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The State of Proteins not with Standing, Translational Velocity is Vital for their Function

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Authors' contributions

This work was carried out in collaboration between both authors. Author IIU conceptualised and derived all equations, analysed and discussed the result while author AOO supervised the experimental process and thesis from where the data was obtained. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Targeting of macromolecules by means of translational diffusion for therapeutic reason is generally of interest. But translational velocity that enables directional delivery of any molecule on target is given less attention.
Objectives: The objectives of this research are to: 1) determine the value of the cohesion factor affecting solutes, 2) determine the translational velocity of porcine pancreatic (PPAA) - and human salivary (HSAA) – alpha amylase and ions, 3) rederive the effective kinetic energy (*K.E.*) of solutes, 4) determine the thermodynamic parameters for a folded to an unfolded transition and 5) give reasons why the velocity of solution components is generally very important.
Methods: A theoretical research and experimentation using Bernfeld method.
Results and Discussion: The *K.E.* of solution components as re-derived is « $3k_BT$ / 2 (where k_B and T are Boltzmann constant and Kelvin temperature respectively.). The velocities of hydrolysis of

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the substrate with the sucrose-treated PPAA were generally higher than those of HSAA. The values of conformational entropy change (ΔS_{conf}) for PPAA were generally higher than those of HSAA. Expectedly the values of ΔS_{conf} were positive.

Conclusion: In conclusion, the square root of the cohesion factor is larger than 22.4 exp (+3) 310.15 K / 273.15 K / 18, accounting for the translational velocity (*u*) in solution being « gas phase velocity (*U*). The translational diffusion *D* and *u* remain respectively, a function of the hydrodynamic radius of the solutes in particular and the magnitude of *D*; unfolding of proteins decreases the values of the parameters. Overall, unfolding is entropy driven. Without the mobility of solution components at desired velocity directional delivery of small molecules to site of need such as intrinsically disordered proteins *etc* may remain impossible.

Keywords: Alpha amylases; thermodynamic parameters; cohesion factor; translational velocity and diffusion coefficient; effective kinetic energy; unfolded and intrinsically disordered proteins.

1. INTRODUCTION

There have been much research activities on the diffusion of macromolecules [1,2]. There could be many reasons for such research activities. Lee [2] noted the difficulty of high molecular weight substrates in reaching the immobilised enzyme via diffusion. The complex and volume-occupied environments which are generally termed 'crowded' and/or 'confined' affects the function of the enzyme [3]. This is explained based on the view that in crowded conditions, an intracellular phenomenon, which can be created *in vitro*, non-specific interactions between macromolecules may hinder diffusion - a major process determining metabolism, transport, and signaling [3]. There are also much concern about the state of proteins (folded, unfolded, misfolded/native-like, and intrinsically disordered proteins) [4-6] under normal and altered biological conditions. However, less concern has been expressed for the translational velocity of the macromolecule. The changes in the velocity of hydrolysis of substrate occasioned by the change in the structure of the enzyme due to the effect of destabilisers and stabilisers and the associated hydrodynamic and thermodynamic changes are adopted as the references for the elucidation of the implication of the abnormal state of proteins as carriers, transporters or vehicles for the transport of drugs to targets. With increase in temperature, molecular motion increases, enabling molecules to slide past each other [7]. In line with kinetic theory, every particle in solution retains its random motion upon attainment of uniform concentration. This implies individual motion which according to Van Oijen [8] cannot be measured. The pathological conditions due to abnormal state of proteins, as reported in literature are Alzheimer's and Parkinson's diseases; type 2 diabetes, cystic fibrosis, and some forms of emphysema etc [9]. It is believed that the adverse conditions are due to proteinaceous deposits in the tissues and organ affected [10-12]. This is obvious because according to Zeleznak and Hoseney [7], much lower kinetic energy (a decrease in velocity in particular) does not allow the molecules to slide pass each other. There are also intrinsically disordered proteins (IDP) some of which have biological function such as cell-signaling and transcription [13,14] while some are implicated in the etiology of several disease states [15]. The protein aggregation presently considered a pathway alternative to protein folding where intermolecular, rather than intramolecular interactions are prevalent [9] cannot permit individualised motion for appropriate function. Intermolecular interaction is very likely when there is a decrease in an intermolecular motion occasioned by increased hydration of misfolded, unfolded, and partially folded protein otherwise called native-like unfolded proteins. Previous attempt in the determination of translational velocity of a protein, both folded and unfolded, seemed to yield an overestimation of what the real value may be [16]. It is on account of this issue that the postulated equation in a research thesis [16] for the determination of the velocity of a solute in an aqueous solution is revisited so as to relate it to another equation in literature [17]. Similar to concern expressed elsewhere [17], it may be assumed that the velocity of hydroxonium ion in solution under electric field gradient is < 4.0 exp (− 7) m/s. Then one may wish to know the likely velocity of a soluble macromolecule like protein in solution under ambient condition. The derived equation is presented in the theoretical section. It is hoped that unfolding and refolding and consequences, changes in translational velocity/diffusion coefficient associated with digestive enzymes can give an insight into the implication of misfolded, unfolded and intrinsically disordered

proteins. Thus, the objectives in this research are to: 1) determine the probable value of the cohesion factor affecting molecular motion in solution, 2) determine the translational velocity of a very important macromolecule, the native and unfolded enzyme and micronutrients (minerals such as calcium, sodium, potassium, hydroxonium, and chloride ions), the cofactors, under ambient assay condition, 3) rederive the effective kinetic energy of soluble particle in solution, 4) determine the thermodynamic parameters implicit in the transition from a folded to an unfolded state of a protein and 5) give reasons why the velocity of solution components is generally very important.

2. THEORETICAL BACKGROUND

In this section a review of literature concerning the equation for the determination of translational velocity of solution component and the effective kinetic energy is to be carried out. In previous research [17], it was postulated that the velocity of any soluble particle in solution is much lower than the velocity of any other particle of the same molar mass in a gas phase under ambient temperature and standard pressure condition.

2.1 Review of Previous Translational Velocity Equation and a Likely Alternative Equation

The following equation may be applicable.

$$u = U/\phi \quad (1)$$

Where u is the reduced velocity of a particle in solution/liquid and U is the velocity if the particle is an ideal gas; ϕ is cohesive factor which reduces the kinetic energy of the particle in solution/liquid. Therefore, U is = $\sqrt{(3k_BT/m)}$ where k_B, T and m is the Boltzmann constant, thermodynamic temperature, and mass of a particle respectively. Then one can postulate that

$$3\, k_BT/\phi^2 d = k_BTu/D \quad (2)$$

Where d is the diameter of the particle or twice the radius (r).

Equation (2) simplifies to

$$1/\phi^2 d = u/D \quad (3)$$

Meanwhile, according to Einstein equation,

$$3\, k_BT/\phi^2 d = 6\pi\eta ru \quad (4a)$$

$$3\, k_BT/\phi^2 = 6\pi\eta ru.\ 2r \quad (4b)$$

Since $r = k_BT/6\pi\eta D$ and substituting the latter into Eq. (4b) gives

$$3\, k_BT/\phi^2 = 6\pi\eta u.2.\ (k_BT/6\pi\eta D)^2 \quad (5)$$

Making u subject of the formula gives

$$u = 3\times 6\pi\eta D^2/2k_BT\phi^2 = 3k_BTD^2/2rD\phi^2k_BT. = 3D/2\phi^2 r \quad (6)$$

The previous speculation is that $\phi^2 = V_M/V_1$ where V_M and V_1 are molar gas volume and volume of water respectively at ambient temperature and pressure. The results from Eq. (6) appear to be an overestimation of the practicable values. In order to relate the effective kinetic energy previously derived [17] to Einstein equation, there is a need to rederive the equation in literature by simply deleting 3 wherever it appears in the series of steps leading to final equation. Doing so, also gives the terminal velocity (u) of dissolved and dispersed solute (or solvent) expressed as $u = D/L$ where $L = V_1^{⅓}$ and V_1 is the molar volume of water if a cube model is adopted.

2.2 Rederivation of Effective Kinetic Energy

The following equations are both re-statement and re-derivation in order to link Einstein original equation for diffusion coefficient in solution given as, $D = \langle X^2\rangle/2t = K_BT/\ 6\pi\eta r$ (where $\langle X^2\rangle$ is the mean square displacement and t is time of transit; η and r are the viscosity coefficient of water and radius of the particle or solute respectively) to effective kinetic energy. First, the interparticle distance is given as in previous publication [17] as:

$$l = \left(\sqrt[3]{D}\right)^2 . \sqrt[3]{\frac{4m_2L}{k_BT}} \quad (7a)$$

Where, l, D, k_B, T, m_2, and L are the interparticle distance that needs to be covered, diffusion coefficient, Boltzmann constant, thermodynamic temperature, mass of a molecule of the solute, and the cube root of the molar volume of the aqueous solvent.

$$= \left(\sqrt[3]{\frac{k_BT}{6\pi\eta r}}\right)^2 . \sqrt[3]{\left(\frac{4m_2L}{6\pi\eta rD}\right)} \quad (7b)$$

Equation (7b) is in line with the original Einstein equation, $6\pi\eta rD = K_BT$ and $D = K_BT/6\pi\eta r$.

Where, η and r are the viscosity coefficient of water and radius of the particle or solute respectively.

Simplification gives

$$= \sqrt[3]{\left(\frac{4m_2L}{D}\right)} . \frac{\left(\sqrt[3]{k_BT}\right)^2}{6\pi\eta r} \quad (7c)$$

Substituting $6\pi\eta rD$ for K_BT gives

$$= \sqrt[3]{\left(\frac{4m_2LD}{6\pi\eta r}\right)} \quad (7d)$$

The solute particle kinetic energy is given as in previous publication [17] as

$$\xi_p = 4m_2D^2/l^2 \quad (8a)$$

Where, the parameter ξ_p is the solute particle kinetic energy.

Substitution of Eq. (7d) into Eq. (8a) gives

$$\xi_P = \frac{4m_2D^2\times\left(\sqrt[3]{(6\pi\eta r)}\right)^2}{\left(\sqrt[3]{4m_2LD}\right)^2} \quad (8b)$$

Simplification gives

$$= \sqrt[3]{4m_2}\left(\sqrt[3]{D}\right)^4\left(\sqrt[3]{\frac{6\pi\eta r}{L}}\right)^2 \quad (8c)$$

Taking D to be $K_BT/ 6\pi\eta r$ and substituting the latter into Eq. (8c) gives after rearrangement

$$= \frac{\sqrt[3]{4m_2} . \left(\sqrt[3]{k_BT}\right)^4}{\left(\sqrt[3]{6\pi\eta rL}\right)^2} \quad (8d)$$

According to Šoltésová et al. [18] several modifications of the equation were proposed, so as to be applicable to small and nonspherical molecules. One of such modification gave the equation such as $D = k_BT/ c\pi\eta r$. Where, c is a numerical factor dependent on the ratio between hydrodynamic radius of the solute and the solvent [18].

In this research such concern regarding size or minimum sphericity of solute molecule is immaterial because whatever alternatives to $c\pi\eta r$, K_BT/D can be substituted into Eq. (8d) to give

$$\xi_P = \sqrt[3]{4m_2}\left(\frac{k_BTD}{L}\right)^{2/3} \quad (8e)$$

It should be unmistakably restated that while generally the kinetic energy of matter may be $3K_BT/2$, such is the case where there is a very weak intermolecular interaction (as may be applicable to ideal gas) be it attractive or repulsive otherwise like ethyl ether, liquid water as well as water in the body fluid will vanish within a short period of time at the speed of a gas. However, Moyer and Abramson [19] referred to a molecule such as a protein molecule as a particle having a mean kinetic energy equal to $3RT/2N_A$, where N_A is Avogadro's number. Thus one may incorrectly opine that the speed of such a molecule should be ridiculously equal to $(3\ RT/M_2)^{½}$.

In a previous publication [17], L was speculatively taken to be $V_1^{⅓}$ where V_1 is the molar volume of water. There is a need to state that miscible solvents like water and ethanol with different boiling points is an expression of different binding energy or cohesive force. Hence at 78ºC, ethanol escapes into vapour phase leaving almost the same volume of water at standard pressure. But for a common salt solution, the evaporation of water at 100ºC leaves behind the entire salt particles; this suggests much greater cohesive force between salt particles than between water molecules. If relevant parameters are substituted into Eq. (8e) it will reveal that the effective kinetic energy is « $3\ K_BT/2$.

Done with what is considered as appropriate equation of effective kinetic energy of solution components, the cohesion factor needs to be determined as follows:

$$\phi^2 = \frac{3k_BT}{\sqrt[3]{4m_2}.\sqrt[3]{(k_BTD/L)^2}} \quad (9a)$$

Simplification gives

$$\phi^2 = 3.\sqrt[3]{k_BTL^2/4\ m_2D^2} \quad (9b)$$

$$\phi = \sqrt[2]{\left(\sqrt[3]{\frac{27\ k_B\ T\ L^2}{4\ m_2D^2}}\right)} \quad (9c)$$

The next derivation is for the determination of d hitherto regarded as a diameter in a research (thesis) [16].

$$\frac{D}{L} = \frac{3D}{\phi^2 d} \quad (10a)$$

Substitution of Eq. (9b) into Eq. (10a) and rearrangement gives

$$d = \sqrt[3]{\frac{4\, m_2 L\, D^2}{k_B\, T}} \quad (10b)$$

The value that is obtainable from Eq. (10b) ≠ *r*. This section ends with the comment that Eq. (9b) only serves to show that the effective kinetic energy is « $3k_BT/2$. The values are necessarily excluded because the translational velocities to be calculated partly illustrate the issue vividly. Equation (9c) is needed in order to compare the result from it with initial result of calculation based on informed assumption in literature [16]. The values of HSAA and PPAA are, to two decimal places, 1501.07 and ~ 1501.07 respectively; both figures are > than (22.4 exp (3) (310.15/273.15)/18)^{½} = 1413.01). Since the values of $D_{310.15K}$ for the native enzymes are > than the values for unfolded, it is obvious that ϕ for the latter should be > than that of the native as expected in the light of Eq. (9c). Since the ions in solution are hydrated their molar mass should be much greater than the dry ionic mass. Until such molar mass is known for each, the value of ϕ cannot be definitely calculated.

2.3 Determination of the Hydrodynamic Radius of Cosolute-Treated Enzyme

In order to roughly estimate the value of the radius of the osmolyte-treated enzyme, the following equation is stated here based on the assumption that the velocity of amylolytic action of the enzyme is directly proportional to translational diffusion coefficient; this is sequel to the proposition that the translational velocity of any solution component towards a target is directly proportional to the translational diffusion coefficient [16,17].

$$V = Ж\, D \quad (11)$$

Where, Ж and *V* are the proportionality constant and velocity of catalytic activity of the enzyme respectively. Meanwhile,

$$r_U = k_BT/6\pi\eta D_U \quad (12)$$

Where r_U and D_U are the radius of the unfolded protein and its diffusion coefficient respectively. If the volume of the unfolded is taken to be equivalent to the volume of a sphere similar to the shape of molten globules [4] the expression for $V_{ol(U)}$ (the volume of the unfolded protein), and $V_{ol(N)}$(the volume of the folded) should be the usual equation given as $4\pi r^3/3$ where *r* can be for the folded and unfolded given respectively as r_n and r_u. This seems to be in line with the view of Fitter [20] regarding *V* which the author sees as the accessible volume of the conformational space occupied by the corresponding state, the folded and the unfolded. The implication is that the equation of the volume for the unfolded is more complicated and needs to be unavoidably given as:

$$V_{ol(U)} = \frac{4\pi}{3}\left(\frac{k_BT}{6\pi\eta D_U}\right)^3 \quad (13)$$

The postulation is that the velocity of catalysis is inversely proportional to the volume of the protein in the presence of additive. One should recall that the volume or area of space occupied by the unfolded enzyme is larger than for the folded enzyme. However, one cannot rule out the possibility of partially folded or partially unfolded enzyme that retains native-like function [4]. The determination of K_{eq} follows after the review of its equation shortly. One of the objectives remains the determination of thermodynamic parameters. Hence the information about the hydrodynamic radius of the folded (extra-folded) and unfolded enzyme is necessary. The equation according to Fitter [20] for the determination of conformational entropy change (ΔS_{conf}) is

$$\Delta S_{conf} = 3RT\ln\frac{r_U}{r_N} \quad (14)$$

Occupying the base line is the native state whose probability is one in the absence of any cosolute. Where a treated enzyme is the case using single or binary mixture of cosolute, there may be fractions of unfolded and folded enzyme; the fraction of the native form may be the concentration available in the reaction mixture due to the effect of protecting osmolyte. Hence, the velocity of catalysis for such treated enzyme may be greater than the untreated native enzyme due to the increase in the population of the native-like enzyme. Therefore, if the fraction of the native-like enzyme is less than 1, then there is a decrease in its concentration and an increase in its conformational entropy.

An increase in the fraction (F_N) of the native-like enzyme is equivalent to an increase in the concentration of the native enzyme; this may be reflected in an increase in the velocity of

amylolysis to values either approaching the native-like velocity without treatment or exceeding the velocity of the native enzyme for the protecting osmolyte-treated enzyme in the presence of a destabilising osmolyte. With the understanding that as the fraction of the folded enzyme increases, the molar concentration of the folded enzyme increases and *vice versa*. As such, the conformational entropy change (ΔS_{conf}) can be given as

$$\Delta S_{conf} = RT \ln \frac{1}{F_N} \quad (15a)$$

$$= RT \ln \frac{1}{1-F_U} \quad (15b)$$

Where, F_U is the fraction of the unfolded enzyme. Therefore, if F_N in Eq. (15a) is increasing for whatever reason, the magnitude of the conformational entropy change should be decreasing; on the contrary if F_N is decreasing, the values of ΔS_{conf} should be increasing. This should be applicable to Eq. (15b) if F_U is increasing. A decrease is expected if F_U is decreasing. This analysis implies that

$$\ln \frac{1}{F_N} = \ln \frac{1}{1-F_U} = \Delta S_{conf} \approx 3RT \ln \frac{r_U}{r_N} \quad (16a)$$

$$\frac{1}{F_N} = \frac{1}{1-F_U} \approx \left(\frac{r_U}{r_N}\right)^3 \quad (16b)$$

Where, r_N is the hydrodynamic radius of the native protein. Equations (16a) and (16b) need to satisfy the condition that unfolding does not necessarily proceed to the secondary let alone primary structure. It is not certain whether extra-folding can reduce the radius of folded protein to values less than the native value. But if so, Eq. (14) where r_U is > r_N, cannot be valid for such situation. Then one may consider equation such as

$$\Delta S_{conf} = RT \ln \frac{r_{<N}}{r_N} \quad (17)$$

Where $r_{<N}$ is the radius of an extraordinarily folded protein to radius less than the native radius. Expectedly the free energy for the transition from folded to unfolded protein requires the determination of the equilibrium constant on the assumption of two state-model.

2.4 Further Insight on the Equation of Equilibrium for the Transition from Folded to Unfolded Protein

The substantial part of this section had been treated in a recent paper [21]. However, minor but very important issue partly based on the original idea of Bolen and Baskakov [22], needs to be examined in order to derive a useful equation of equilibrium. The determination of the fraction of the unfolded enzyme (F_U) and the fraction of the folded enzyme (F_N) depends on if the velocity of hydrolysis for instance, of the native enzyme treated with a stabiliser in a binary mixture of the former and a destabiliser is higher than the velocity of the same enzyme treated with a destabiliser only. It is possible too that despite this condition stated the velocity of catalytic action is lower than the velocity of the untreated native enzyme but higher than the velocity of destabiliser-treated enzyme. As in literature [21], the equations addressing the first case are given as

$$F_N = \frac{V_{OBS} - V_N}{V_{OBS} - V_{MIN}} \quad (18)$$

$$F_U = \frac{V_N - V_{MIN}}{V_{OBS} - V_{MIN}} \quad (19a)$$

$$\frac{F_U}{F_N} = K_{eq} = \frac{V_N - V_{MIN}}{V_{OBS} - V_N} \quad (19b)$$

The order, $V_{OBS} > V_N > V_{MIN}$ may be a possibility. In this case, Eq. (19b) is appropriate; V_{OBS} (the velocity of catalysis as observed) may be decreasing/increasing but it must be > V_N (the velocity of catalysis of the native enzyme) within the concentration range of the cosolute; V_{MIN} is the velocity of catalysis of the unfolded enzyme. The 2nd case is illustrated with

$$F_U = \frac{V_N - V_{OBS}}{V_N - V_{MIN}} \quad (20)$$

$$F_N = \frac{V_{OBS} - V_{MIN}}{V_N - V_{MIN}} \quad (21a)$$

$$K_{eq} = \frac{V_N - V_{OBS}}{V_{OBS} - V_{MIN}} \quad (21b)$$

The catalytic velocity, V_{OBS} for the treated enzyme may < V_N for the native untreated enzyme but > V_{MIN} for the unfolded enzyme. In this case Eq. (21b) is suitable. The value of V_{OBS} may be increasing or decreasing but it must < V_N and > V_{MIN}. The original equation [22] is

$$K_{eq} = \frac{V_{OBS} - V_N}{V_N - V_{Min}} \quad (22)$$

The issue with Eq. (22) is that if the value of V_{OBS} is increasing with increasing concentration of the cosolute and it is always > V_N, the implication is that K_{eq} should also be increasing rather than decrease as expected if the enzyme catalytic

activity is decreasing; increasing K_{eq} (F_U /F_N) means that F_U is increasing even with increasing velocity (v_{OBS}) of catalysis. Thus whenever $v_{OBS} - v_N$ is < $v_N - v_{MIN}$, Eq. (22) should be applicable because in all cases, K_{eq} is < 1 if $F_U < F_N$, and it is > 1 if $F_U > F_N$. The choice of either Eq. (19b) or Eq. (21b) depends on whether or not v_{OBS} is < v_N. The result in this research will clarify the issue shortly.

3. MATERIALS AND METHODS

3.1 Materials

As stated elsewhere [21], the chemicals used were: Sucrose (St Lious France); raw (native) potato starch (Sigma Chemicals Co, USA); ethanol, hydrochloric acid and sodium chloride (BDH Chemical Ltd, Poole England); 3,5-dinitrosalicylic acid (DNA) (Lab Tech Chemicals, India); Tris (Kiran Light Laboratories, USA); porcine pancreatic alpha amylase (EC 3.2.1.1) (Sigma, Adrich, USA); crude human salivary amylase; all other chemicals were of analytical grade and solutions were made in distilled water.

3.2 Methods

As stated elsewhere [21], 0.01 g of PPA was dissolved in 20 mL of distilled water to give 500 μg/L while potato starch was prepared by dissolving 1 g in 90 mL of tris-HCl buffer, 5 mL of 6% (W/W) NaCl (aq.) and 5 mL of distilled water to give 1 g/100 mL. The preparation of crude human salivary alpha amylase is as described elsewhere [23]. The determination of velocity (v) as $C_3 \rightarrow 0$ is as in previous investigation [21]. Assay for the generation of the velocities of the hydrolysis of starch is according to Bernfeld method [24]; 1 mL solution of 3, 5-dihydrosalicylic acid is added to the reaction mixture to terminate the action of the enzyme at the end of the duration of assay. Then the reaction mixture is heated in a water bath for 5 minutes for colour development, cooled in cold water, and diluted with 9 mL of distilled water before taking spectrophotometric reading. Spectrophotometric readings were taken at 540 nm with extinction coefficient equal to 181.1 /M/cm. The Gibbs free energy for the folding to unfolding transition is given as:

$$\Delta G = -RT \ln K_{eq} \quad (23)$$

The determination of entropy change for the same transition is according to Eq. (15 a). Then the enthalpy change (ΔH) is given as:

$$\Delta H = -RT \ln K_{eq} + RT \ln \frac{1}{F_N} \quad (24)$$

The determination of translational diffusion coefficient, on the assumption of the occurrence of a native-like unfolded state with relics of sphericity (*i.e*. the so-called molten globule [4,5]) is determined for the folded and unfolded enzyme according to Einstein-Stokes method as shown in Eq. (10). The translational velocity needed for the vectorial delivery of digested balanced diet and activated drugs to desired target is determined according to the method in literature [17] but in a modified form given as

$$u = D/L \quad (25)$$

3.3 Statistical Analysis

The velocities of hydrolysis were determined in triplicates. A method described by Hozo et al. [25] was used to determine the mean and standard deviation.

4. RESULTS AND DISCUSSION

At this juncture, it has become imperative to state that neither exclusion nor binding interaction does not require any form of translational velocity upon which vectorial targeting of a target (receptor, substrate, toxin, enzyme *etc*) depends. Most importantly is the observation that exclusion may not always be the only way to fold an unfolded protein [26-28].

4.1 Velocity of Hydrolysis of Substrate and Change Resulting from Effect of Osmolyte

The binding of small molecule to misfolded protein is said to promote the folding of misfolded proteins which are forced to take-up definite conformation [26-28]. Both translational velocity and the state of the enzyme can affect the velocity of hydrolysis of the substrate. Generally there was transition from folding to unfolding due to the binding interaction of ethanol which appeared to be generally opposed by sucrose when the protein is in a ternary solution.

The implication of changes in the conformational state (folding to unfolded transition to be specific) of a protein is investigated using the assay of two hydrolytic enzymes as model. The likelihood of misfolding cannot be ruled out, though such is better verified instrumentally. A clear case of the existence of a mixture of an unfolded and a folded enzyme is exemplified by the lower

velocities of hydrolysis of substrate with PPAA (Table 1a) and HSAA (Table 1b).

4.2 The Radii of Unfolded Protein and Corresponding Translational Velocity and Diffusion Coefficient

Following the determination of the hydrodynamic radii of the enzymes according to Eq. (16b), as shown in Table 2a for PPAA and Table 2b for HSAA against the backdrop of velocities of hydrolysis of the substrate, it was possible to determine both translational diffusion and translational velocity of the biomolecules. Based on the well known equation, Einstein-Stokes equation, the translational diffusion coefficient values (Table 3a) and translational velocity values (Table 3b) of unfolded PPAA are lower than those for the folded due expectedly, to the longer hydrodynamic radius of the unfolded enzyme. The translational diffusion and velocity (Table 4 respectively) of HSAA showed similar trend with those of PPAA.

The same parameters where determined for selected important biological ions (Table 4). The interest in translational velocity lies in the fact it is an expression of vectoriality which ends Brownian motion so as to deliver on target; hence the report in literature [29] and similar concern elsewhere [30] which indicates that targeting alpha-synuclein (and perhaps tau and the Ab peptide) by small molecules represents a promising approach to the development of therapeutic treatments of Parkinson's disease and related conditions cannot be an overstatement.

Despite the view that there may be criteria for the dependence of the over-all rate of enzyme catalysed reaction on medium viscosity [31], there seem to be a contradictory suggestion that a number of enzymes will exhibit no appreciable dependence of over-all rate on the medium viscosity. As stated elsewhere [16], where Brownian motion ends at the beginning of any intermolecular electrostatic/hydrophobic perturbative influence, there vectorial motion begins. This is relevant to both diffusion controlled and non-diffusion controlled enzyme catalysed reactions.

The reoccurring word is target which arises if in particular a small molecule is advancing towards a larger molecule. The frequency of collision between the bullet molecule and the target is a function of time which in turn is a function of velocity defined earlier in the text (Eq. (25)). It is not disputable that small molecules in particular are always in random motion in solution and, as such, there cannot always be a direct collision with the target; this implies that longer time is often taken before reaching a target. This is illustrated as follows: In the first place, there is a minimum distance (l_0) between bullet and target particle that elicits mutual perturbative effect leading to either attraction or repulsion. This presupposes minimum bimolecular volume (V_{min}) of the two molecules (the smaller and bigger molecules); but due to random motion the bullet molecules sweeps out a volume (V_{max}) which is »V_{min}. The implication is that, what should have remained the minimum distance, l_0 increases to a maximum distance (l_t). The equation linking these parameters is:

$$l_t = \sqrt[3]{V_{max}/V_{min}} \cdot l_0 \quad (26)$$

If the total time taken to cover such distance (Eq. (26)) is *t*, then Eq. (25) is combined with Eq. (26) to give

$$Dt/L = \sqrt[3]{V_{max}/V_{min}} \cdot l_0 \quad (27)$$

Rearrangement of Eq. (27) gives

$$t = \sqrt[3]{V_{max}/V_{min}} \cdot l_0 L/D \quad (28)$$

The magnitude of l_0 and V_{min} should be seen as a characteristic of a given bullet-target pair so that they are constant parameters. Therefore, the total time expended before collision is directly dependent on the magnitude of the cube root of V_{max} and inversely proportional to *D* (Eq. (28)). It should be emphasised that Einstein relation such as $t = \langle X^2 \rangle / 2D$ which is well known is useful but the time calculated seem to be the time spent over a root mean square displacement unlike Eq. (28) which is relevant to the total time spent over all the distance covered in random motion before hitting the target. Further to this is the observation that the volume (V_{max}) swept out is related to the number of jumps *N* and the area of swath Ã (the collision cross section) by the relation [32].

$$V_{max} = N \check{r} \tilde{A} \quad (29)$$

Where, ř is the average length of a jump. Thus given that *N* ř is total distance covered in a random displacement (or jump) in a total time *t* the velocity is definitely *N* ř/*t* which does not take into account the mean root square displacement. Delivery of digested food and drugs to the

desired site depends ultimately on the velocity of transit.

Citing other workers Loman et al. [1] report that equipment such as dual-focus fluorescence correlation spectroscopy (2fFCS) allows the measurement of precise absolute values of the translational diffusion coefficient of macromolecules close to the infinite dilution limit, while rotational diffusion is usually determined by static or dynamic fluorescence anisotropy measurements. The lack of relevant equipment

Table 1a. Velocity of hydrolysis of starch with PPAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	***v*/U/ml**	***v*/U/ml**	***v*/U/ml**
~1.25	285.00 ± 95	223.00 ± 12.2	219.00 ± 14
~2.40	154.00 ± 0.00	135.00 ±1.3	129.00 ± 7.6
~3.23	120.00 ± 2.8	142.00 ± 4.7	146.00 ± 1.9
~5.28	40.00 ± 5.4	49.00 ± 3.5	64.00 ± 5.5

The data is obtained from the thesis [Udema]. PPAA denotes porcine pancreatic alpha amylase; v denotes the velocity of amylolysis

Table 1b. Velocity of hydrolysis of starch with HSAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	***v*/U/ml**	***v*/U/ml**	***v*/U/ml**
~1.25	114.00 ± 11.8	77.00 ± 10.9	72.00 ± 3.0
~2.40	90.00± 3.3	70.00 ± 23.8	66.00 ± 34.3
~3.23	82.00 ± 12.6	65.00 ± 24.1	61.00 ± 44.6
~5.28	74.00 ± 40.3	61.00 ± 44.6	60.00 ± 40.3

The data is obtained from the thesis [Udema]. HSAA denotes human salivary alpha amylase; v denotes the velocity of amylolysis

Table 2a. Translational diffusion coefficient of PPAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	***D*/10^{-11} m^2/s**	***D*/10^{-11} m^2/s**	***D*/10^{-11} m^2/s**
~1.25	11.24	11.95	11.99
~2.40	9.76	11.20	11.98
~3.23	7.24	-	11.18
~5.28	6.27	-	7.66

D is the translational diffusion coefficient; PPAA denotes porcine pancreatic alpha amylase. The value of D for the native PPAA is ~ 1.39 exp (− 10) m^2/s

Table 2b. Translational velocity of PPAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	***u*/10^{-9} m/s**	***u*/10^{-9} m/s**	***u*/10^{-9} m/s**
~1.25	4.29	5.10	5.12
~2.40	3.73	4.78	5.11
~3.23	3.21	-	4.77
~5.28	2.78	-	3.27

u is the translational velocity of PPAA; PPAA denotes porcine pancreatic alpha amylase. The value of u for the native PPAA is ~ 5.32 exp (− 9) m/s

Table 3a. Translational diffusion coefficient of HSAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	***D*/10^{-11} m^2/s**	***D*/10^{-11} m^2/s**	***D*/10^{-11} m^2/s**
~1.25	12.64	9.37	9.52
~2.40	10.97	8.19	8.72
~3.23	10.07	7.08	7.93
~5.28	6.22	5.84	7.86

D is the translational diffusion coefficient of HSAA; HSAA denotes human salivary alpha amylase. The value of D for the native HSAA is ~ 1.31 exp (– 10) m^2/s

Table 3b. Translational velocity of HSAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	***u*/10^{-9} m/s**	***u*/10^{-9} m/s**	***u*/10^{-9} m/s**
~1.25	4.83	3.58	3.64
~2.40	4.19	3.13	3.33
~3.23	3.84	2.70	3.03
~5.28	2.37	2.23	2.80

u is the translational velocity of HSAA; HSAA denotes human salivary alpha amylase. The value of u for the native HSAA is ~ 4.99 exp (– 9) m/s

Table 4. Calculated translational diffusion coefficient and velocity of selected ions

IONS	$D_{(310.15\ \mathrm{K})}$/exp (-9) m^2/s	$u_{(310.15\ \mathrm{K})}$/ exp (-7) m/s
$H_3O^+_{(aq)}$	~ 12.83	4.89
$K^+_{(aq)}$	2.69	1.03
$Na^+_{(aq)}$	1.83	~0.70
$Ca^{2+}_{(aq)}$	~1.09	~ 0.42
$Cl^-_{(aq)}$	~ 2.79	~ 1.07
$OH^-_{(aq)}$	7.24	2.76

$D_{(310.15\ K)}$ *and* $u_{(310.15\ K)}$ *are diffusion coefficient and translational velocity at 310.15 K*

may be a challenge, but there are theoretical approaches in line with two aspect of this research. In this research translational diffusion given as $D_{trans} = k_B T/6\pi\eta r$ is of greater interest as against rotational diffusion given as $D_{rot} = k_B T/8\pi\eta r_{rot}$ [1].

Tzafriri *et al* [33] found that tissue deployment of slow dissolving crystalline drug particles results in temporally and spatially more uniform drug delivery to interstrut (strut - brace made-up of a rod used to prevent compression in medical practice) zones. This finding may appear to oppose the notion that highly mobile enzyme, drug or substrate is desirable for the optimisation of function. This may not necessarily be the case because slow dissolving drugs possess greater degree of interaction even in solution leading to lower randomness making delivery on target very likely coupled with the sustained concentration gradient. With larger V_{max}, the duration of transit becomes longer for a very soluble solute. For the less soluble, taking longer time for total dissolution, V_{max} may not be very large because of interaction that occurs between the solute molecules. Therefore, speed cannot be totally ignored or precluded. One may add that the importance of translational velocity cannot be overemphasised in that despite many wrong collisions, there are molecular process of a momentary attachment, very rapid rotation, and a moving away unless the specific forces that cause the two surfaces to fit and bind are prevalent [34, 35]. This is where rotational diffusion coefficient becomes relevant. In this research the ions notably Ca^{2+}, Cl^{-1}, H_3O^+, Na^+

etc in their hydrated state have influence on the function of relevant enzymes. Hence their velocities can for instance influence the rate of enzymatic function. This is applicable to ethanol and sucrose whose translational velocities and translational diffusion were not stated in this research.

4.3 Thermodynamic Consideration

However, it needs to be made clear that preferential binding of the stabilising osmolyte is largely unfavourable unlike preferential binding of destabilising osmolyte in line with the principle of solvophobicity and solvophilicity of Bolen and Baskakov [21]. In this research however, the digestive enzymes' response to the presence of a cosolute which can be described as relatively small molecules compared to the macromolecules, is investigated unlike similar issue in literature in which functionally different molecule is the case. Such other molecules are alpha-synuclein (aSyn) [29], transthyretin [26, 27], glucocerebrosidase-17 [28] *etc*. These other molecules are regarded as target for small molecules as to imply a need for binding leading to stability of such misfolded proteins. This again, is against the well known effect of preferential binding which includes unfolding and its concomitant increase in conformational entropy due perhaps to the emergence of substates of the protein. In this regard, the view that the "continuum of models" could be used to describe how the unbound ensemble of a disordered protein is modulated by the binding of a small molecule [36] deserves examination. Also of interest is the view that disordered proteins spans between two extreme cases with intermediate case, depending on whether the conformational entropy of the protein decreases or increases [36]. Koshiba & Kobashigawa [6] refer to intermediate state in the transition from folded to unfolded protein; such intermediate was seen to be native-like in structure. One extreme is the conformational entropy collapse upon binding of the small molecule contrary to the usual effect of preferential binding of osmolytes; the other extreme is a further conformational entropy expansion (more disorder) thus favorably contributing to the free energy of binding of the small molecule which is in line with general effect of destabilisers [37,38]; the intermediate structure is one in which the conformational entropy of the protein remains approximately constant, which is referred to as "isentropic shift" [36]. Recall the issue of molten globules stated earlier [4-6].

Regardless of the presence of destabiliser which may cause unfolding, the model equations *e.g*. Eq. (16b) seems to provide means to calculate the fraction of unfolded protein. Hence as shown in Table 6a, there was increasing radius of the enzyme (PPAA) with increasing concentration of ethanol with each fixed concentration of sucrose. The conformational entropy change for PPAA is shown in Table 6b. Similar trends were observed for HSAA as shown in Table 6a and Table 6b with respect to the increasing radii and conformational entropy change respectively. The magnitudes differ for both enzymes.

There is a need to state categorically against the backdrop of kinetic theory that there is always intermolecular and intramolecular motion at temperatures above absolute zero; the motions may be vibrational within the complex molecular structure and translational. This explains in part, why extreme cold adapted enzymes (from psychrophiles) exhibits conformational flexibility essential for catalytic function [39,40]. Therefore, the presence of protein-unfolding osmolyte merely increases the conformational space of the enzyme; this pre-existing conformational state is also supported by the view that many proteins do not adopt a unique fold in native conditions, but rather exist as an ensemble of distinct conformations in dynamic equilibrium [13,14]. This is in addition to the concern that the structure-function paradigm must be substituted by the structure-(dynamics)-function, as proteins are flexible entities, and thus move intra-molecularly and inter-molecularly [41]. The result in this research merely confirms the fact that excessive unfolding leads to loss of function or at most a decrease in rate at which a function is executed (Table 1). Expectedly the conformational entropy changes for both enzymes were positive. The effect of ethanol even in the presence of sucrose was more pronounced with HSAA (Table 2) than with PPAA (Table 1); this translates into the fact that there was generally longer hydrodynamic radius of HSAA (Table 6a) than PPAA (Table 5a). The same general pattern is observed with respect to the conformational entropy change, Tables 6b and 5b for HSAA and PAA respectively.

The existence of different substates corresponding to an increase in the conformational space may not always be detrimental to function considering the issue of adaptation to different environment [39,40], despite the observed decrease in the velocity of catalytic action of the two enzyme [Tables 1a and

1b] due to the effect of ethanol, a preferentially binding (or targeting) osmolyte. The binding of two molecules occurs spontaneously when it is associated with an overall decrease in free energy ($\Delta G < 0$), (where ΔG indicates the difference between the free energy of the final state and that of the initial state [42]. *Ipso facto*, the value of K_{eq} (U/N) >1 is indicative of the spontaneity of the unfolding pathway. Thus the negative magnitude of the free energy may ultimately determine the magnitude of either exothermicity or the endothermicity of the conformational change. Since unfolding implied in positive conformational change always leads to positive entropy, the magnitude of the exothermic change depends mainly on the magnitude and sign of the free energy. The results for PPAA, Gibbs free energy change and enthalpy change, are shown respectively in Table 8a and Table 8b while the results for HSAA, Gibbs free energy change and enthalpy change, are shown respectively in Table 8a and Table 8b.

The unfolding is more feasible (spontaneous as exemplified by the negative free energies) at higher [ethanol] with lower [sucrose] than at lower [ethanol] (Table 7a). Since the enthalpy changes (Table 7b) were endothermic, it may be inferred that the unfolding was mainly entropy-driven. Unlike the unfolding of PPAA, the unfolding of HSAA was more spontaneous except at lower [ethanol] with the lowest [sucrose] (Table 8a). An apparent paradox seem to be the case considering the known effect of sucrose [43] whose higher concentration within the concentration range in this research could have opposed the effect of ethanol. Like PPAA, unfolding of HSAA can be said to be entropy-driven considering the observation the fact that the enthalpy changes (Table 8b) were all endothermic. This is to imply that the unfolded state is not stable in the presence of a stabiliser, sucrose in this research.

4.4 Usefulness of Translational Velocity

The usefulness of translational velocity for targeting cannot be overemphasised. It is very valuable during and after digestion of food (assimilation), transport into cells and anucleate cell (Red blood corpuscles), activation of drug, drug delivery to targets *etc.* Any drug that is highly mobile and binds to a poison molecule, reducing the mobility of the latter could be one of the useful application of not just diffusion coefficient, but the rate of displacement. This definitely should be of concern to the pharmacokineticist who for instance is concerned with current trends in drug delivery via two approaches such as: Passive and active targeting approaches [44].

Table 5a. Hydrodynamic radius of unfolded PPAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	$r_U/10^{-9}$ m	**$r_U/10^{-9}$ m**	**$r_U/10^{-9}$ m**
~1.25	3.01	2.83	~ 2.88
~2.40	3.46	3.08	2.88
~3.23	4.67	-	3.09
~5.28	5.39	-	4.51

r_U is the hydrodynamic radius of PPAA; PPAA designates porcine pancreatic alpha amylase; the hydrodynamic radius (r_N) of the native enzyme is 2.42 $\times$ 10^{-9} m

Table 5b. Conformational entropy change of PPAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	ΔS_{conf}/ kJ/mol.K	**ΔS_{conf}/ kJ/mol.K**	**ΔS_{conf}/ kJ/mol.K**
~ 1.25	~1.30	0.82	~ 0.96
~ 2.40	2.39	1.49	~ 0.97
~ 3.23	~4.70	-	~ 1.50
~ 5.28	5.81	-	4.42

ΔS_{conf} is the conformational entropy change of PPAA; PPAA designates porcine pancreatic alpha amylase. The dash may be as a result of error in assay or artifact leading to departure from a regular trend observed with other fixed concentration of sucrose

Table 6a. Hydrodynamic radius of unfolded HSAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	$r_U/10^{-9}$ **m**	$r_U/10^{-9}$ **m**	$r_U/10^{-9}$ **m**
~1.25	~ 2.67	3.62	~ 3.57
~2.40	3.07	4.14	3.89
~3.23	~ 3.35	~4.80	4.28
~5.28	5.46	~ 5.82	4.38

r_U is the hydrodynamic radius of HSAA; HSAA denotes human salivary alpha amylase; the hydrodynamic radius (r_N) of the native enzyme is ~ 2.58 × 10^{-9} m

Table 6b. Conformational entropy change of HSAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	ΔS_{conf}/ kJ/mol.K	ΔS_{conf}/ kJ/mol.K	ΔS_{conf}/ kJ/mol.K
~ 1.25	0.12	~ 2.49	~ 2.37
~ 2.40	~ 1.22	3.53	3.04
~ 3.23	1.88	~ 4.66	3.78
~ 5.28	5.66	6.15	~ 3.96

ΔS_{conf} is the conformational entropy change of HSAA; HSAA denotes human salivary alpha amylase

Table 7a. Calculated gibbs free energy change for the folding-unfolding transition of PPAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	ΔG/ kJ/mol	ΔG/ kJ/mol	ΔG/ kJ/mol
~ 1.25	1.09	4.58	~ 2.06
~ 2.40	– 1.09	~ 0.64	2.03
~ 3.23	–4.24	-	0.61
~ 5.28	– 5.53	-	– 0.51

ΔG is the calculated Gibbs free energy change of PPAA; PPAA denotes porcine pancreatic alpha amylase

Table 7b. Calculated enthalpy change for the folding-unfolding transition of PPAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	ΔH/ kJ/mol	ΔH/ kJ/mol	ΔH/ kJ/mol
~ 1.25	~ 2.39	5.40	~ 3.02
~ 2.40	~1.30	~ 2.13	~ 3.00
~ 3.23	0.45	-	2.11
~ 5.28	~ 0.29	-	0.51

ΔH is the calculated enthalpy change of PPAA; PPAA denotes porcine pancreatic alpha amylase

No form of intermolecular interaction, be it attractive, repulsive, hydrophobic etc can ensue outside the region of mutual perturbative influence. The quantification of interactions (which is a function of vectorial motion) between biomolecules is essential for understanding the molecular basis of biological processes (rate of digestion, passive transport *etc*) [45]. Here there is need to define interaction in the light of Kirkwood-Buff solution theory [46]. This implies that such interaction may be negative or positive; however, since targeting is of interest the interaction needs to be positive being a case of preferential binding to the target. Here again, a

Table 8a. Calculated gibbs free energy change for the folding-unfolding transition of HSAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	Δ*G*/ kJ/mol	**Δ*G*/ kJ/mol**	**Δ*G*/ kJ/mol**
~ 1.25	~ 7.87	−1.25	~ −1.06
~ 2.40	1.30	− 2.77	~ − 2.10
~ 3.23	− 0.18	− ~4.20	− 3.11
~ 5.28	− 5.36	− 0.25	− 3.33

ΔG is the calculated Gibbs free energy change of HSAA; HSAA denotes human salivary alpha amylase

Table 8b. Calculated enthalpy change for the folding-unfolding transition of HSAA as a function of [Ethanol] with different fixed [Sucrose]

[Ethanol]/mol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L	[Sucrose]/ mmol/L
	3.57	**~14.29**	**28.57**
	Δ*H* / kJ/mol	**Δ*H* / kJ/mol**	**Δ*H* / kJ/mol**
~ 1.25	~ 7.99	~1.24	1.31
~ 2.40	2.52	~ 0.76	~ 0.95
~ 3.23	~1.70	~ 0.42	~ 0.68
~ 5.28	0.30	~ 0.25	~ 0.63

ΔH is the enthalpy change of HSAA; HSAA denotes human salivary alpha amylase

target cannot be reached without translational motion of the bullet molecule. Hence a great concern has be shown via both original research and review in the area of drug delivery [47] which is impossible without translational velocity. Hence the claim that the amount of cellular uptake of nanoparticles in upright and inverted cultures depended on the rate of diffusion/sedimentation of the nanoparticles has become very pertinent [48].

One way in which ligands may bind disordered regions, consists of using relatively weak enthalpic interactions to ensure specificity, but relying on entropic factors for increasing the binding free energy" [42]. The simple mathematical model applied in this research may be useful in this regard for the determination of the conformational entropy change. In order words since aggregates are inimical to the biological function of protein, a recipe for organic diseases, conformational flexibility occasioned by the binding of such molecules to the aggregates may be correctively useful for biological function and health [42]. It is not certain however, if a psychoactive drug such as ethanol can serve such purpose as to promote the axiom that a little wine is good for the body. If this axiom is applicable, both alcohol and aldehyde dehydrogenases may not allow a prolonged life-span of ethanol for the actualisation of its effect on protein aggregates.

Ethanol diffuses faster into the blood stream if taken before meal or long after meal [49-51]; this may account for long residence time of alcohol (ethanol) in various section of the small intestine [52]. The benefit of this information is that strong alcoholic beverage, both local and foreign needs to be rationally fortified with nutritional materials like partially digested protein apart from carbohydrate such as sucrose, that can preferentially bind the molecules of ethanol, retaining them in the small intestine for a reasonable period of time; this might reduce the intoxicating effect of ethanol since its mobility may be reduced. This suggestion remains speculative until further investigation at *in vitro* and clinical level.

5. CONCLUSION

In conclusion, this research has shown that, the square root of the cohesion factor is larger than 22.4 exp (+3) T_i / 273.15 / 18, accounting for the translational velocity (u) in solution being « gas phase velocity (u_{gas}). The translational diffusion D and u remain respectively, a function of the hydrodynamic radius of the solutes in particular and the magnitude of D; unfolding of proteins decreases the values of the parameters. The derived *K.E.* remains « $3k_BT$ / 2. The spontaneity of unfolding depends on the positive magnitude of ΔS_{conf}; the enthalpic dependence of unfolding also depends on magnitude of ΔS_{conf}; but overall,

unfolding is entropy driven. Without the mobility of solution components at desired velocity directional delivery of end product of digestion, delivery of drugs or small molecules to site of need such as intrinsically disordered proteins *etc* may remain impossible.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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