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A conceptual electrophoresis-mediated protein identification method by frictional coefficient during motion under electrical and magnetic interference

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ABSTRACT

Protein Identification (PI) based on conventional Mass Spectrometry (MS) is prone to errors. These errors are a result of the inability to solubilize the protein, interference of detergents, usage of wrong protease enzymes, inability to cleavage of the target protein by proteases, and interference of endogenous or exogenous contaminant molecules and calibrants. Conventional Mass Spectrometric PI is solely based on ionization and mass trajectory of protein in a vacuum. Therefore, it is crucial to develop and modify the existing technique not to be entirely dependent on the ionization and mass of the protein molecule in a vacuum. Other than the mass and an overall electrostatic charge of a protein, characteristic steric hindrance on a protein while travelling through a Polyacrylamide Gel matrix, can be used for accurate identification of proteins in Mass Spectrometry. A concept for a modified Mass Spectrometric method based on a Polyacrylamide Gel platform, to utilize the steric hindrance and friction coefficient of the moving protein under the influence of electric field and magnetic fields is proposed in the article. The theoretical approach of the concept is based on the laws of motion, electrical fields, electromagnetism, and Fleming's Left Hand Rule. In this concept, on a Polyacrylamide Gel platform, in an electric field, protein is accelerated to a constant velocity characteristic of the protein introduced to a magnetic field where the radius of the circular trajectory and ratio of mass and overall surface charge of the accelerated protein is measured. This article directs attention to the modification of Mass Spectrometry to improve reliability and accuracy in protein identification.

KEYWORDS:

Mass Spectrometry, Lorentz Equation, Fleming's Left-Hand Rule, Centrifugal Force, Steric Hindrance, Ultra-Thin Polyacrylamide Gel Electrophoresis, Friction Coefficient, Electrophoretic Mobility

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Introduction

Mass Spectrometry (MS) is a methodology widely used in the identification of proteins. In MS, proteins are subjected to ionization and their mass is calculated depending on their trajectory of them (proteins), in a vacuum (Steen & Mann, 2004). But it is stated that intact proteins are very difficult to work with and peptides are used in conventional MS analysis rather than using the intact protein. Other than this, protein is difficult to handle and there are reports of detergents used to solubilize the protein interfering with MS. This may result in wrong identification using conventional MS for proteins. If the researcher needs to identify a specific protein, data regarding the sequence information will be needed and the limit where MS is most efficient in retrieving sequence information is up to ~ 20 amino acid residues long (Fenn et al., 1989; Horn et al., 2000; Mørtz et al., 1996; Sze et al., 2002). Information obtained from tandem-MS spectra is often incomplete and the intervening peaks which may or may not be belonging to the analysis of the spectra might render confusing and misleading results. As an example, a mass difference of 114 Da can be found between the two large peaks of the output spectra, but a very small peak corresponding to 57 Da can also be found between the two large peaks of the spectra. This specific region or region part of the spectrum might be corresponding to the amino acid of one Asparagine (residue mass = 114 Da) or two amino acids of Glycine (residue mass = 57 Da) (Bustamante et al., 2005). During practical scenarios, as computer algorithms are not reliable in determining the amino acid sequence of the proteins, experts can only interpret at least certain parts of the tandem-MS spectra. (Bustamante et al., 2005). Proteins are digested before analysis and identification using conventional MS using protease enzymes (Bustamante et al., 2005). Usage of the wrong protease enzyme for the cleavage of proteins is a major limiting factor in the MS identification of proteins (Lubec & Afjehi-Sadat, 2007). Several researchers even try to overcome the limitations incurred during the digestion of hydrophobic residues (Fischer & Poetsch, 2006). It is possible that all proposed enzymes used in conventional MS identification may fail to cleave an individual intact protein properly by forcing the researchers to utilize chemical cleavage (Li et al., 2001; Van Montfort et al., 2002). During conventional MS identification of proteins, several endogenous and exogenous contaminant molecules can hinder the identification process (Lubec & Afjehi-Sadat, 2007). These contaminants can introduce chemical noise (Krutchinsky & Chait, 2002). Internal or external calibrants with known or calculated molecular masses are used in all conventional MS methods and all existing MS are dependent on calibrant molecules (Bantscheff et al., 2002; Chamrad et al., 2003; Gobom et al., 2002; Levander et al., 2004; Moskovets et al., 2003). Miscalibration is one of the major drawbacks in protein identification by existing MS methods. Sometimes, the signal of calibrants used may get masked by the analyte peptides/proteins or the calibrant signal may get partially overlapped with the analyte signal. This results in a false assignment of spectra (Lubec & Afjehi-Sadat, 2007). In general Mass Spectrometers in today's use measure the mass-to-charge ratio (m/z) of the proteins that are ionized in gaseous phases. But since proteins and peptides are non-volatile and polar biomolecules, specialized procedures are implemented to transfer proteins and peptides to the gas phase without extensive degradation of the

molecule. Advanced methods like Matrix Laser-Assisted Desorption Ionization (MALDI) require peptide molecules to be co-crystallized onto a metal plate with a solid phase matrix. There are several challenging frontiers with regard to the MALDI technology. One of them is its bias towards low molecular compounds (Noberini., 2016). Apart from MALDI, Fourier Transform Ion Cyclotron Resonance (FTICR) Mass Spectrometry has shown some unreliability in producing detectable signals. On certain occasions, even molecules with the same mass-charge ratio (m/z) and initial velocity are created at random time intervals and in random phases. In other words, at random points on their circular trajectory in the magnetic field. Therefore, the incoherent motion of such molecules is unable to produce a macroscopic signal which can be detected (Knight et al., 1985).

During the analysis of biologically related proteins such as the analysis of chemokine oligomers, MS is used to measure the mass-to-charge ratio in the gaseous phase. However, to obtain reliable information on the chemokines to be biologically significant, oligomers that are in the solution should be transferred to the gas phase intact. This is achieved by the technique named "electrospray" (Rostom & Robinson, 1999). In addition, the parameters related to the ionization source of MS should be tuned to make the transfer of the protein from the solution to the gas phase to be gentle to the maximum extent. During Mass Spectrometry, ions are collided with the neutral gas molecule to facilitate the desolvation of the ions. But this may disassemble non-covalent complexes too. Lowering the energy packed in the collisions of the ions with the neutral gas molecules helps to maintain the native complex during the transfer of the proteins from solution to gas phase intact. But this will reduce the sensitivity of the procedure. Lowering the desolvation potential may result in the formation of protein solvent clusters. The formation of solvent clusters retains several solvent molecules and buffer ions bound to the protein or the polypeptide. This phenomenon creates the serious issue of proteins having several mass-to-charge ratios at the same time, resulting in broad and less resolved peaks in the spectrum. Another problem is that the folded proteins or polypeptides retain less charge than the unfolded or linear proteins. This results in a much higher mass-to-charge ratio in folded proteins or polypeptides. These mass-to-charge ratios are above the scan range of many Mass Spectrometers available on the commercial scale. To adapt the commercially available Mass Spectrometers to scan these higher mass-to-charge ratios, some specialized software and hardware are needed (Sobott et al., 2002).

Above mentioned drawbacks are evident in protein identification by conventional Mass Spectrometry, and it is crucial to modify the existing Mass Spectrometric technique to minimize the interference of calibrant molecules, dependency on sequence information available on computer algorithms, reducing the utility of proteases for pretreatment of the protein, minimize chemical noise by contaminant molecules, minimize the interference of detergents in identification. Furthermore, the proposed methodology of this article does not demand or require specialized software or hardware to operate (Sobott et al., 2002). The proposed method of the article eliminates the necessity of transformation of the proteins in the native solvent to the gaseous phase intactly eliminates the bonding of the proteins or polypeptides with solvent molecules and buffer ions (Wang et al., 2013). The proposed

methodology in this article eliminates the extensive procedure such as co-crystallizing the protein into a metal plate. Techniques such as FTICR have more than one detectable peak for the same compound use to the dependency on the mass-to -charge ratio of the protein molecule. Other than the molecular mass of polypeptides and proteins and the charge of the ionized protein, characteristic steric hindrance created on protein while moving through a Polyacrylamide Gel matrix, can be used in Mass Spectrometry. Since each protein has its molecular size, shape and characteristic residues that may interfere with the Polyacrylamide Gel matrix, it is an excellent parameter that can be used for the identification of proteins. Steric hindrance creates friction on the protein molecule and its surrounding liquid. The friction coefficient created by the protein molecule on its surrounding in a Polyacrylamide Gel environment is characteristic of a protein molecule. There is a research gap for modifications of existing Mass Spectrometric methods for protein identification and incorporation of new parameters of proteins during identification. The concept proposed in this article relies on additional parameters such as the frictional coefficient of peptide or protein molecule in combination with the behaviour of peptides and proteins under the influence of electric and magnetic fields, other than the mass-to-charge ratio that is abundantly used in modern Mass Spectrometric techniques in protein identification. Furthermore, the proposed methodology completely eliminates the use of mass-to-charge ratio of a protein during its identification. The proposed concept in this article is based on the theoretical foundations of forces and equilibriums generated on the protein molecule under the influence of electric and magnetic fields, and protein- Polyacrylamide Gel interactions.

Purpose

The purpose of this concept article is to introduce a modification to Mass Spectrometric principles by incorporating characteristic forces acting upon protein molecules moving in a Polyacrylamide Gel matrix for accurate identification of proteins. The proposed method uses the friction coefficient of the protein in a Polyacrylamide environment to identify the protein. This concept article intends to minimize the gap for the development of new protein identification techniques and modification of existing MS technologies. Furthermore, directing attention towards the importance of protein Polyacrylamide Gel interactions and potential uses of these interactions to improve the accuracy and reproducibility of molecular biological techniques is also a secondary purpose of this concept article.

Background and Rationale

Proteins with an overall induced or native electrostatic charge can move within an induced electric field during Gel Electrophoresis (Bustamante et al., 2005). Therefore, an accelerated charged protein molecule moving at a constant velocity entering a strong magnetic field acts as excellent candidate molecule that can perform a circular motion inside a magnetic field. When the charged protein molecule moves in the electric field, the electric field exerts an external force on the charged protein molecule forcing the protein molecule to accelerate in the electric field (Coronel-Escamilla et al., 2016). If the accelerated protein with an entering velocity is introduced to a magnetic field, an external force acts on the

charged protein molecule by the magnetic field, where the motion of the charged protein molecule is governed by the Lorentz equation (Giuliani, 2008). Due to the motion of the charged protein molecule due to the magnetic field, an external force acts perpendicular to the direction of motion of the charged protein molecules as dictated by Fleming's Left Hand Rule (Wang et al., 2013). Therefore, the behaviour exerted by the charged protein molecule inside the magnetic field can be defined as a circular motion (Gradov et al., 1995). If the charged protein molecule is entering into the magnetic field from outside of the field, the protein molecule can perform only a segment (arc movement) of a circular motion. If the protein molecule is projected to the magnetic field within the magnetic field itself, it can perform a full circular trajectory. The charged protein molecule can perform the full circular trajectory only if the magnetic field is distributed in a large surface area.

For accurate prediction of the trajectory of the charged protein, alongside the above-mentioned external forces, protein – Polyacrylamide Gel matrix interactions should be taken into consideration (Viovy, 2000). Based on the above predictions, manipulations on biomolecules such as proteins are performed under the influence of electric and magnetic fields (Coronel-Escamilla et al., 2016).

Based on the above theory, a hypothesis was formed; "if protein a molecule moving in a Polyacrylamide Gel matrix, shows measurable changes characteristic to the protein under the influence of electric and electromagnetic fields, those measurable changes can be used for identification of the protein".

A platform to observe the measurable changes of the protein molecule in electric and magnetic fields should be formed. A Polyacrylamide Gel platform can be used in the proposed method of this article. Several parameters were taken into consideration when proposing the Gel platform. Compatibility with proteins that is subjected to identification, compatibility with the detection method that is being used to identify the trajectory of the protein in the magnetic field, simple method of preparation and manipulation of the Gel platform. Among the number of polymers used for Electrophoresis, Polyacrylamide is proposed to be the polymer of interest. Polyacrylamide is a neutral polymer in nature and Gel composition has a few per cent of cross-linkers (e.g., Bis-acrylamide) (Righetti, 1989; Viovy, 2000).

To apply the above-mentioned principles, a protein molecule should have an overall charge. Since some proteins do not possess a strong overall charge, a strong overall charge should be induced in the protein molecule. A strong surfactant molecule can be used to induce an overall electric charge to the protein. Sodium Dodecyl Sulphate (SDS) was recognized to be the most suitable surfactant to be used for this. SDS transforms the protein molecule into a linear uniformly negatively charged polyelectrolyte to be used for the analysis. Apart from that, SDS possess highly effective protein solubilization properties. Millimolar concentrations of SDS have the ability to bind to proteins (Long & Ajdari, 1996; Michaux et al., 2008)

Before charged protein molecule with a mass m_p, enters the magnetic field and performs the circular trajectory, the protein molecule should in accelerated from a stationary state to the entering constant velocity (V_p) characteristic of the protein. Therefore, consider

a protein molecule subjected to an external electric field with the magnitude of E_p, in a Polyacrylamide Gel platform where the SDS-induced overall charge q_p. The charged protein molecule travels from the negative electrode to the positive electrode of the electric field. Apart from the external force acting on the charged protein molecule by the electric field, additional interferences on the protein are created by the Polyacrylamide polymer molecules. During the subjection of a protein molecule to an external electric field in a Polyacrylamide Gel platform, the protein molecule is subjected to friction by the surrounding fluid and steric forces created by the Polyacrylamide Gel platform also (Viovy, 2000).

Electrophoretic Force (F_{el}) acting on the protein molecule is given by,

$$F_{el} = q_p E_p \quad \text{---------(1)}$$

By Newtonian laws of motion, since F_{el} is acting on the protein molecule, the protein gains an acceleration (a_{el}).

$$m_p a_{el} = q_p E_p \text{ and } a_e l = \frac{q_{pE_p}}{m_p} \quad \text{-------(2)}$$

To get the initial velocity distribution and initial acceleration of the charged proteins in the electric field, consider the protein molecule travels in the Polyacrylamide Gel a total distance of L.

The total length (L) of the Polyacrylamide Gel that the charged protein molecule migrates is divided into n number of smaller units of equal lengths. The length of a smaller unit is l_S. All the small units of lengths are numbered $l_s^1, l_s^2, l_s^3 \ldots \ldots \ldots l_s^n$. .

$L = nls$ -----(3)

If the time taken by the charged protein molecule to migrate the first unit length (l_s^1), is t_s^1. Therefore, the velocity V_p^1, for this length of (l_s^1), of the charged protein molecule is,

$$V_p = \frac{l_s^1}{t_s^1} \quad \text{---------(4)}$$

Likewise, for all the n number of length units, the velocity for each length can be given (from V_p^1 *to* V_p^n). To measure the overall total acceleration (a_O) of the charged protein in the Polyacrylamide Gel, the following relationship is used.

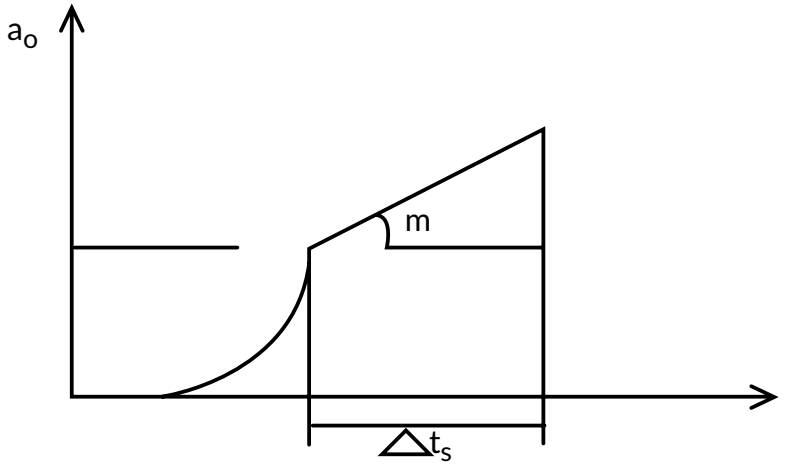
$$a_0 = \frac{(V_p^n - V_p^{n-1}) + (V_p^{n-1} - V_p^{n-2}) + (V_p^{n-2} - V_p^{n-3}) \ldots\ldots\ldots\ldots\ldots (V_p^2 - V_p^1)}{t_s^1 + t_s^2 + t_s^3 + t_s^4 + \cdots \ldots\ldots\ldots t_s^n} \quad \text{-------(5)}$$

Total acceleration achieved by the protein can also be given as the sum of all unitary accelerations $(a_0^1 \; to \; a_0^n)$ of the protein within l_s units.

$$a_0 = a_0^1 + a_0^1 + a_0^2 + a_0^3 + a_0^4 \ldots\ldots\ldots\ldots a_0^n \quad \text{-------(6)}$$

By examining the unitary accelerations, the time in which the charged protein molecule gained constant velocity can be recorded and that velocity is considered as the entering constant velocity of Vp.

Figure 3.1- Gradient m gives the constant velocity of the charged protein molecule, and the linear graph gives the time it reached constant velocity. Δt_s indicates the time duration that the protein molecule was at the constant velocity.



When the protein molecule accelerated to the final constant entering velocity of V_p in the Polyacrylamide Gel matrix can then enter the strong magnetic field. Moving protein with an overall charge of q_p through a magnetic field within a time of tm is like an electric current (l_p). Since protein molecules are negatively charged (negative charges are induced by SDS), the direction of the current (l_p) is opposite to the direction of movement of the protein.

$$I_p = \frac{I_p}{t_m} \quad \text{--------(7)}$$

Consider a charged protein molecule with Sodium Dodecyl Sulphate (SDS-induced) induced overall charge of q_p and, mass mp, entering a strong homogenous magnetic field B_o with a constant velocity of V_p. If the protein molecule enters with an entering angle of Θ_p, goes l distance within a time te, inside the strong magnetic field B_o, exerting an electric current of l_p [from equation (7)], it is evident that force exerted on the protein (F_m) is,

$$F_m = B_0 I_p l \sin\Theta_p \quad \text{---------- (8) (Giuliani, 2008)}$$

Constant velocity (V_p) of the protein molecule into the strong magnetic field can be defined as; distance travelled (l)within a time (t_e) as,

$$V_p = l \times t_e \quad \text{----------(9),}$$

Therefore,

$$l = V_p \times t_e \quad \text{---------(10),}$$

And if the protein molecule enters perpendicular to the strong magnetic field B_o, $\Theta = 90^0$, and $\sin 90^0 = 1$,

From equations (8), (9) and (10), it can be derived that,

$$F_m = B_o \times q_p\, t_e \times V_p\, t_e \sin \Theta_p \quad \text{----------(11),}$$

Therefore, when the charged protein molecule enters the magnetic field perpendicular to it, the external force (F_m) applied on the protein molecule is given by,

$$F_m = B_0 q_p V_p \quad \text{----------(12)}$$

Polyacrylamide Gel matrix should be uniformly spread throughout the region of the magnetic field used in the experimental set-up. The region where the protein molecule enters the magnetic field from the electric field is termed the "horizon", in this article.

As explained above, the behaviour of the charged protein molecule is predicted to be the behaviour of a charged particle governed by the Lorentz equation and Fleming's Left Hand Rule (Coronel-Escamilla et al., 2016; Giuliani, 2008; Q. Wang et al., 2016; Wang, et al., 2013). But certain modifications and introduction of new forces acting on the protein and surrounding fluid were introduced, as described by Viovy, (2000), in equations (12), (13) and (14s) below. Charged protein molecule performs a circular motion inside the strong magnetic field. When the protein molecule is introduced within the strong magnetic field, the protein molecule is only expected to perform a full circular trajectory. Since the proposed method only expects to perform a segment of the trajectory of the protein movement, protein is introduced into the strong magnetic field from outside of the field.

The motion of the charged protein molecule and the surrounding fluid in the Polyacrylamide Gel matrix is subjected to an external force (F_{ext}) and an electric field (E_p), is obtained by superimposing flows obtained in pure electrophoretic ($F_{ext} = 0$) environment and pure non-electrophoretic environment ($E_p = 0$). The mobility of the protein is given by the balance of forces, where μ0 indicates the electrophoretic mobility of the protein in a free liquid and $\mathcal{S}$ indicates the frictional coefficient of the protein (Viovy, 2000).

$$F_{ext} - \mathcal{S}\,(V_p - \mu_0 E_p) = 0 \quad \text{--------(13)}$$

Since the protein is now in non-electrophoretic environments ($E_p = 0$), F_{ext} becomes, as follows,

$$F_{ext} = \mathcal{S} V_p \quad \text{---------(14)}$$

From equations (13) and (14), the resultant force(F_R) acting on the protein molecule can be given as,

$$F_R = (F_m) - (F_f) \text{-------(15) or } F_R = B_0 q_p V_p - \mathcal{S} V_p$$

Based on equation (11) and the effect of Fleming's Left Hand Rule, the protein molecule is moving in a circular trajectory, where the radius of the trajectory is R_p, inside the magnetic field. Therefore, the centrifugal force acting on the protein molecule is,

$$B_0 q_p V_p = \frac{mpVp2}{R_p} \quad \text{--------(16)}$$

Therefore, $R_p = \frac{mpVp}{B_0}$ --------(17)

During the protein's circular motion trajectory, where the radius of the trajectory is R_p, and the constant velocity of the protein is V_p, is under an equilibrium, centrifugal force (F_c) acting upon the protein is equal to the resultant force(F_r) on the protein molecule.

Therefore,

$$F_R = \frac{mpVp2}{Rp} \quad \text{------(18), or}$$

$$B_0 q_p V_p - \text{Ƨ} V_p = \frac{mpVp2}{Rp} \text{ or } Vp\,(B_0 q_p - \text{Ƨ}) = \frac{mpVp2}{Rp}$$

Equation (18) can be rearranged as, $B_0 q_p V_p - \text{Ƨ} V_p = \frac{mpVp2}{Rp}$

Divide both sides by V_p,

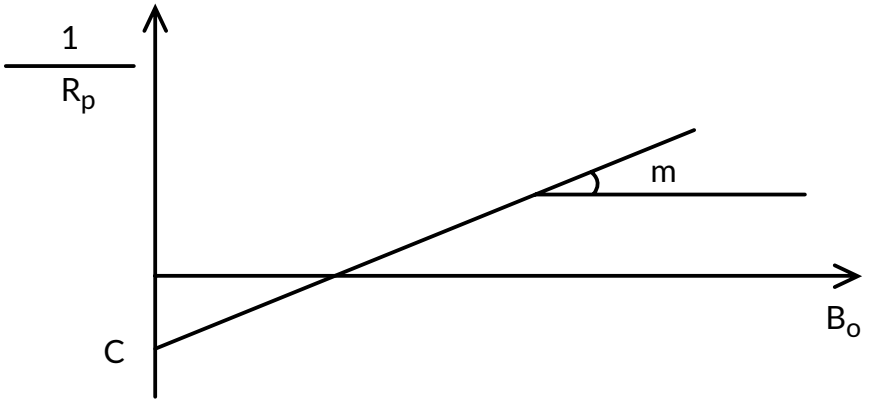
$$B_0 q_p - \text{Ƨ} = m_p V_p R_p \quad \text{------(19)}$$

Divide both sides of equation (19) by $m_p V_p$,

$$\frac{1}{Rp} = B_0 q_p\, m_p V_p - \text{Ƨ}\, m_p V_p \quad \text{--------(20)}$$

Equation (20) is in the form of $y = mx - c$ linear graph equation.

Figure 3.2- The expected linear graph of the magnitude of the electromagnetic field against the radius of the circular trajectory of the charged protein. Intersection c is $\text{Ƨ}\, m_p V_p$ and the gradient m is $B_o\, q_p\, m_p\, V_p$



Based on equation (20), the magnetic field of B_o can be changed and the radius R_p of the circular trajectory of protein can be measured $(\frac{1}{Rp})$. A linear graph can be constructed using data and gradient m and intersection c of the graph is,

$$m = B_0 q_p m_p V_p \quad \text{--------(21)}$$

$$c = \text{Ƨ}\, m_p V_p \quad \text{-------(22)}$$

Using gradient m, the overall charge of the protein induced by SDS can be calculated.

Divide the intersection c by gradient m,

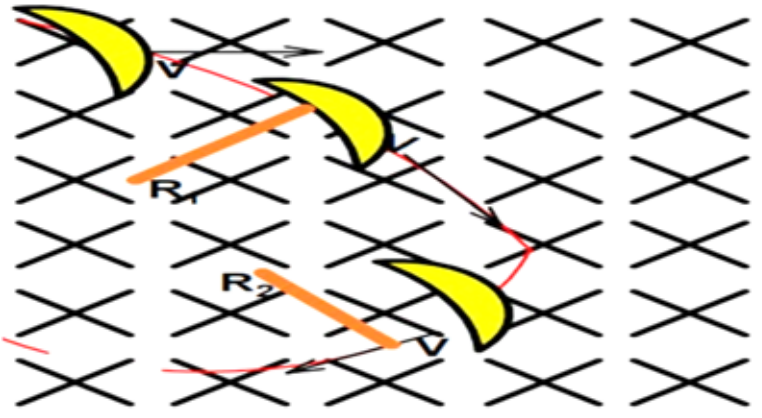
$$\frac{c}{m} = \frac{8\,mpVp}{B_0 q_p m_p V_p} \quad \text{---------(23)}$$

Therefore,

$$\frac{c}{m} = \frac{8}{B_0 q_p} \quad \text{--------(24)}$$

Therefore, using equation (23) the experimental value for the frictional coefficient (8) of the charged protein molecule that needed to be identified is calculated. Reference values of friction coefficient (8) can be obtained for known proteins and the values can be used for the identification purposes and characterization purposes of an unknown protein. Because the friction coefficient (8) of a protein is characteristic of a protein, it is an excellent parameter to be used to identification of an unknown protein.

Figure 3.3-R_1 and R_2 are the radii of the trajectory of the moving protein (yellow colour) in velocity of V in changing magnetic field.



Based on the above-mentioned hypothesis, equations and equilibriums, a suitable methodology is proposed.

Methodology

Architecture of the Protein Friction Coefficient (8) Measurement Assembly

The proposed system of the concept article has two main counterparts. They are,

(1) SDS PAGE Protein Acceleration Set Up; To accelerate the protein solution to enter constant velocity into the magnetic field.

(2) Electromagnet System; Used to create the magnetism for the measurement of the radius of the circular trajectory of the protein molecule. A Polyacrylamide Gel slab is spreading from the loading wells of the SDS PAGE Protein Acceleration Set Up to the Electromagnet System.

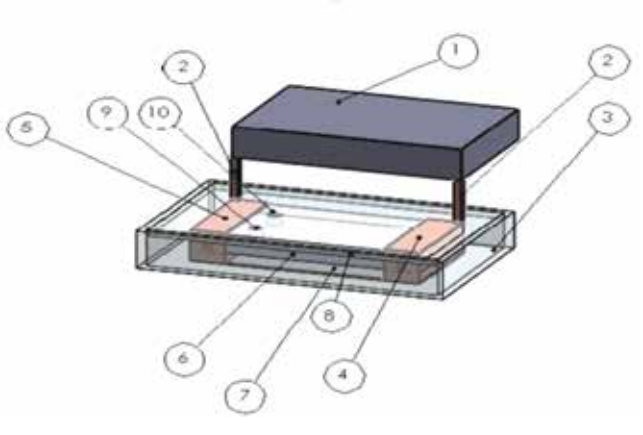
SDS Page Protein Acceleration Set Up

Gel slab can be prepared by following the protocols and chemical reagents for Ultrathin-Layer SDS Polyacrylamide Gel Electrophoresis (UTLSDS PAGE) (Heukeshoven & Dernick, 1985). The only structural difference from the standard set-up is that the system has only two loading wells and their horizontal orientation.0.1% SDS can be used for the PAGE set-up to denature the protein mixture that is needed to be identified (Perrett, 2000).

The gel slab consists of two wells. One loading well is used to load the protein solution and another well to load the protein ladder comprising a polypeptide chain of different chain lengths and molecular weights. Polyacrylamide Gel matrix stretches from the SDS PAGE Protein Acceleration Set Up into the Electromagnet where a strong magnetic field is induced on the moving protein. The stretch of the Polyacrylamide Gel matrix between the electric field of the SDS PAGE Protein

Acceleration Set Up and the magnetic field of the Electromagnet is termed as 'horizon' in the article. The total length of the Polyacrylamide Gel matrix of SDS PAGE Protein Acceleration Set Up is divided into smaller and equal units of length, with a dedicated timer. A plastic strip is located at the horizon to stop protein ladder molecules from entering the Gel matrix where the Electromagnet system is located.

Figure 4.1.1.-Theoretical set up-SDS PAGE PROTEIN ACCELERATION SET UP.



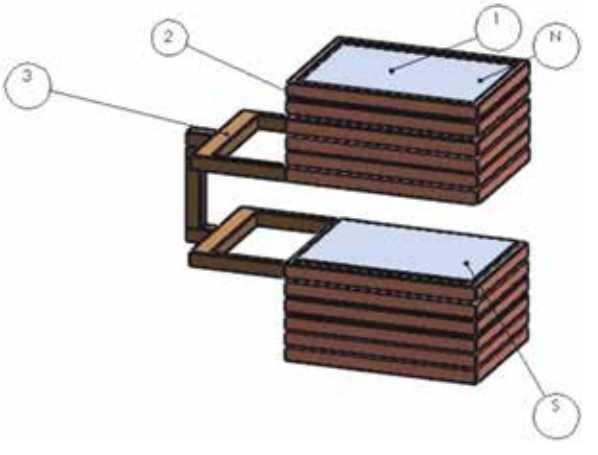
Label Number	Components
1	Power supply
2	Connectig wires
3	Buffer tank with Tris HCL
4	Positive electrode
5	Negative electrode
6	Polyacrylamidegel
7	Bottom glass layer
8	Top glass layer
9	Loading well for proteins
10	Loading well for poptide ladder

Electromagnet System

The proposed magnetic material of the Electromagnet System is 'Superperm 80', with a density of 8.74 $g\ cm{-}3\,/$ and resistivity of 55 μΩ/cm. The composition of Superperm 80 by the percentage of weight is, 80% Ni (Nickel), 16% Fe (Iron), and 4% Mo (Molybdenum) and it is processed for high initial permeability (Luborsky et al., 1978). Electromagnet System consists of two Superperm 80 blocks (Block A and Block B, respectively) acting as two separate electromagnets. The north pole (N) of Block A is faced towards the Polyacrylamide Gel matrix and is stationed above the Polyacrylamide Gel matrix and the South Pole (S) of Block B is faced towards the Polyacrylamide Gel matrix and is stationed below the Polyacrylamide Gel matrix as shown in the figure 4.1.2. The Polyacrylamide

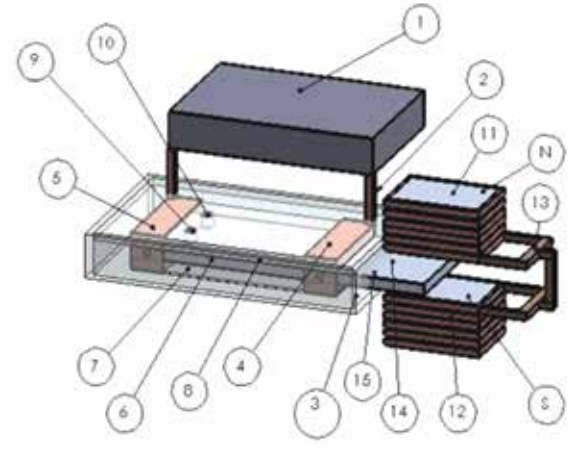
Gel matrix of the Electromagnet System is divided into a grid with measurable *x* and y coordinates. Both blocks are wound tightly by binding coils to form an electromagnet. Both blocks of Superperm 80 are shaped as shown in Figure 4.1.2. Electricity to the magnetic material is given by a battery to the winding coils to magnetize Superperm 80 blocks. Winding coils are made from Copper (Cu) (Son & Lee, 2012). Cu wires are insulated with polytetrafluoroethylene (PTFE) or known as Teflon, which is resistant to heat (Son & Lee, 2012). Insulation is within 0.1mm of thickness (Wardoyo et al., 2020).

Figure 4.1.2.- Theoretical set up ELECTROMAGNET SYSTEM.



Label names	Components
N	North pole electromagnet
S	South pole electromagnet
1	Superperm 80
2	Teflon coated copper winding
3	Insulator handle

Figure 4.1.3.- Theoretical set-up for the Protein Friction Coefficient(β) measurement assembly.



Label names	Components
1	Power supply
2	Connecting wires
3	Buffer tank with TrisHCL
4	Positive electrode
5	Negative electrode
6	Polyacrylamide gel
7	Bottom glass layer
8	Top glass layer
9	Loadijg well for proteins
10	Loadijg well for peptide ladder
11	Superperm 80
12	Teflon coated copper winding
13	Insulator handle
14	Polyacrylamide layer for electromagnetic system
15	Horizon layer
N	North pole electomagnet
S	South pole electomagnet

Measurement of Protein Friction Coefficient ($\mathfrak{S}$)Values of the Unknown Protein

The following steps are proposed to measure the $\mathfrak{S}$ values of an unknown protein molecule.

Acceleration of the Protein Molecule in the Electric Field by Ultrathin-Layer SDS Polyacrylamide Gel Electrophoresis (UTLSDS-PAGE).

Two fractions of the samples of the protein that needs to be identified are prepared and purified by electroelution (Sá-Pereira et al., 2000). One fraction of the sample protein is loaded into the loading well of the Polyacrylamide Gel matrix and the protein ladder is loaded into the other well using a micropipette. Bromophenol Blue is used as the tracking dye. Standard UTL SDS PAGE is carried out as described by Heukeshoven & Dernick, (1985), by supplying an electric current to the Gel block using electrodes. After the protein ladder and the protein solution is accelerated to the entering constant velocity of V_p, characteristic of the protein that is subjected to identification, the protein solution enters the horizon of the Gel block. The protein ladder solution is then stopped at the horizon by a plastic strip. The Time taken by the protein molecule to migrate from one unitary length of the Polyacrylamide Gel matrix is measured using an accurate timer. Migration of the protein molecule along the length of the Polyacrylamide Gel matrix is tracked by the movement of the tracker dye, Bromophenol Blue. Based on equations (5) and (6), constant acceleration (a_o) for the protein can be calculated by drawing a graph by determining the point at which the protein has gained a_o taking the acceleration gained by the protein molecule across unitary lengths of SDS PAGE Set Up. A graph can be drawn by taking the acceleration gained by the protein molecule across unitary lengths of SDS PAGE Set Up. as x axis and the time taken by the protein molecule to migrate across unitary lengths of the Polyacrylamide Gel matrix of SDS PAGE Protein Acceleration Set Up as x axis. Entering constant velocity (V_p), of the protein molecule is obtained from the gradient m of the graph. The time period (Δt_s) in which the protein migrates under constant acceleration (a_o) is also obtained from the graph and from the equations (25) and (26) below.

Constant entering velocity (V_p) of the protein molecule is obtained from the below equation of motion of constant acceleration.

$$V_p = u + a_0 \Delta t_s \quad \text{--------(25)}$$

Where u = Initial velocity, a_o = acceleration, t_s = time. Since the protein is in stationary state, ($u = 0$ and $V_p = a_o \Delta t_s$ -------(26).

The mass (m_p) of the protein molecule can be used to roughly estimate by the direct comparison of the electrophoretic picture of the protein ladder with polypeptides with known molecular weights. The remaining fraction of the purified volume of protein by electroelution is used for accurate measurement of the molecular mass of the protein, Size Exclusion High-Performance Liquid Chromatography (Size - HPLC) by two detector approximation is used as described by Folta-Stogniew & Williams,(1999) and experimental value is converted to mass of the protein molecule to be used in equations derived above for mass calculation of the protein molecule.

Protein Friction Coefficient ($\mathfrak{F}$) Measurement of the Protein Molecule During Circular Trajectory in the Magnetic Field

After the accelerated protein molecule enters the magnetic field of the Electromagnetic System, by changing the strength of the magnetic field (B_o), by a known value and plotting the radius of the circular trajectory of the protein on the Gel matrix Rp, the linear graph is developed ($y = mx + c$). Based on equations (22) and (23), the friction coefficient ($\mathfrak{F}$) and the overall electrostatic charge (q_o) are calculated based on the intersection m and gradient m of the graph. The radius of the circular trajectory of the protein on the Gel matrix R_p is calculated based on the radius of the track of the circular trajectory of the protein based on the location of the tracker dye, Bromophenol Blue on the Polyacrylamide Gel matrix. Obtained friction coefficient values ($\mathfrak{F}$) of the unknown protein are compared with the calculated library of reference values of $\mathfrak{F}$ of known proteins for identification.

Discussion

If the protein solution contains any foreign impurities, they can interact with the protein. Sometimes foreign matter can interact with the protein and bind with the protein irreversibly. If the mass of the impurities is m_0. The new mass (m_n) of the molecular complex (protein+ impurities) can be taken as

m_n = Mass of the impurities (m_0) + Mass of the protein molecule (m_p) ------(27)

According to equation (26) entering the constant velocity (V_p) of the protein into the magnetic field is directly proportional to the constant acceleration (a_o) of the protein molecule.

$$V_p \propto a_0 \quad \text{----------(I)}$$

according to equation (1), where the overall electrostatic charge of the protein is q_p and electric field strength is E_0, the electrophoretic force (F_{el}) acting on the protein molecule is, $F_{el} = q_p E_p$

According to Newtonian law of motion, where the mass of the protein molecule is M_p, the acceleration a_o of the charged protein molecule is, $m_p a_o = q_p E_p$. Therefore,

$$a_0 = \frac{qpEp}{m_p} \quad \text{----------(28)}$$

When considering relationship (I),

$$V_p \propto \frac{qpEp}{mp}$$

Therefore, the mass of the protein molecule is inversely proportional to the entering constant velocity of the protein molecule.

When considering equation (27),

$$V_p \propto \frac{qpE0}{(mp+mo)} \quad \text{----------(II)}$$

Therefore, when a foreign contaminant gets attached to the protein, entering constant velocity of the protein may change, interfering with accurate identification. The usage of pure solutions of proteins devoid of contaminants is important.

In the SDS PAGE Acceleration Set Up, consider the overall electrostatic charge induced by SDS is q_p, the mass of the protein is m_p. The force exerted on the protein molecule by Electrophoresis (F_{ep}) is equal to the force generated by the external electric field (F_{ef}) and friction per Kuhn length of the Polyacrylamide polymer is $\mathfrak{F}k$ (Viovy, 2000).

Therefore,

$F_{ef} = F_{ep}$ ----------(29)

Under these conditions, the constant entering velocity of the protein in the Polyacrylamide Gel before entering the magnetic field is given by the following formula (Viovy, 2000).

$$V_p = \frac{Fep}{Ff} = \frac{qpEp}{\mathfrak{F}k} \quad \text{----------(30)}$$

From equation (24), $u + a_o\Delta t_s = \frac{Fep}{Ff} = q_pE_p\,\mathfrak{F}k$ -------(31)

Since the protein molecule starts acceleration from a stationary state, $u = 0$,

Therefore, $a_o\Delta t_s = \frac{Fep}{Ff} = q_pE_p\mathfrak{F}k$ -------(32)

and $a_o\Delta t_s \propto \frac{1}{\mathfrak{F}k}$ ------(III)

According to the relationship (III), the product of constant acceleration (a_o) of the protein and the time of acceleration(Δt_S) is inversely proportional to friction per Kuhn length of the Polymer($\mathfrak{F}k$). Since $\mathfrak{F}k$ of the Gel polymers is characteristic to them, it is important to use a uniform constitution for the Polyacrylamide Gel matrix in the identification process and take reference $\mathfrak{F}$ values for the known proteins.

From equation (23), the ratio of the gradient m and the intersection c of the graph (Figure 3.2), it is evident that the mass-to-charge ratio has no effect in the proposed methodology of the concept paper. If further clarifies this statement, consider the equation (23).

$$\frac{c}{m} = \frac{\mathfrak{F}\,\cancel{mp}\,\cancel{Vp}}{B_0 q_p \cancel{m_p V_p}} \quad \text{---------(23)}$$

Here, the mass-to-charge ratio, $\frac{mp}{q_p}$ (mass of the protein is m_p and the overall charge is q_p) of the protein that is subjected to identification is removed within the equation due to cancellation of parameters of mass (m_p),velocity (V_p) of the protein, nullifying its effect on identification. This is one of the most striking features of the proposed theory and methodology of this concept article.

Unlike DNA molecules, proteins and peptides express differences in the overall electrostatic charge-to-mass ratios. Furthermore, depending on the pH value of the environment in which the protein or the polypeptide resides, the charge of the molecule

can be ranging from basic from to anionic form and from being hydrophobic to being hydrophilic. In addition to these proteins and polypeptides can have different shapes ranging from globular conformation to linear conformation with varying degrees and tendency of cross-linking on them. Sodium Dodecyl Sulphate (SDS) binds to the backbone of proteins, which is hydrophobic in a regularized manner and denatures the protein. This bonding of SDS and the denaturation occurs regardless of the protein. Therefore, it applies universally to identify any kind of protein and polypeptide in the proposed methodology of this article.1.4g of SDS is capable of binding with 1g of the protein that is needed to be identified for denaturation, irrespective of the type of protein. The SDS-protein/polypeptide complex has a linear configuration during the identification process. SDS-protein/polypeptide complex has an overall negative charge on the protein/polypeptide and migrates in a single direction (Perrett, 2000).

Therefore, in the proposed methodology of this article, the protein molecules that are subjected to identification have an overall negative charge uniformly spread across the entire molecule. Since the negative charges are present in all the proteins of the mixture that is subjected to identification, there are only repulsion interactions among the protein molecules in the UTLSDS-PAGE. This hinders the cross-linking of the proteins in UTLSDS-PAGE to a great extent. Furthermore, the existence of cross-linking of protein products in PAGE is very low (Wittig & Schägger, 2009).

Identification of the protein only depending on the constant velocity of the protein molecule during electrophoresis is highly debatable and prone to errors. That is because several protein molecules have very similar (not equal) molecular mass. Although two proteins have very similar molecular masses, they may possess very different amino acid sequences in polypeptide chains. As an example, isomers of the Human Growth Hormone (hGH) can be given. The molecular mass difference between the two isomers of the Human Growth Hormone may come to as small as 2kDa (Bustamante et al., 2005). Therefore, this article proposes a concept that uses both electric and magnetic fields to identify the proteins. To improve accuracy and reliability, the proposed concept utilizes multiple variables such as magnetic field strength, the radius of the circular trajectory of the protein molecule inside the magnetic field, constant velocity of the protein molecule in the Electrophoresis and homogenous magnetic field, constant acceleration of the protein in Electrophoresis, the friction coefficient of the protein in the Polyacrylamide Gel environment.

For estimation of the molecular weight of the unknown proteins, a constant set of protein ladders should be used in all protein identification assays to ensure uniformity of the results and reduce bias due to the use of different protein ladder sets to estimate molecular weight. More accurate alternative methods in molecular weight determination and the development of a wide range of protein ladders with known molecular weights where the weight difference can be differentiated even for the small number of amino acid residues is an area for development for this technique.

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