] but very high activity. This means that not just temperature is sufficient in the enhancement of catalytic power of enzymes. Despite lower frequency of collision between enzyme and substrate due to lower translational velocity at low temperature unlike at higher temperatures, the rate of enzyme catalyzed hydrolysis of substrate is high because, the enzyme active site exist in stable conformational state with requisite conformational flexibility [16, 17] needed to achieve adequate mutual electrostatic attraction for the substrate. But at higher temperature unfavourable to the psychrophiles, the rate of hydrolysis → zero because higher translational terminal velocity is not enough in the face of the loss of the capacity to electrostatically attract the substrate to active site region due to unfolding. This is unlike the mesophiles and thermophiles that need higher temperatures to lessen the rigidity of the 3-dimensional structure. The important deduction from the foregoing is that complex formation must be preceded by unidirectional motion of a bullet molecule towards the target.

There is no doubt that diffusion is important factor that influence the rate of catalytic action [4, 6, 18-19]. Diffusion has been seen as a vital means by which macromolecules and nanoparticles in the extracellular matrix can deliver therapeutic agents into tumour tissues [19]. Effective rate constants that depend both on the macromolecular-ligand relative diffusion constant and on the substrate and product concentration [19] are ultimately predicated on the terminal translational velocity. There is also the finding that diffusion can be significantly hindered by electrostatic interactions between the diffusing particles and charged components. Consequently neutral particles have been implicated to diffuse faster than charged particles; yet it is suggested that optimal particles for delivery to tumours should be initially cationic to target the tumour vessels and then change to neutral charge after existing the vessel [18]. Several deductions can be made from these finding and proposition. There is attraction between polar (if not charged)

bullet molecule and its target. However, not all targets are desirable and if that is the case there may be delay before the bullet molecule hit the correct target. This hindrance leading to delay is buttressed by Kim and Yethiraj [20] who observed that the reaction rate always decrease as the volume fraction of crowding agents is increased due to reduced diffusion coefficient of reactants. Force of attraction imposes directionality thereby reducing randomness or precisely the entropic factor as may be vividly illustrated with figure (7). If enzyme rotates about its axis [1], it may present its active site to the substrate in a way that can lead to strong enzyme-substrate attraction. There is also the possibility of the active site being deviated from the appropriate effective contact with the substrate justifying the suggestion that, the enzyme must be in specific alignment with substrate to ensure stable complex formation. But if the enzyme rotate about its axis, it must be undergoing translational motion quantified as velocity beginning from the point where $(2Dt)^{½} = t(\xi_p/m_2)^{½}/2$ in the face of mass movement of solution components before specific contact (be it effective or non effective) can be made with the substrate. Rotational motion or diffusion about an axis without linear displacement unlike "rotating wheel of a vehicle" that, can be seen as purely an analogy, may lead to zero collision frequency. A combination of Einstein-Brownian model and classical Newtonian model for the determination of linear displacement linked by translational diffusion coefficient or indirectly by $k_BT/6\pi\eta r_p$ is more appropriate than rotational diffusion coefficient that is not coupled to any form of linear displacement or motion.

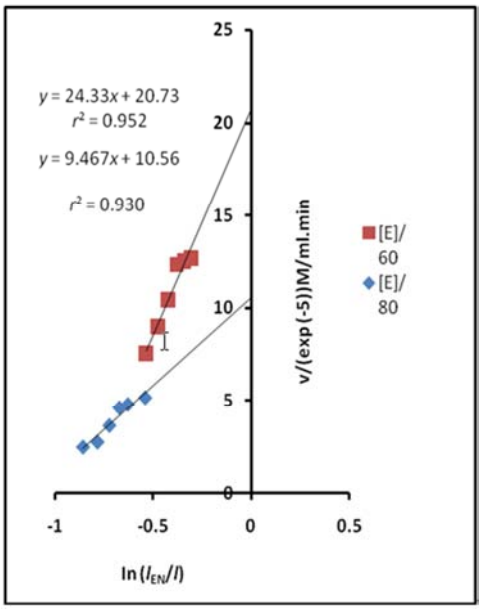

Figure 7. *Plot of velocity (v) of hydrolysis versus natural logarithm of relative interparticle distance (Rel. lnt) illustrating the implication of concentration on reaction velocity. Rel. lnt = l_{EN}/l where for the purpose of description, l_{EN} is the interparticle distance for which the time of coverage based on Einstein and Newtonian model is equal and l is calculated interparticle distance based on reaction volume (V_{ol}), $(V_{ol}/N_A\Sigma n)^{1/3}$ while Σn and N_A are the sum of the number of moles of substrate and enzyme and Avogadro's number; The concentrations of the enzyme is [E]; (◆) and (■) are (0.1g/l)/80 and (0.1g/l)/60 respectively.*

$\ln(l_{EN}/l)$ is thermodynamically analogous to $\Delta S/3k_B$ where ΔS is entropy that results from wide interparticle distance as in diluted solution as against concentrated solution or reaction mixture. Hence there is a negative correlation between v and $\Delta S/3k_B$ or $\ln(l_{EN}/l)$. Specific binding occurs through sites that must be properly aligned for the reaction to occur which has been described in a review by Kim and Yethiraj [20] as anisotropic reactivity. This is partially similar to the claim that a ligand must diffuse into and then through the crevice in order to reach the active site [21]. However, at the point of entry into the active site random diffusional motion must give way to directional motion apart from the compulsory fact that the lighter molecule must be the bullet molecule from microscopic point of view in comparison with the much larger target molecule. For instance molar mass of potato starch is reported to be 1×exp (+6) g/mol [22]. This may however, represent low molecular weight starch compared with 7.62 exp (+7) g/l [23]. *Aspergillus oryzea* alpha amylase with molar mass~52 kDa is several folds (~19-fold) less than value reported for potato starch by Blom and Schwaz [22]. It is better imagined what the case should be if the mass of the enzyme is compared with the molar mass reported for native potato starch by Xie *et al*. [23]. Thus in the face of the same thermal energy, the enzyme should reasonably be the bullet molecule. But this demands that there must be collision made possible by translational velocity (which is ≠ zero before extra accelerative force induced by electrostatic attraction) a vectorial character as against random diffusional motion that lacks unidirectionality. At the point where collision is most probable, Brownian motion gives way to directional motion.

Beginning from possibility to what may be an impossibility, are the values of l_{EN} ≅3.15 exp (-8) m which is < the range, 5.492-7.445 exp (-8) m when [E] = 2.4 exp (-8) mol/l and, ≅ 4.04 exp (-8) m which is also, < the range, 5.49-6.91 exp (-8) m when [E]~3.21mol/l. This is reasonable because it indicates the trend towards an end to randomness or Brownian motion and the beginning of trend in vectorial motion. This further corroborates the fact that there should be reasonable concentration of the substrate at lower range in particular and upper range well above the concentration of the enzyme [24]. The value of l_{EN} above arises from the use of $(\xi_p/m_2)^{½}$. But with the use of $(3k_BT/m_2)^{½}$, l_{EN} ~ 8.97Å. This is a reflection of non-interacting particles that need narrower interparticle distance for attractive interaction to occur as it is the case for gaseous reactant which is totally different from the situation in solution where there is strong solvent-solute interaction in addition to solvent resistance to motion. Hence as stated earlier, it should be understood that $(\xi_p/m_2)^{½} \rightarrow 3D/L$ is evidence of deceleration due to solvent resistance. Therefore, the direct use of $(3k_BT/m_2)^{½}$ may not be applicable to solutes in solution. The values of Σn, [S] and n_s which are approximately 2.57 exp (-8) mol/ml, 25.73 g/l, and 2.57 exp (-8) mol/ml respectively (using $(3k_BT/m_2)^{½}$ to generate figure (3)) remain mere illustration without practicability for reason already adduced; ≅ 1.653 exp (-8) mol/ml, 16.51 g/l (this is ≈ 1.65 times the highest concentration prepared), and 1.651 exp (-8) mol/ml respectively are values resulting from the use

of $(\xi_p/m_2)^{½}$ to generate figure (2) when [E] = 2.4 exp (-8) mol/l and, ~1.480 exp (-8) mol/l, ~14.76 g/l, and 1.476 exp (-8) mol/l when [E] = 3.21 exp (-8) mol/l; what is impossible arising from the use of $l_{EN} \cong 0.036$ m (this amounts to interparticle distance at infinite dilution) is Σn~-0.011 mol/ml. The reason advanced earlier remains tenable.

The question as to what the ratio of enzyme concentration to substrate concentration should be has attracted concern. Huggins and Russell [25] advised that "enzyme preparations are diluted so that not more than 35 mg (≈ 44%) of the starch will be hydrolyzed in the allotted time". Citing other authors, Huggins and Russell [25] report other limits, 40% and 30%. The issue being discussed is also in agreement with the demand that $[E_T]/([S_T] + K\text{m}) \ll 1$ in other that standard quasi-steady state assumptions (sQSSA) be valid. "The agreement between the sQSSA solution and the numerical solution is quite good when $[E_0] \leq 0.01[S_0]$" [26]. But this mainly guarantees linearity at the initial stage in particular where according to Butterworth *et al* [24], kinetic measurements appear to be more accurate. From this result using [E] = 2.4exp(-8) mol/l the lower limit for the concentration of the substrate may be increased to about 5 g/l as one of 7-8 different concentrations in which the upper range may be ≥ 16.51g/l but definitely > the initial 10 g/l. In the same vein, with [E] = 3.2 exp (-8) mol/l, there may be upward adjustment by one g/l in [S] such that the new [S] may range from 6-≥ 14.76 g/l. The alternative is the use of lower [E] (on nano-mole scale) so as to ensure absence of substrate exhaustion.

4. Conclusion

The main objective of this research, the derivation of a mathematical model for the determination of translational velocity (found to be ~8.43 exp (-9) m/s) of solutes in aqueous solution and the minimum interparticle distance covered for which the time determined according to two methods, Einstein approach and Newtonian approach, seems to be emphatically achieved. The kinetic energy of solution components is $\ll 3k_BT$ because of cohesive energy. Bullet-like motion with vectorial character ultimately delivers the enzyme as the bullet to the much larger target-like large molecular weight starch (or starch granule) or drug as bullet targeted against pathogen. At the point where mutual attraction occurs between two solutes, random diffusional motion gives way to directional motion. This is important because it ensures effective collision for complex formation. Further research may be carried out to determine the duration of various aspects of enzyme catalyzed reaction viz: transit of the enzyme before contact with enzyme, bond breaking and bond making-catalysis-, and product departure.

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