**Paper 9: Techniques Used In Molecular Biophysics I**

**Module 3: Viscosity and its Applications in biology and medicine**

**Introduction:**

Viscosity is one of the characteristic bulk property of a liquid. It is a measure of liquid's resistance to flow which arise due to friction between the internal layers of fluid as they slip past each other. When there is a strong intermolecular forces of attraction between molecules in a liquid, there is a larger resistance in the movement of layers past one another that leads to increased viscosity. Example glycerol or honey has a much higher viscocity than water. The fact that viscosity actually make one type of liquid to flow faster than the other as the comparison between water and honey. When the liquid flows over a fixed surface the molecule of liquid at the surface remain stationary. The velocity of upper layer increases as the distance from the stationary fixed layer increases. This kind of gradation in velocity from one layer to other is known as laminar flow. If we choose any layer in the flowing liquid the layer above it will try to accelerate the flow of liquid while the layer below will try to retard the flow of liquid. Thus only a part of fluid moves that force other layer to flow along with it causing an internal friction that ultimately leads to reduced rate of flow. Hydrogen bond and van der wall forces are strong enough to cause viscosity. Glass is an extremely viscous liquid as can be seen from the window pane of old building that have thick glass in the bottom of window pane compare to top.

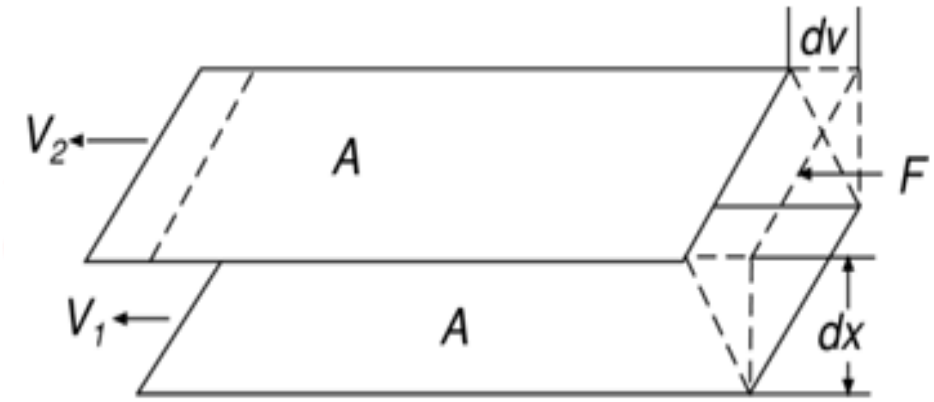
Viscosity can be observed in gases as well in the form of resistance to flow, change shape or movement.

## Objectives:

- Introduce to the property **of** viscosity
- Relationship between viscosity and temperature
- Explain how to measure viscosity and Viscometry technique
- Applications of viscometery to measure compositions in a mixture

- Applications to biopolymers, like DNA , protein for Molecular weight determination, structural characterization and
- Role of viscosity and clinical applications

It is important to understand some basic term and interrelation between viscosity, shear stress, and shear rate. If one suppose fluid as a deck of card on a table and if one pushes horizontally on the top card , each card experience a slipping friction with one another and thus the card will progressively slip over each other. More the friction , less the card slip horizontally at a given instant under a horizontal push. Continuing the analogy of the deck of cards, shear stress is the horizontal pushing force divided by area of card (Force/Area). Isaac Newton defined viscosity by considering the model represented in the figure below.




Two parallel planes of fluid of equal area A are separated by a distance dx and are moving in the same direction at different velocities v1 and v2. The horizontal distance of displacement per sec of a card beyond its immediate lower neighbor divided by its thickness is called shear rate or velocity gradient and viscosity (the analogy of friction) as the shear stress divided by shear rate. Newtonian fluids are those for which the ratio of shear stress / shear rate is not dependent on either shear stress or shear rate and viscosity remains the same whatever the shear rate at which it is measured. Newton assumed that the force required to maintain this difference in speed was proportional to the difference in speed through the liquid, or the velocity gradient. To express this, Newton wrote:

$$\frac{F}{A} = \eta \frac{dv}{dx}$$

- Hagen–Poiseuille in 1844 propose an equation for viscosity of liquid that flow through tubes called Poiseuille's equation.

$$\eta = \frac{\pi r^4 P t}{8VL}$$

……..Equation 1

Where η is called the viscosity coefficient, V is the volume of the liquid, t is the time of flow of liquid L is the distance travelled by the liquid during time t and P is the hydrostatic pressure. The unit of viscosity is called the Poise (P) also known as kg/m s (or Pascal-seconds, Pa s).Different types of viscometer are being used for measuring viscosity including Ostwald viscometer, Falling sphere viscometer, Falling piston viscometer, Oscillating piston viscometer, Vibrational viscometers, Rotational viscometers, Bubble viscometer etc. Among all of them Ostwald viscometer is the commonly used viscometer that will be discussed in detail in this module.

**2. Intrinsic viscosity**

Intrinsic viscosity is a measure of a solute's contribution to the viscosity of a solution. Intrinsic viscosity is defined as

$$[\eta] = \lim_{\phi \to 0} \frac{\eta - \eta_0}{\eta_0 \phi}$$

where is the viscosity in the absence of the solute and is the volume fraction of the solute in the solution. **As defined here, the intrinsic viscosity is a dimensionless number**

## 2.0. Ostwald's viscometer

One of the most elementary ways to measure viscosity is **by Ostwald viscometers** named after Wilhelm Ostwald (also known as glass capillary Viscometer).It is also

known as U-tube viscometer and used to measure the viscosity of fluid with known density.An Ostwald Viscometer consists of a capillary tube and two reservoir bulbs. The upper bulb of viscometer is filled with liquid by suction and allowed to flow through the capillary to reaches the lower bulb after accurately measuring the volume from two marks (one above and one below the upper bulb). The viscometer is then put into a water bath which equilibrates the temperature of the test liquid to maintain a constant temperature. Lastly, the time taken for the liquid to reach lower bulb through capillary of known diameter is recorded which is proportional to the kinematic viscosity. The capillary viscometry is used to measure viscosity of newtonian fluid.

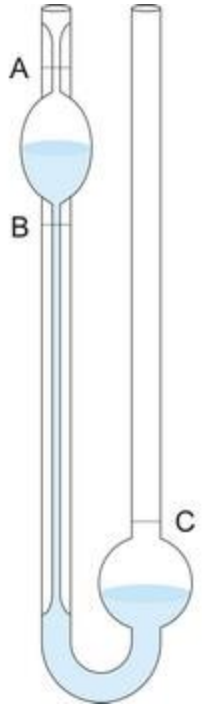


Figure1.An Ostwald viscometer Source:

In an Ostwald viscometer the measured distance the liquid travels, L, the radius, r and the volume of liquid, V will always be constant. Equation (1) can be written as

$$\eta = K\,pt \text{ where } K=\frac{\pi r^4}{8VL}$$

The Oswald viscometer must be calibrated with a reagent of known viscosity such as water. One can calculate the viscosity of test sample after knowing the value of viscosity of a known sample by the following realtion. where $\eta_1$ and $\eta_2$ are viscosity coefficients of the liquid and water, and $\rho_1$ and $\rho_2$ are the densities of

liquid and water and t1 is the time for the sample to travel distance from upper bulb to lower bulb and t2 is the time flow of the reference sample.

$$\eta_1 = \eta_2 \cdot \frac{\rho_1 t_1}{\rho_2 t_2}$$

## 2.1. Temperature Dependence of Viscosity

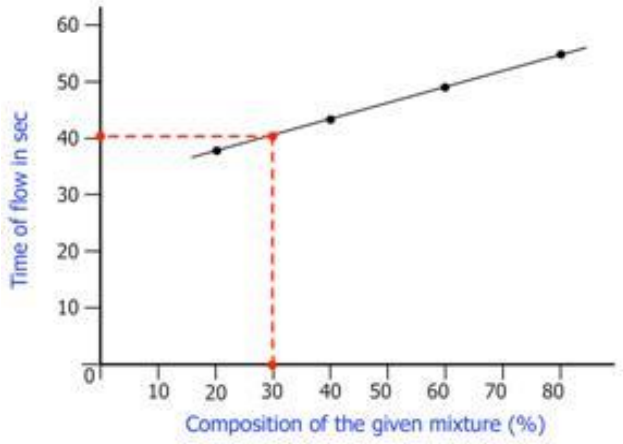
We have already discuss Arrhenius equation for temperature dependent in reaction rates in previous module. For the viscosity of liquids, as the temperature increases the viscosity decreases according to following relation

$$k = A\, e^{-E\eta/RT}$$

Where A is Arrhenius constant, R is the gas constant, Eη is the activation energy for flow, T is the temperature of the liquid using an absolute scale . A plot of η vs. 1/T should be linear with slope Eη /R if the fluid viscosity exhibits Arrhenius-like behaviour.

## 2.2 Determination of unknown composition

With the help of Ostwald viscometer, one can estimate the unknown composition of a mixture. First of all the viscosities of mixtures of different known compositions are measure. After that a graph is plotted with viscosity against the compositions of the different mixtures. From the graph, the composition of th unknown mixture corresponding to the viscosity can be determined



# 3.0. Applications of viscometry

The viscosity is an important property of liquids utilized in various paint, oil and varnishes industry, household products etc. In the field of Biomedical engineering and medical research the study of intrinsic viscosity of biopolymer has been of great interest for many years. Intrinsic viscosity is a measure of a solute's contribution to the viscosity of a solution. It should not be confused with inherent viscosity, which is the ratio of the natural logarithm of the relative viscosity to the mass concentration of the polymer. Intrinic viscosity of a solute is measured with the help of Ubbelohde viscometer. Various application of intrinsic viscosity in protein include rough estimates of the number of subunits in a protein fiber, determining quaternary structure of protein, insight to molecular structure and interactions in solution etc. We will now discuss the major applications of viscosity to biopolymer structure determination ,estimation of molecular weight, Structural characterization of DNA, protein etc

## 3.1. Average Molecular Weight of Polymer

Molecular weight (MW) is one of the most important determinant in characterizing a polymer. Molecular weight of various polymer can be determined by different techniques. One of the rapid and simplest method for determining the molecular weight of polymer is viscosimetry. The Mark–Houwink equation, gives a relation between intrinsic viscosity (η ) and molecular weight (M)

$$\eta = KM\alpha$$

where K and α are constants depend on a polymer–solvent–temperature system. By plotting log [η] versus log molecular weight, these constants are determined .

### 3.2 Viscosity of polymer solution :

The physical properties of a polymer sample in solution depend on temperature, solvent and concentration. At low concentrations, the polymer chains are separated from each other and each chain occupies a spherical volume of radius of gyration. In this solution, the polymer-polymer interactions are small and the polymer coil volume is determined by polymer-solvent thermodynamic interactions.The

hydrodynamic volume occupied by a given polymer mass is the intrinsic viscosity, [è], which is a parameter that can be determined by dilute solution viscosity measurements. The determination of intrinsic viscosity of polymer has been found useful in probing the molecular weight, radius of gyration, overlap concentration, and pore size of concentrated polymers. These parameters are useful in many applications of biotechnology, analytical chemistry, and separation science. For example in agarose gel electrophoresis of DNA and SDS PAGE electrophoresis of protein , the property of sieving matrix critical for separation time calculations, band resolution and dynamic range is determined by measuring intrinsic viscosity values.

Measure efflux times(time it takes for the solution level to drop) (t) for solutions of a polymer solution at varying concentrations. For comparison measure the efflux time (to) of the pure solvent, with no polymer dissolved in it.

**Intrinsic Viscosity of solution**

The value of intrinsic viscosity is also useful for determining the solubility of polymer in different solvent for application in drug-excipient interactions, drug permeation through the skin and development of transdermal patches. Moreover, the size of micellar clusters , degree of hydrophobic associations and hydrolysis, can be determined from intrinsic viscosity measurements. Additionally intrinsic viscosity measurement are important for determining the structure of biological macromolecular and interaction with solution.

The intrinsic viscosity of polymer solution is determined from measuring the intrinsic viscosity of solution at different concentration through a capillary tube as in Oswald viscometer by measuring the time of flow of solution or by recording the force required to rotate two concentric surfaces separated by the solution. A plot of reduced or inherent viscosity versus polymer concentration is used to extract the intrinsic viscosity. The other approach detects the intrinsic viscosity using light scattering and imaging techniques. From the scattered intensity or particle position data the mean square displacement of scattering particles is calculated. Stokes-Einstein equation is then used to determine the intrinsic viscosity. Recently a microchip based technique is developed for measurements of intrinsic viscosity and viscosity of polymer and biopolymer solutions such as

proteins and DNA solutions in various buffer conditions such as ionic strength, pH, and surfactants. The key component of the powerful microchip viscometer include accurate pressure control for dilution, precise microchip geometry, and calibrated fluorescence signal for intrinsic viscosity measurements.

**Relative viscosity :**

$$\frac{\text{efflux time of solution}}{\text{efflux time of our solvent}} = \text{relative viscosity}$$

$$\frac{t}{t_0} = \eta_r$$

**Specific viscosity of a polymer solution using the following relation**

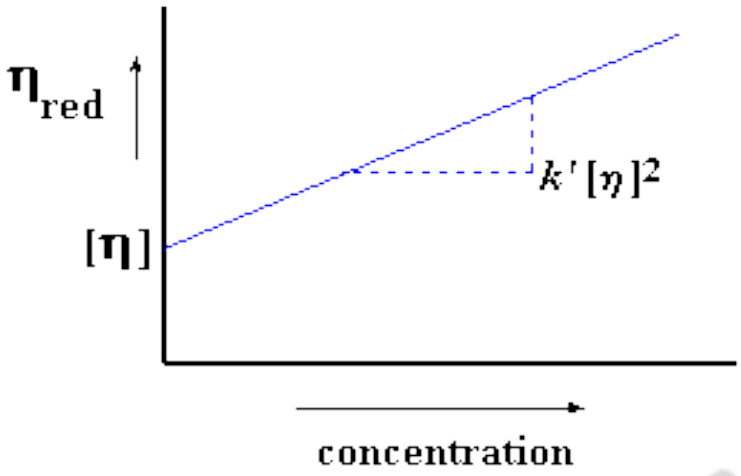
$$\frac{(\text{efflux time of solution} - \text{efflux time of solvent})}{\text{efflux time of our solvent}} = \text{specific viscosity}$$

$$\frac{t - t_0}{t_0} = \eta_{sp}$$

**Reduced Viscosity : Calculate the *reduced viscosity* from relative and specific viscosity**

$$\frac{\text{specific viscosity}}{\text{concentration}} = \text{reduced viscosity}$$

$$\frac{\eta_{sp}}{c} = \eta_{red}$$




A plot of reduced viscosity vs. concentration.
We call the $y$-intercept $[\eta]$, or the intrinsic viscosity.
The slope is related to $[\eta]$, it's equal to $k'[\eta]^2$.

$$y = mx + b$$

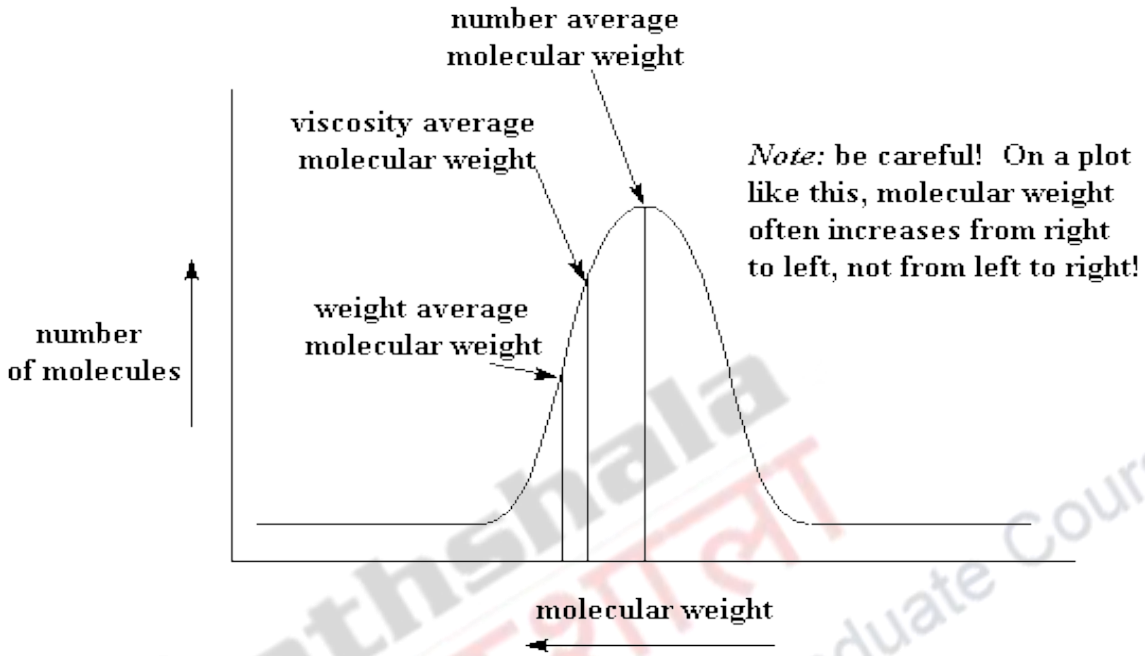
$$\eta_{red} = k'[\eta]^2 c + [\eta]$$

**From intrinsic viscosity [n] we will calculate molecular weight from Mark–Houwink equation**

$$[\eta] = K'M^a$$

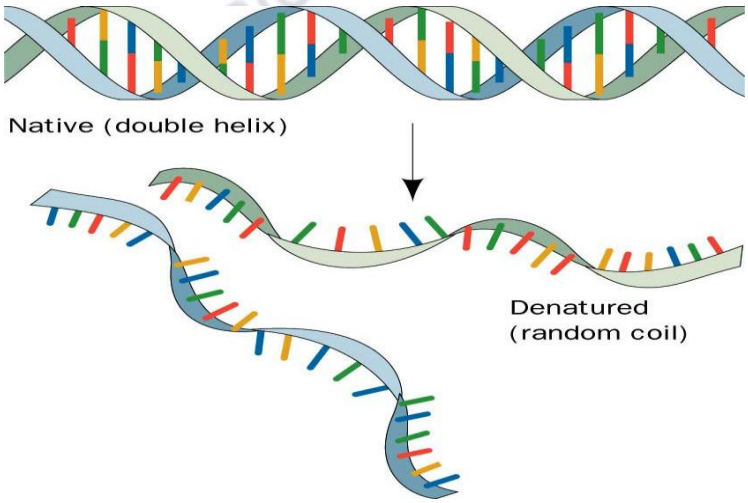
*M* is what we call the *viscosity average molecular weight* and *K'* and *a* are the *Mark-Houwink constants* . The molecular

weight obtained by measuring the viscosity is a close to number average or the weight average molecular weight.

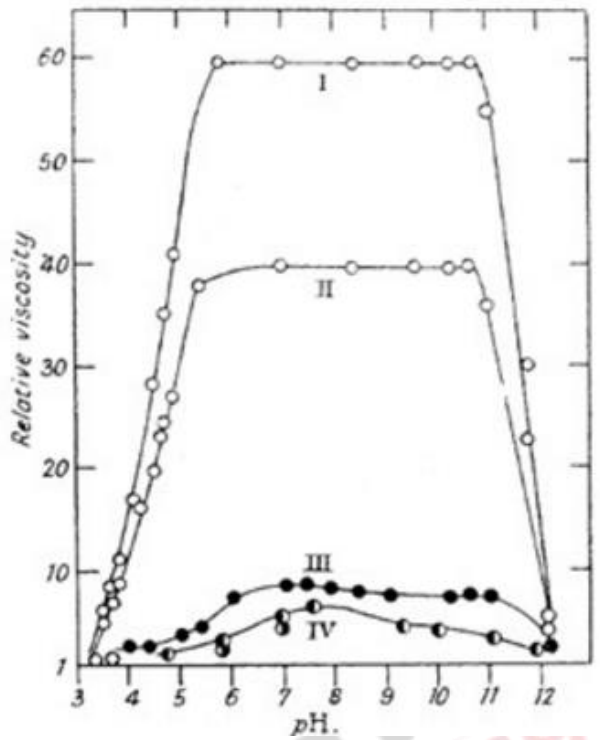



## 3.2. Structural characterization of DNA, protein etc

Viscosity is a key parameter for characterising the behaviour and flow of biological samples. Upon isolation of genomic DNA one can see double stranded DNA fragments resembling a long highly assymtrical rod having high intrinsic viscosity.



Intrinsic viscosities of DNA are dependent on the source.For example PBS- DNA had the highest intrinsic viscosity. Upon denaturation of DNA the two strand of DNA separate out to form two single strands that can adopt different conformations in a form of a random coil. This form of DNA has a lower intrinsic viscosity than the double stranded DNA. The viscosity behavior of single-stranded DNA has been studied in presence of different ionic strength and pH. And it has been observed that the intrinsic viscosity of DNA is influenced more by ionic strength than pH. At ionic strengths below 0.001 the intrinsic viscosity of single-stranded DNA is greater than that for double-stranded DNA. At ionic strengths above 0.001, the reverse is true. Viscosity measurement has been used to monitor progressive denaturation of DNA in presence of DMSO. Viscosity is a a far more sensitive mean of observing denaturation of DNA than UV melting. In presence of 40% DMSO , DNA viscosity is very sensitive to ionic strength after and before denaturation. Viscosity measurements before and after denaturation has revealed double strand break by sonication and single strand break after UV irradiation in presence of hydrogen peroxide. Contamination by slight protein reduce the intrinsic viscosity of DNA.At pH 7.0 and temperature between 20 to 25ºC native DNA is highly viscous. When DNA sample is subjected to extremes of pH and temperature(90º) viscosity decrease sharply indicating a structural change in DNA.The transition from double-stranded DNA to the single-stranded form can be detected by decrease in the viscosity of the DNA solution. Such measurement are also useful for detecting unusual DNA structure such as DNA triplex , Quadruplex etc.




Creeth, J.M., Gulland J.M. & Jordan, D.O. (1947) J. Chem. Soc. 1141-1145

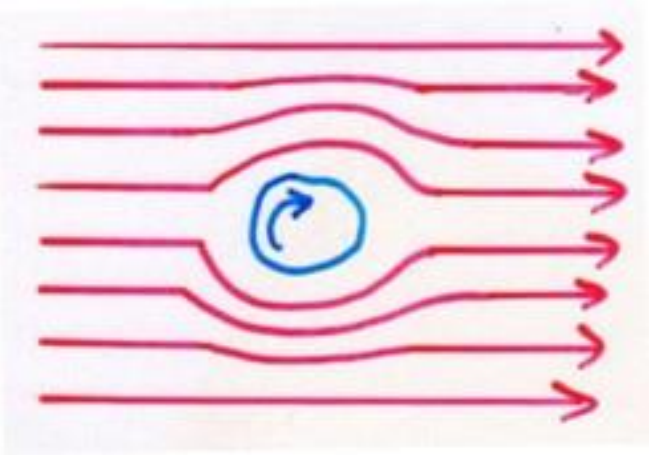
*The variation of the viscosity of solutions of various specimens of deoxypentose nucleic acid.*

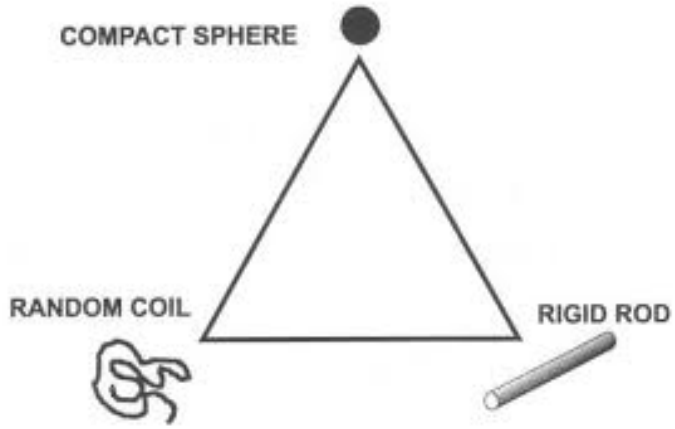
*Tetrasodium salt of deoxypentose nucleic acid of calf thymus, ○ :*
*I (applied pressure 3000 dynes/cm.²), II (applied pressure 7000 dynes/cm.²).*
*Tetrasodium salt of deoxypentose nucleic acid after alkaline treatment, III, ● ; after acid treatment, IV, ◐.*
*Tetrasodium salt of deoxypentose nucleic acid of calf thymus supplied by Professor Caspersson, IV, ◑.*

A dissolve macromolecule will increase the viscosity of a solution because it disrupt the streamline of the flow. Intrinsic viscosity of biomolecule depend upon various factors such as shape, flexibility,

Proteins :See below the viscosity of globular and rod like proteins given in the table. And you will understand why the shape and Mwt paly imp role in viscosity

| | M (g/mol) | [η] (ml/g) | |
|---|---|---|---|
| Glucose | 180 | 3.8 | GLOBULAR |
| Myoglobin | 17000 | 3.25 | |
| Ovalbumin | 45000 | 3.49 | |
| Hemoglobin | 68000 | 3.6 | |
| Soya-bean 11S | 350000 | | |
| Tomato bushy stunt virus | $10.7 \times 10^6$ | 3.4 | |
| Fibrinogen | 330000 | 27 | RODS, COILS |
| Myosin | 490000 | 217 | |
| Alginate | 200000 | 700 | |






Haug Triangle represesnt the three extreme conformation of macromolecule. Each extreme has its own characteristic dependence of intrinsic viscosity on macromolecule.

The stability and viscosity of concentrated therapeutic protein solutions is of great relevance to the pharmaceutical industry. Stability of protein plays an important role in protein drug formulation, storage shelf life and drug efficacy. An unstable proteins unfold and aggregate to cause an increase in the viscosity of the protein. Aggregated proteins mark an immune response leading to serious side effects. This makes viscosity a key ingredient in determining protein stability, an integral part of protein formulation. Stability of protein depends on concentration, pH, temperature and many other factors. Upon denaturation at high temperature protein unfolds and loses tertiary and secondary structures. As unfolding progresses, proteins begin to aggregate, which could lead to the precipitation or formation of a gel-like structure.

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## Clinical applications of viscometry

The viscosity is an important property of liquids utilized in various pharmaceutical industry and medicine. The stability and viscosity of concentrated therapeutic protein solutions is of great relevance to the pharmaceutical industry.

- The viscosity of some medications has been modified foe easier application , such as in liquids used to remove warts, for easier application. The cough syrup made by drug companies are viscous to coat and sooth the throat . Different occupation need to adjust viscosity of the substance to suitable application.
- Xanthan gum is an example of a viscosity modifier used in liquid formulations. Buffers may be used to regulate pH, whilst surfactants or co-solvents are used to enhance solubility and antioxidants or chelating agents are used to enhance stability.
- In the field of Biomedical engineering and medical research the study of hemorheology has been of great interest for many years. It plays an important role in atherosclerosis. Hemorheological properties of blood include RBC definability and aggregation , plasma viscosity, whole **blood viscosity.**. The whole blood viscosity is an important key that determine the blood flow during physiological as well as pathological condition. Due to a number of parameter that affect blood flow such as lumen diameter, pressure, compliance of vessels, whole blood viscosity, peripheral vascular resistance the significance of viscosity has not been fully appreciated yet . **Pathological conditions :**

- Heart Disease : During myocardial infarction and ischemic heart disease the viscosity of blood is measured by various research group . It has been shown that there is direct relation between viscosity and coronary arterial disease . **It has been reported that whole blood viscosity is found to be higher in patients with peripheral arterial disease than that in healthy contro**ls. Stroke , hypertension
- Other investigation reported elevated two or more rheological parameters in strokes which included red blood cell (RBC) ,whole blood viscosity, RBC rigidity, plasma viscosity,plate aggregation, and hematocrit. **In essential hypertension the whole blood viscosity and plasma viscosity were significantly high than in healthy ones.**
- Smoking patients :
  Hemorheological studies have shown that there is a relationship between whole blood viscosity and smoking, age, and gender. And it was found that smoking and aging might cause elevated blood viscosity.
- Male vs female blood viscosity
  Additionaly male blood shows higher blood viscosity, RBC rigidity and aggregability than premenopausal female bloodBlood viscosity itself can throw light on : RBC deformability and aggregation ,plasma viscosity, hematocrit, .

  **CSF**

  Other than RBC Cerebrospinal fluid (CSF) has critical value for the diagnosis of acute meningitis and discrimination of bacterial and aseptic meningitis when the protein content of CSF increases.

  **Urine :**

  Viscosity measurement of urine is critical for accurate modelling of fluid mechanics and heat transfer during hyperthermia treatments of bladder cancer.

## Summary :

- Viscosity is a measure of liquid's resistance to flow which arise due to friction between the internal layers of fluid as they slip past each other. When there is a strong intermolecular forces of attraction between

molecules in a liquid, there is a larger resistance in the movement of layers past one another that leads to increased viscosity

- Viscosity is temperature dependent, upon heating the viscosity of liquid decrease and in case of gases viscosity increase with increase in temperature
- Viscosity is measured with the help of viscometer.
- In the field of Biomedical engineering and medical research the study of intrinsic viscosity of biopolymer has been of great interest in rough estimates of the number of subunits in a protein fiber, determining quaternary structure of protein, insight to molecular structure and interactions in solution , biopolymer structure determination ,estimation of molecular weight, and structural characterization of DNA, protein etc
- The transition from double-stranded DNA to the single-stranded form can be detected by decrease in the viscosity of the DNA solution. Such measurement are also useful for detecting unusual DNA structure such as DNA triplex , Quadruplex
- The stability and viscosity of protein solutions is of great relevance to the pharmaceutical industry. An unstable proteins unfold and aggregate to cause an increase in the viscosity of the protein.
- **It has been reported that whole blood viscosity is found to be higher in patients with peripheral arterial disease than that in healthy contro**ls. Viscosity of other body fluids like urine, CSF etc are helpful in pathological conditions.
-