Birla Institute of Technology & Science, Pilani
Hyderabad Campus
Department of Biological Sciences

BIO F110: BIOLOGY LABORATORY

LABORATORY MANUAL

Name: _____

ID No: _____

Section No: _____ Group No:_____

Instructor: _____

Biology Laboratory Safety Guidelines

Students are instructed to strictly follow the guidelines given below:

Attire:

1. Shoes must be worn in the lab.
2. Lab coat is compulsory during experiment.
3. Shorts and half pants are not allowed in the lab.
4. Long hair must be tied back.

Handling of Chemicals and Equipment:

1. Consider all chemicals to be hazardous. Know those chemicals that you are using.
2. Avoid contact of chemicals with your skin or eyes. If such contact occurs, inform the instructor/ lab technician and as a first aid measure, immediately flush the eyes with copious amounts of water.
3. Do not use flammable reagents near fire.
4. Be careful while pipetting. Use pipette bulb only and pipetting through your mouth is strictly prohibited.
5. Always pour acids into water and not the other way around.
6. Excess reagents should not be returned to stock bottles. Dispose excess reagents and chemical waste as instructed by the instructor/ lab technician
7. Never taste or directly smell chemicals.
8. Follow the directions carefully while using instruments.

Conduct:

1. Please come on time, in case you are late by more than 5 minutes, it may impact evaluation of your observation. In case you are late by more than 20 minutes, you will not be evaluated for your result and observation, but you can continue with the experiment.
2. Eating and drinking are strictly prohibited in the laboratory.
3. Keep you work area clean. Put paper trash and broken glass, if any, in the dust bins. Clean you work place before leaving laboratory, otherwise marks will be deducted for that experiment.
4. Avoid spills. If you do spill something, clean up the area immediately, taking adequate precautions. Inform the instructor/ lab technician immediately.
5. Keep the area around instruments clean and debris-free.
6. Always wash your hands thoroughly before you leave the laboratory.

List of Experiments

S. No.	Title of the Experiment	Date	Marks	Remarks/ Sign
1	Measurement of total protein content in the given sample			
2	Measurement of glucose content in the given sample			
3	Separation of chlorophyll pigments using paper chromatography			
4	Observation of permanent slides			
5	Study of the phenomenon of plasmolysis in onion peel			
6	Identification of mitotic stages in the given plant tissue sample			
7	Determination of ABO-Rh blood types			
8	Measurement of total cholesterol levels in serum			
9	Micrometric measurement of microorganisms			
10	Extraction of DNA from banana			

Experiment No. 1

Measurement of total protein content in the given sample

Theory:

Protein acts as body builder and made up of amino acids with peptide bond. Amino acids [RCH (NH_2) COOH] are amphoteric molecules containing an amine group, a carboxylic acid group and a side chain that varies between different amino acids. Amino acids are classified by the properties of their side chain into two groups, aliphatic and aromatic amino acids. Phenylalanine, Tryptophan, Tyrosine and Histidine are aromatic amino acids. In this experiment, protein content would be measured by Lowry's method, in which protein samples (rich with aromatic amino acid) are treated with Biuret reagent (Reagent C) and subsequently with Folin-Ciocalteu reagent (Reagent D). In Biuret reactions, Cu^{2+} in alkaline condition reacts with nitrogen present in the peptide bonds of the proteins and form chelates with copper ions by reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}). This chelate compound in presence of Reagent D gives a characteristic blue color due to reduction of phosphomolybdate (present in reagent D) by aromatic amino acids present in the protein. The intensity of the blue color of the solution is directly proportional to the amount of aromatic amino acids present in the sample. This intensity of blue color is measured as absorbance/ optical density (OD) by calorimeter at 640 nm.

Photometry: Colorimeter and Spectrophotometer

Colorimeter is form of photometry, which deals with the measurement of light absorption of coloured substances in solutions. The analytical instrument which measures the intensity of color in terms of light absorbed at specific wavelengths is known as colorimeter. Colorimetric procedures are limited to the visible portion of the spectrum whereas spectrophotometric procedures however involve use of Ultraviolet, visible and Infra-red portions of the spectrum. The working of spectrometers and colorimeter are based on Beer's and Lambert's laws.

Beer's law: It states that the optical density (OD) of a solution is directly proportional to the concentration of the solution.

Lambert's law: It states that the optical density (OD) of a coloured solution is directly proportional to the path of light i.e., (diameter of the cuvette).

Note: If the diameter of cuvette is doubled the OD will also be doubled but since cuvettes of the same diameter are used for most analytical purposes so automatically Lambert's law is observed. According to Beer and Lambert's law: $A \propto CL$, $A = KCL$

Where,

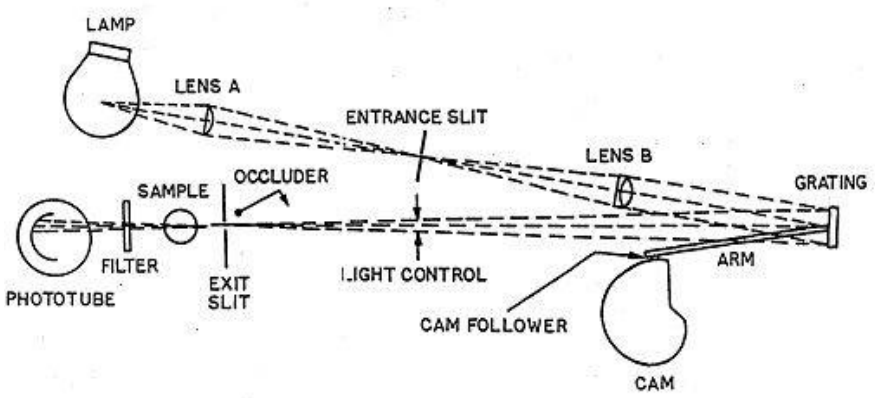
A= Absorbance or Optical density

K= Molar extinction coefficient constant (depends on the characteristic of the solution) C= Concentration of the coloured solution

L= Path of light through the coloured solution It can also be written conveniently as:

A= log Io/I and the ratio I/I_0 is called transmittance (T) of the coloured solution A= log 100/T = log 100 - log T = 2- log T

Where I_0 is incident light and I is the transmitted light (light emerging from cuvette containing solution)



By converting this expression through logarithmic form:
Alternative form of Beer- Lambert's law $T= 10^{-kcl}$
Log T = - KCL
- log T = KCL
O.D. = KCL

O.D. is constant for a particular solution and path length is also constant as the diameter of cuvette is fixed therefore the optical density is directly proportional to the concentration. Absorbance is also called optical density. A = KCL
Molar extinction coefficient is the extinction (O.D. or absorbance) given by a substance concentration 1 mol/litre in a path length of 1 centimetre. Graphs of absorbance vs. concentration, or log %T vs. concentration are known as Beer's Law plots. They are made by measuring the light absorbed by solution of varying concentration. The cell width and the wavelength of the wavelength of the light are maintained constant. If a linear plot is obtained (showing that the Beer- Lambert relationship holds for the solution at that wavelength), it than may be used to determine the concentration of unknown solutions.

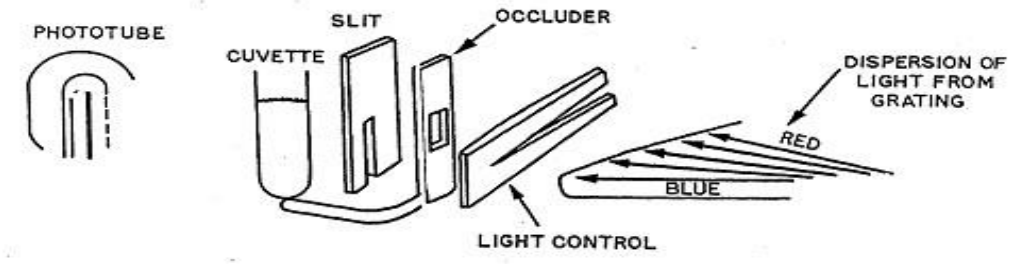
According to Beer and Lambert's law the absorbance of a solution containing light absorbing material depends on the follow factors:

(1) The nature of the substance
(2) The wavelength of light
(3) The path of light
(4) The amount of coloured material in the light path.

Description of the Spectronic 20/Colorimeter:
It is device that measures the intensities of the light before (Blank) and after it has passed through a colored solution. A colorimeter consists of mainly six parts:

1. A light source
2. A condensing lens to render the light rays parallel
3. A filter to generate monochromatic light (radiations of single wave length based either upon refraction by a prism or diffraction by a grafting)
4. A sample holder (Cuvette)

5. A photo cell to convert light energy (Photon) into electrical energy
6. A galvanometer with digital read out to measure the electrical energy (current) thus generated



A light from a tungsten lamp (composed of wavelength between 400 and 650 nm) passes through a slit, a condensing lens, a filter and finally emerges as a parallel beam of monochromatic light. The monochromatic light passes through a sample solution and the transmitted light falls on the photo cell. The photo cell converts the transmitted light energy into electrical energy, which is amplified and measured by the galvanometer. The galvanometer is calibrated to read the absorbance /Transmittance directly.

Working of colorimeter

Requirements:

1. **Reagent A:** Dissolve 20 g Na_2CO_3 and 4 g NaOH in 1 litre of distilled water.

2. **Reagent B:** Dissolve 0.5 g $CuSO_4.5H_2O$ and 1 g sodium potassium tartarate in 80 ml of distilled water. Make up the volume to 100 ml. (**Precaution:** Do not shake this reagent before use. Only its supernatant should be used).

3. **Reagent C:** Take 50 ml of reagent A and add 1 ml of reagent B. it should always be freshly prepared.

4. **Reagent D:** Diluted (1N) the commercially available Folin's and Ciocalteu reagents

5. **Protein Standard Solution:**

   5.1.Stock Solution: Dissolve 50 mg of bovine serum albumin (BSA) in 50 ml of distilled water (Concentration: 1mg/ml) and store in refrigerator

   5.2.Working Standard: Dilute the stock solution 5 times so that final protein concentration is 200µg/ml.

Procedure:

- Take 6 clean test tubes and mark them as B (Blank), S1, S2, S3, S4 (different concentrations of working standard) and T (test solutions). Fill in these tubes in the following way:

Sl. No.	Water (ml)	Working Standard (ml)	Un known Test sample (ml)	Final Conc. (µg/ml)	Reagent C (ml)	Incubate for 10 min. at RT	Reagent D (ml)	Incubate for 20 min. at RT in dark	OD 640 nm
Blank	1	---	----	0	5	"	0.5	"	0
S1	0.75	0.25	-----	50	5	''	0.5	''	
S2	0.50	0.50	-----	100	5	''	0.5	''	
S3	0.25	0.75	-----	150	5	''	0.5	''	
S4	---	1	-----	200	5	''	0.5	''	
T	----	----	1	?	5	''	0.5	''	

- Plot the values of OD 640 nm against different concentrations of working standard protein solution in the graph paper for preparation of standard curve.
- Match the OD of T (test sample) on the standard curve. The corresponding concentration on X-axis will be the actual protein concentration (in (µg /ml) in the given sample.

Results

Protein concentration in the given sample is_____µg/ml.

Marks Obtained: _____

Signature of Instructor: _____

Review Questions

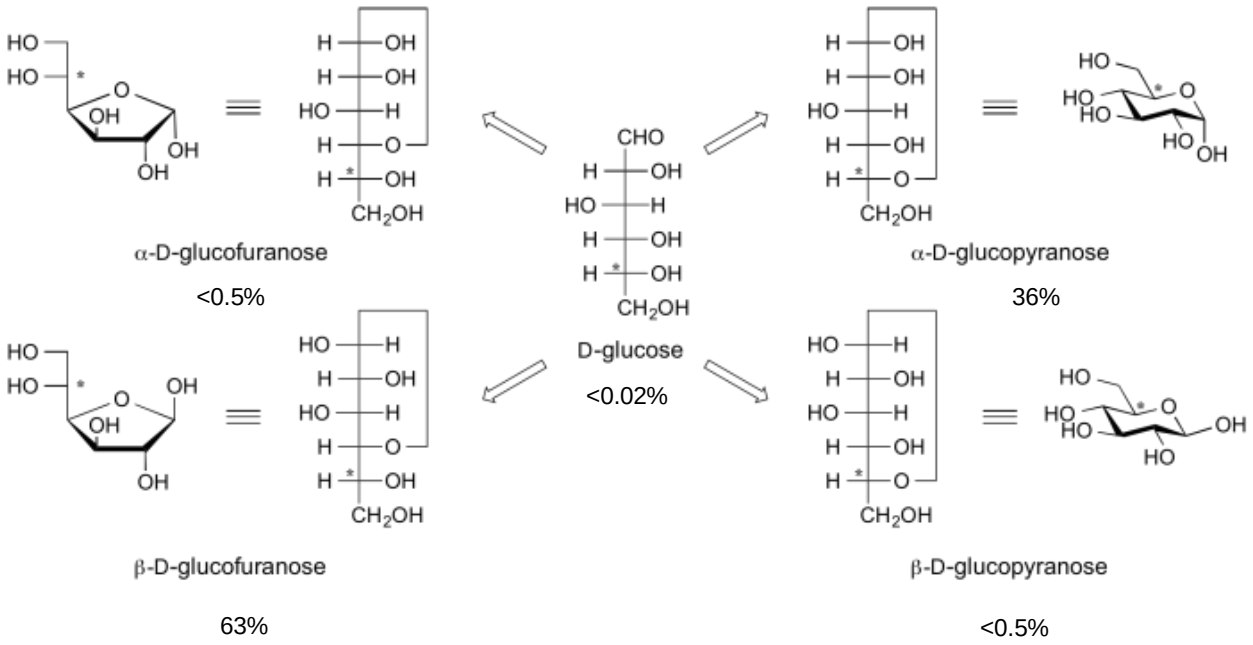
1. Name three aromatic amino acids which help in protein estimation by Lowry's method.
2. What protein have you used in Lowry's method to obtain your standard curve and why?
3. Why does a too densely colored sample fail to give proper results using the instrument?
4. Why Blank is used in colorimetric estimation of protein?

Experiment No. 2

Measurement of glucose content in the given sample

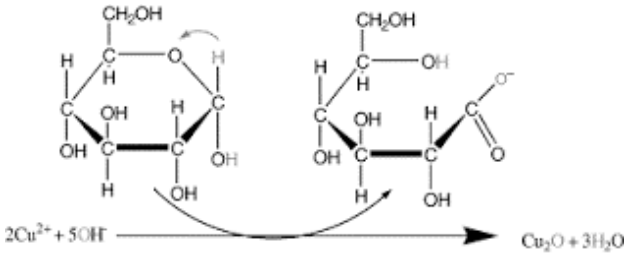
Theory:

Glucose is a primary source of energy in most organisms. It is broken down stepwise (during the processes of glycolysis and Kreb's cycle) to give CO_2 and water, and 7300 kCal of energy per mole. Excess glucose is stored as glycogen in animals and as starch in plants. In human, a constant level of glucose in maintained in blood by the interplay of hormones such as insulin, glucagon and epinephrine. Glucose determination is mainly useful in diagnosis of diabetes mellitus, the condition in which blood glucose levels are elevated (hyperglycemia). Other diseases like hyperthyroidism and hyperpituitarism also leads to hyperglycemia. Hyperglycemia occurs frequently as a result of over dosage of insulin anti-diabetes treatment. If untreated, it may lead to coma. Glucose is an aldohexose, belonging to the carbohydrate family, and is found in large quantities throughout, the living world. It is the primary fuel for living cells. Dietary sources include plant starch, lactose, maltose and sucrose. In nature, glucose exists as D-glucose (dextrose). Glucose is a simple sugar (monosaccharide). In solution, it exists as open chain form (less than 1%), and cyclized ring forms –α- D-glucose (36%) and β-D-glucose (63%).



Fischer projections and Haworth conformational projections of D-glucose

Glucose is a reducing sugar and is able to function as a reducing agent, because free or potentially free, aldehyde group is present in the molecule. This aldehyde group is readily oxidized to gluconic acid at neutral pH by mild oxidizing agents and enzymes. This property is utilized in detecting and quantitating glucose in biological fluids such as urine or blood. The cuprous oxide formed is brownish in color. For optical quantification, this compound is reacted with phosphomolybdic acid, to give blue color, whose intensity is proportional to concentration of Cu_2O and thereby glucose.



The oxidizing agent used here is tartrate complex of alkaline copper (II) sulphate, known as Fehling's solution. A precaution needs to be taken when used with blood, since it reacts with blood proteins and may give false results, hence blood is deproteinized using tungstic acid before use.

Yet another method of glucose estimation, which is more rapid the Folin-Wu's method, is the GOD/POD method. Here, glucose oxidase (GOD) is the oxidizing agent peroxidase (POD) catalyzes the subsequent reaction to produce red coloration.

Requirements:

1. Alkaline copper sulphate:

Reagent A: 2% Na_2CO_3 in 0.1 N NaOH.

Reagent B: 0.5% $CuSO_4$ in 1 % sodium potassium tartrate.

Reagent C: alkaline copper sulphate solution: Mix 50 ml of reagent A with 1.0 of reagent B. Prepare fresh.

2. Phophomolybdic acid:

Take 35gm molybdic acid and add 5gm Na-Tungstate and dissolve it in 100ml water. To this add 200ml of 1N NaOH (made by dissolving 8g NaOH in 200ml water). Boil this solution for 30-45 minutes. When you observe a brown colour then cool the solution and make up to 350ml by adding distilled water. Finally add 125ml of 85% phosphoric acid and make up the volume to 500ml.

3. Glucose standard 100mg/100ml= 1mg/ml (working).

Procedure:

Take 5 test tubes and mark them as B, S1, S2, S3, S4 and T (B=blank, S=standard and T=test solution). Fill in these test tubes in the following way:

	B	**S1**	**S2**	**S3**	**S4**	**T**
Standard solution	-	0.50 ml	1.00 ml	1.50 ml	2.00 ml	-
Water	2.0 ml	1.50 ml	1.00 ml	0.50 ml	-	-
Test solution	-	-	-	-	-	2.0 ml
Concentration (mg/ml)	0.0	0.25	0.5	0.75	1.00	?

1. Add 2ml of alkaline copper solution in each tube and keep in a boiling water bath for 10 minutes.
2. Cool the tubes under tap water and add 2 ml of phosphomolybdic acid solution to all the tubes.
3. Mix the contents by gentle shaking and read the optical density at wavelength of 410nm. Use blank solution to adjust zero setting on the instrument.

Observation:

1. Record the optical density in the following table:

Tube	B	S1	S2	S3	S4	T
OD_{410nm}						

2. Prepare standard curve and match the optical density of the test sample. The corresponding concentration on X-axis will give the concentration of glucose present in the blood.

Results:

The concentration of glucose was found to be _____

Marks Obtained: _____

Signature of Instructor: _____

Review Questions:

1. What is the role of heat treatment at 80°C in glucose estimation from blood serum by Folin Wu's method?
2. What is the normal concentration of blood sugar in human?
3. How the level of sugar is kept constant in vertebrate blood?
4. Why glucose alone is used for intravenous infusion, not fructose or sucrose?
5. What is the difference between reducing and non-reducing sugar?

Experiment No. 3

Separation of chlorophyll pigments by paper chromatography

Theory:

The means to carry out photosynthesis in plants is the green pigment chlorophyll. A whole series of chlorophylls called C*hl* a, b, c, d and e are known. Chlorophyll a is the primary photosynthetic pigment and is found widely distributed in the plant kingdom. Higher plants contain predominantly two types of chlorophylls; chlorophyll a and chlorophyll b. However, chlorophyll c, d and e are encountered only in algae and in combination with chlorophyll a. Chl b is absent in blue green algae and red algae.

Basic molecule of chlorophyll consists of a tadpole like structure with a porphyrin head and a phytol tail. The porphyrin head is formed of four pyrrole rings linked together by methane (-CH=) groups forming a ring system. This produces a sequence of conjugated double bonds (-C=C-C=C-

.e. alternating single and double bonds) in the porphyrin ring. The skeleton of each pyrrole ring comprises of five atoms – four carbons and one nitrogen, which lie in the centre.

In the centre of the porphyrin head is a bivalent Mg^{++} (non-ionic) atom. It is complexed with the nitrogen atoms of the four pyrrole rings by two covalent and two coordinate bonds. In addition to four pyrrole rings, a fifth isocyclic ring (cyclopentanone ring) is also present. It comprises of only the carbon atoms and is chemically the reactive site of the molecule. The porphyrin head bears a

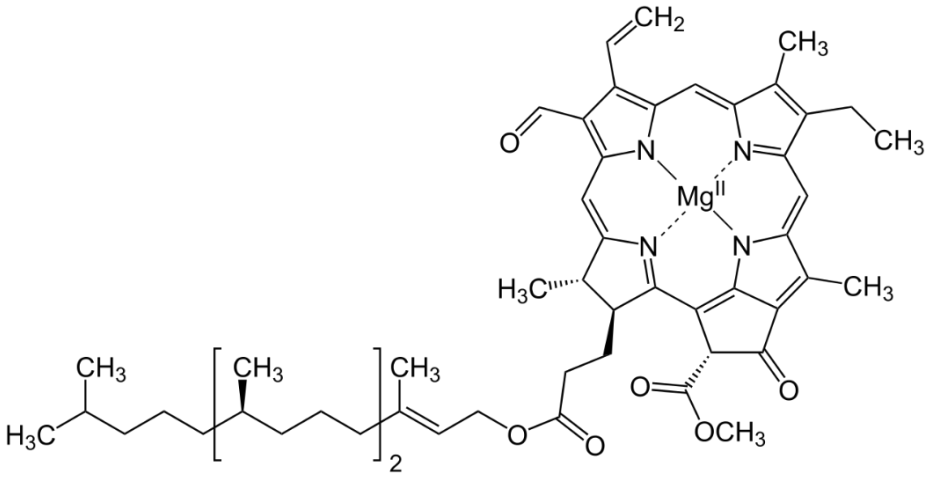
number of characteristic side groups at various points. The identity of the side groups provides the identity of various chlorophylls.

Phytol tail is a 20-C alcohol attached to carbon 7 of the pyrrole ring IV through a propionic acid ester bond. It is considered as the most important side group. The long lipophilic tail is extremely useful in the orientation and anchoring of the chlorophyll molecules in the chloroplast lamellae. Chlorophyll a differs from chlorophyll b in the nature of groups attached at carbon 3 of pyrrole ring II. Chlorophyll a has a methyl group ($-CH_3$) while chlorophyll b has an aldehyde group (-CHO), chlorophyll a has the molecular formula $C_{55}H_{72}O_5N_4Mg$ (mol. Wt. 893) and chlorophyll b $C_{55}H_{70}O_6N_4Mg$ (mol. Wt. 907). Chlorophyll a is blue green while chlorophyll b is yellow green. Chlorophyll a is soluble in petroleum while chlorophyll b is best soluble in methyl alcohol.

Chlorophyll a is called primary photosynthetic pigment since it is responsible for the emission of electrons during cyclic and non-cyclic photophosphorylation. Chlorophyll b on the other hand is an accessory pigment because it transfers the energy absorbed by it to chlorophyll a. Most plants contain 2 or 3 more chlorophyll a than chlorophyll b. Chlorophyll absorbs light near both ends of visible spectrum. The blue and red light, and transmit or reflect green light and that is why the chlorophyll appears green.

These chlorophylls are very effective photoreceptors because they contain networks of alternating single and double bonds. Such compounds are called polyenes. These have very strong absorption bands in the visible region of spectrum, where the solar output reaching the earth is maximal. The peak molar absorption coefficient of Chl a and Chl b are 10^5 cm^{-1} M^{-1}, among the highest absorbed for organic compounds.

Xanthophyll (yellow) and carotene (yellow-orange) are accessory pigments called as carotenoids which absorb light of wavelength other than that absorbed by chlorophyll and help in photosynthesis.



Structure of chlorophyll a

Paper Chromotography:

The basis of all forms of chromatography is the partition and distribution coefficient (K_d) which describes the way in which a compound distributes itself between two immiscible phases. For a compound distributing itself between equal volumes of two immiscible solvents A and B, the value for this coefficient is a constant at a given temperature and is given by the expression:

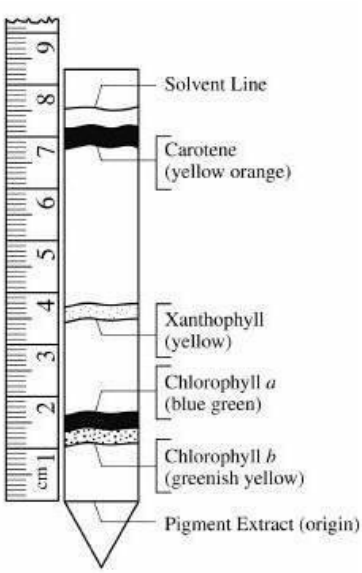
$$\frac{\text{Concentration in solvent A}}{\text{Concentration in solvent B}} = K_d$$

The distribution of a compound can, however, be described not only in terms of its distribution between two solvents, but also by its distribution between any two phases, such as solid/liquid or gas/liquid phases. Thus a distribution coefficient of a substance between silicic acid and benzene might be 0.5, which means that the concentration of the substance in benzene is twice than that in the silicic acid.

Paper chromatography is the method in which the analysis of an unknown substance is mainly done by the flow of solvents on specially designed filter paper. The cellulose fibres of chromatography paper act as the supporting matrix for the stationary phase. The stationary phase may be water, a non-polar material such as liquid paraffin or impregnated particles of solid adsorbent. Paper chromatography separates compounds on paper as solvent carries the mixture up the paper by capillary action. Compounds which are highly soluble in the solvent move along with the advancing solvent front, while less soluble compounds travel slowly through the paper, well behind the solvent front. As a result, different compounds are separated on the basis of their solubility in the chosen solvent.

Rf is defined as the ratio of the distance travelled by compound at its point of maximum concentration to the distance travelled by solvent. Both the distances are measured from the point of application of the sample. Rf value has no unit.

$$Rf = \frac{\text{Distance travelled by substance}}{\text{Distance travelled by solvent}}$$



Separation of four main pigments using paper chromatography

Requirements:

1. Coin
2. Petroleum Ether
3. Acetone
4. Distilled Water
5. Solvent preparation: Mix Petroleum Ether, Acetone and water in the ratio of 3: 1: 1.
6. Spinach leaf
7. Glass rod, Stapler and ruler

Procedure:

Sample Loading:

1. Take the rectangular sheet of Whatman No. 1 filter paper.
2. Mark a pencil line to mark the locus of putting the leaf extract. Make sure to keep this line almost 2 cms from any of the longer side corner.
3. Place the leaf on the pencil line, and start rolling the coin with lesser force, to transfer the leaf constituents on the marked line. Rolling should be done thrice with 1-minute gap between each roll.

Sample chromatographic run:

1. Take 50 ml of solvent in a jar and cover it with lid.
2. Put down the paper into the solvent using a glass rod, making sure that marked line portion does not come in direct contact with the solvent.
3. Run the chromatogram for 15 minutes.
4. Take the paper out and dry at room temperature for 2 minutes and mark solvent front with a pencil.

Different pigments will appear like lines on the paper at different distance from the starting point, measure Rf value and record.

Observation:

Parameter	Distance travelled, cm
Solvent	
Chlorophyll b	
Chlorophyll a	
Xanthophyll	
Carotene	

Retention factor of various pigments is calculated using the formula:

$$Rf = \frac{\text{Distance travelled by pigment}}{\text{Distance travelled by solvent}}$$

Results:

Retention Factor of:

Chlorophyll b: _____

Chlorophyll a: _____

Xanthophyll: _____

Carotene: _____

Marks Obtained: _____

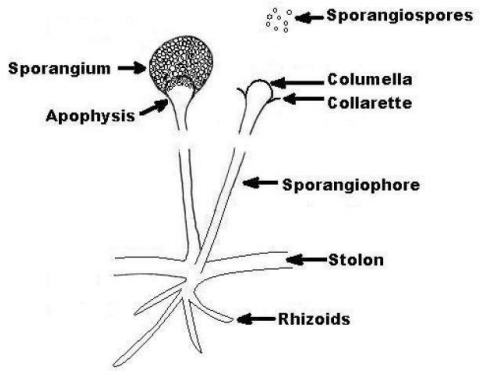
Signature of Instructor: _____

Review Questions:

1. What will happen if plants are exposed to green light?
2. Name the central element present in chlorophyll.
3. What is the partition coefficient?
4. Mention one structural difference between *Chl a* and *Chl b.*
5. What are Carotenoids?

EXPERIMENT No. 4
Observation of permanent slides

Rhizopus:



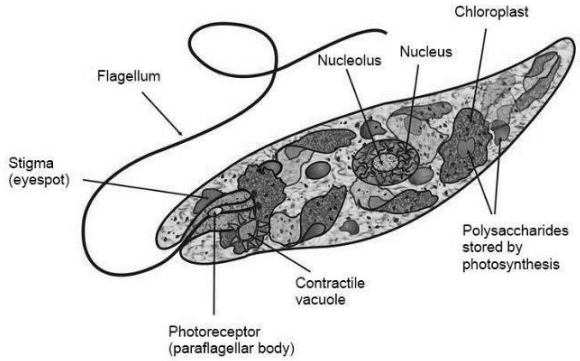
General features:

- *Rhizopus* is a genus of common saprophytic fungi that lives on plants and animals.
- *Rhizopus stolonifer*, is commonly found on bread surfaces and is known as black bread mold.
- *Rhizopus* species grow as filamentous, branching hyphae that generally lack cross-walls.
- They reproduce by forming asexual and sexual spores.
- In asexual reproduction, sporangiospores are produced inside a spherical structure known as sporangium.
- Sporangium is supported by a large columella atop a long stalk, the sporangiophore.
- In sexual reproduction, a dark zygospore is produced where two compatible mycelia fuse.

Observations:

Comments:

Euglena:



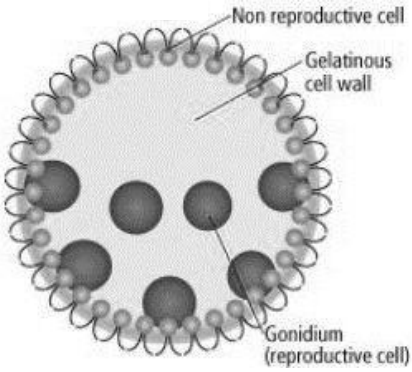
General features:

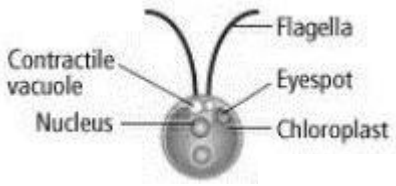
- *Euglena* is a genus of single-celled flagellate eukaryotes, found in fresh and salt waters.
- *Euglena gracilis* has been used extensively in the laboratory as a model organism.
- *Euglena* lacks a cell wall. Instead, it has a pellicle made up of a protein layer supported by a substructure of microtubules.
- *Euglena* has two flagella rooted in basal bodies located in a small reservoir in front of the cell.
- *Euglena* possesses a red eyespot, an organelle composed of carotenoid pigment granules.
- *Euglena* reproduces asexually by binary fission.

Observation:

Comments:

Volvox:





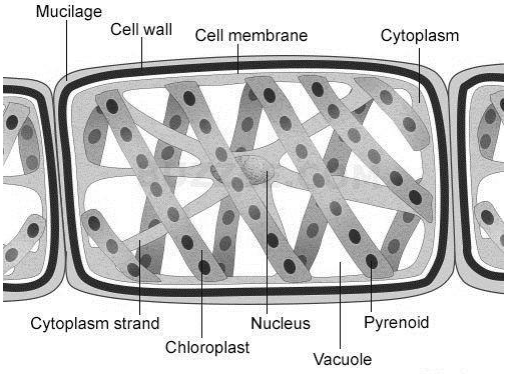
General features:

- *Volvox* is chlorophyte green algae.
- It forms spherical colonies of up to 50,000 cells that live in a variety of freshwater habitats.
- Each *Volvox* colony is composed of two differentiated cell types: numerous flagellate somatic cells and a smaller number of germ cells.
- Adult somatic cells comprise a single layer of flagella facing outward.
- The cells have anterior eyespots that enable the colony to swim towards light.
- The cells of colonies are interconnected by thin strands of cytoplasm called protoplasmates.
- An asexual colony includes both somatic (vegetative) cells, which do not reproduce, and large, non-motile gonidia in the interior, which produce new colonies through repeated division.
- In sexual reproduction two types of gametes are produced.

Observation:

Comments:

Spirogyra:



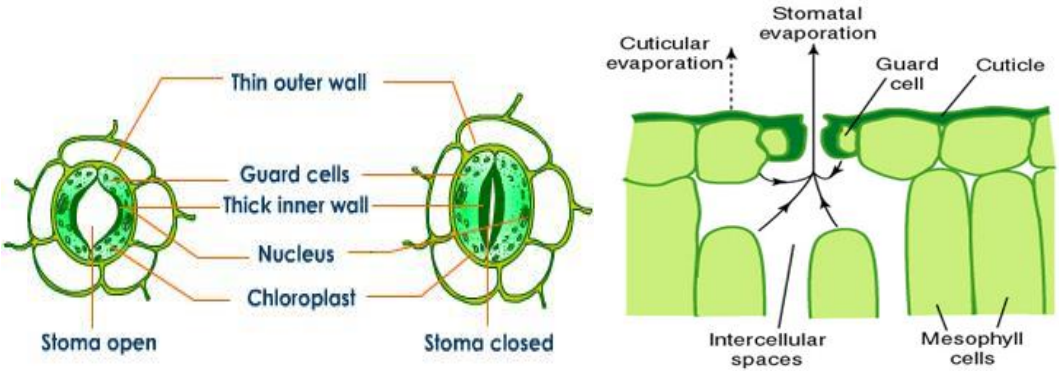
General features:

- *Spirogyra* is filamentous charophyte green algae, commonly found in freshwater.
- It is very common in clean eutrophic water, developing slimy filamentous green masses.
- It is also called as water silk or pond scum because of slippery touch of its thread like filaments.
- Each filament is unbranched and consists of cylindrical cells placed end to end.
- The cell wall is two layered and is made up of cellulose and pectin.
- It can reproduce both sexually and asexually.
- Asexual reproduction takes place by fragmentation.
- Sexual reproduction is of two types: Scalariform conjugation in which two different filaments lined side by side either partially or throughout their length are associated and lateral conjugation in which gametes are formed in a single filament.

Observation:

Comments:

Stomata:



General features:

- Stomata helps in increasing the solute concentration and lower the water potential inside the cell, which results in the diffusion of water into the cell through **osmosis**. This increases the cell's volume and **turgor pressure**.
- **Osmosis** is the movement of water or other solvent through a plasma membrane from a region of low solute concentration to a region of high solute concentration. **Osmosis** is passive transport, meaning it does not require energy to be applied.
- **Turgor pressure** is the force within the cell that pushes the plasma membrane against the cell wall. The pressure exerted by the osmotic flow of water is called turgidity. It is caused by the osmotic flow of water through a selectively permeable membrane.
- **Importance of Turgor pressure** within cells is regulated by osmosis and this also causes the cell wall to expand during growth. Along with size, rigidity of the cell is also caused by turgor pressure; a lower pressure results in a wilted cell or plant structure (i.e. leaf, stalk). One mechanism in plants that regulate turgor pressure is its semipermeable membrane, which only allows some solutes to travel in and out of the cell, which can also maintain a minimum amount of pressure. Other mechanisms include transpiration, which results in water loss and decreases turgidity in cells.
- **Turgidity** is the point at which the cell's membrane pushes against the cell wall, which is when turgor pressure is high. When the cell membrane has low turgor pressure, it is flaccid. In plants, this is shown as wilted anatomical structures. This is more specifically known as plasmolysis. This makes the cell plasmolysed, which results in the closing of the stomatal pores.

Observation:

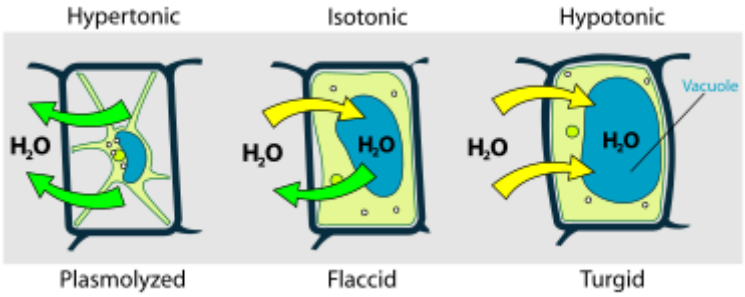
Comments:

Marks Obtained: _____

Signature of Instructor: _____

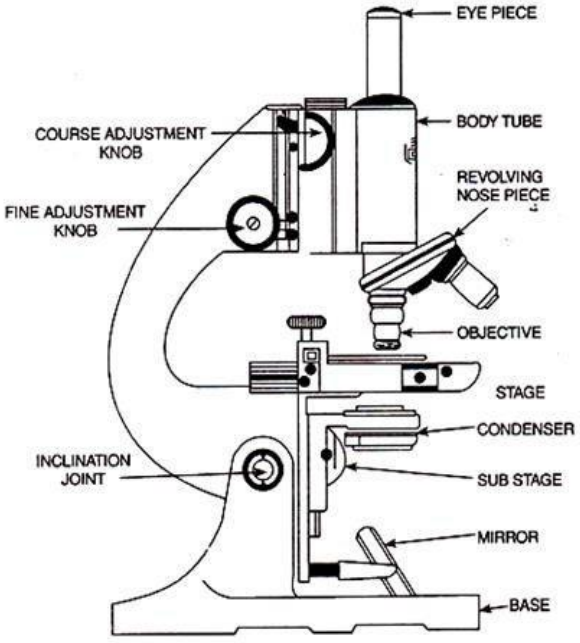
EXPERIMENT No. 5
Study of the phenomenon of plasmolysis in onion peel

Theory:



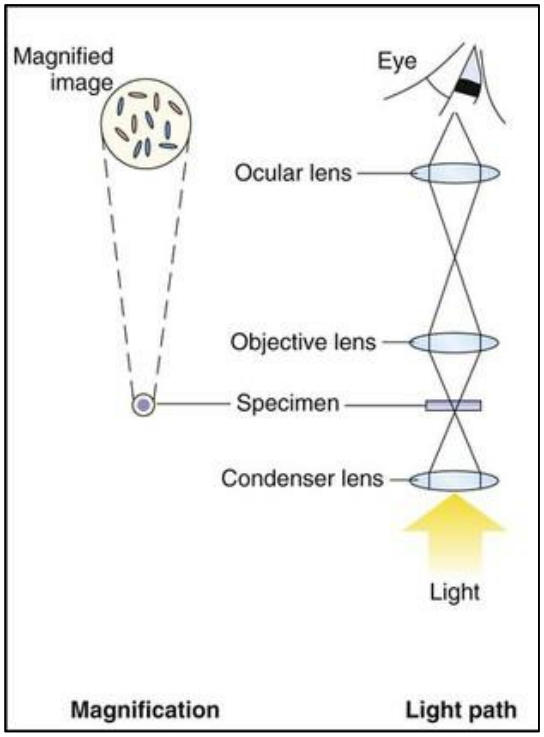
Plasmolysis is the process in plant cells where the plasma membrane pulls away from the cell wall due to the loss of water through osmosis. Plasmolysis only occurs in extreme conditions and rarely happens in nature. The reverse process, cytolysis, can occur if the cell is in a hypotonic solution resulting in a higher external osmotic pressure and a net flow of water into the cell. Through observation of plasmolysis it is possible to determine the tonicity of the cell's environment as well as the rate at which solute molecules cross cellular membrane.
If a plant cell is placed in a hypertonic solution, the plant cell loses water and hence turgor pressure, making the plant cell flaccid. Plants with cells in this condition wilt. Further water loss causes plasmolysis: pressure decreases to the point where the protoplasm of the cell peels away from the cell wall, leaving gaps between the cell wall and the membrane. Eventually **cytorrhysis**– the complete collapse of the cell wall – can occur. **Cytorrhysis** is the permanent and irreparable damage to the cell wall after the complete collapse of a plant cell due to the loss of internal positive pressure. There are some mechanisms in plants to prevent excess water loss in the same way as excess water gain. Stomata and wax lining help retain water in the plant so that the plant does not dry out. The equivalent process in animal cells is called **crenation.**

Microscope:



Microscopes are indispensable in biological studies. Human eye has some intrinsic limitations as a magnifying instrument, eye cannot focus on objects brought closer to it than approximately 25 cm. This is distance of maximal effective magnification. Secondly, to be visible an object must subtend an angle of 1^o or greater at the eye. As can be seen from the figure, the compound microscope consists of three lenses: the objective lens, the condenser and

the eyepiece (ocular) lens. The specimen is mounted on a stage that can be moved at right angles to the microscope axis, which is also referred to as z-axis. Light source is focused by adjusting the condenser so that light passes through the objective ocular lens, which is rigidly connected to the microscope column. Focusing the specimen is done by adjusting distance between specimen and the objective.



Working principle of microscope: The principle function of a microscope is thus magnification. In other words, the microscope increases the apparent angle subtended at the eye by objects within the microscopic field. This property of a microscope is quantified as the Magnification (M). Resolving power specifies the smallest detail that a microscope can resolve in imaging an ideal specimen. The distance between two points in the microscopic field that can just be distinguished from one another is called the minimum resolvable distance, D_{min}. This is defined by the equation:

$D_{min} = \frac{0.5\,\lambda}{N \sin\alpha}$ where, λ is Wavelength of light source; α is the Aperture angle of the objective lens; N is the Refractive index of the medium between the specimen and the objective lens.

In order to be visible through a microscope an object must possess a certain degree of contrast with its surrounding medium. This contrast is a result of the fact that less light is transmitted through the object than through the medium. This decreased light transmission is caused by two factors- light absorbed by the object and light refracted out of the optical path of the microscope by a difference in the refractive index between the object and the surrounding medium. Contrast can be greatly increased by staining procedures: treatment with dyes that bind selectively either to the whole cell or to certain cell components, thus producing a much greater absorption of light. Thus we see that magnification, resolution and contrast are three important factors in microscopy.

Requirements:

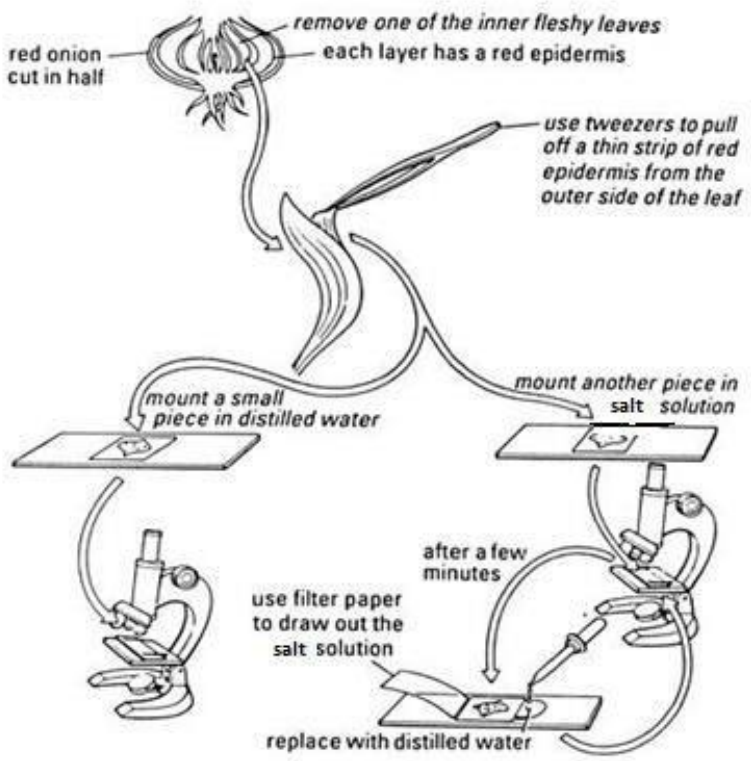
1. One or two pieces of onion, cut into slices approximately 1 cm wide
2. Compound microscope
3. Microscope slides, 1 per specimen
4. Cover slips, 1 per specimen
5. Distilled water
6. Salt solution (Sodium Chloride) 5% w/v
7. Rubber bulb
8. Pipettes
9. Filter paper

Precautions:

1. Onion may irritate some students' eyes to the point of discomfort. Hence onion pieces may be dipped in water to avoid irritation of eyes.
2. Take care with microscope slides and (especially) cover slips which are fragile and break easily. Ensure how to deal with broken glass by consulting with instructor/ lab technician
3. Sodium chloride is described as 'low hazard' on Hazard 47B.

Procedure:

1. Cut 1 cm square of onion. Then peel off a single layer of the red cells from an inner fleshy leaf of the onion.
2. Place the strip on a glass slide. Cover it with a drop or two of distilled water. Add a cover slip.
3. Look at the cells through a microscope, starting with low power lens.
4. Take similar strip of cells from the onion. This time mount the cells with couple of drops of 5% sodium chloride solution.
5. Examine through the microscope and compare the cells that are mounted with distilled water and sodium chloride solution.
6. After a few minutes draw out the sodium chloride solution with a piece of filter paper placed at the edge of the coverslip. Replace it with distilled water added at the other side of the coverslip.
7. Observe the changes that happen to the cells.



Overview of the experiment

Observation:

A. <u>Cells mounted in presence of water</u>
Diagram and General features:

B. <u>Cells mounted in presence of sodium chloride</u>
Diagram and General features:

Results:

Marks Obtained: _____

Signature of Instructor: _____

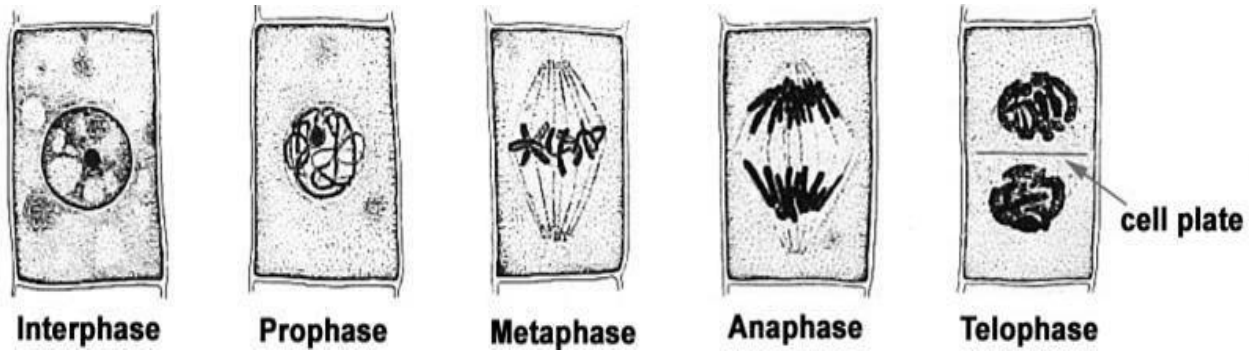
Review Questions:

1. Explain what happened to the cells in sodium chloride solution using biological terms. ***(****Try to include these words: Cytoplasm, Diffusion, Water, Solvent, Dissolved salts, Solute, Cell membrane, Vacuole, Cell wall, Osmosis, Plasmolysis, Turgid, Flaccid, Turgor)*
2. What prevents the plant cells from bursting when they take in lots of water?
3. What is the key role of cell wall/cell membrane?
4. Is there any difference exist between osmosis and plasmolysis? Justify your answer.
5. What would be the response of a plant cell when placed in: a) highly concentrated sucrose solution and b) highly concentrated potassium chloride solution.

EXPERIMENT No. 6
Identification of mitotic stages in the given plant tissue sample

Theory:

Mitosis is somatic cell division. The cell division takes place in two stages; karyokinesis (division of cell nucleus) and cytokinesis (division of cell cytoplasm). The karyokinesis is divided into four phases: prophase, metaphase, anaphase and telophase. In any population of mitotically active cells, only some cells are in dividing phases (karyokinesis) at any one time, while other cells are in interphase (non-dividing phase). The fraction or percentage of dividing cells is defined as the mitotic index (MI). MI is important to determine the duration of mitosis in the cell cycle of growing tissue/cells and also for scaling up of cell culture for various biotechnological purposes. To obtain MI, one should examine a mitotically active cell population, for example, growing root tips of *Alium cepa* (onion). The mitotic stages are generally studied by squash preparation.



Requirements:

Reagents:

1. 10% Hydrochloric Acid- Take 9 ml water and add 1 ml HCl to it slowly.
2. Acetocarmine Stain – Mix 90 ml Acetic Acid with 110 ml distilled water (45% acetic acid). Add 2 gm carmine to 100 ml 45% acetic acid and boil for 30 minutes. Cool and make up the volume of filtered stain to 100 ml by adding 45% acetic acid and store.

Materials:

1. Microscope with 10X, 40X objective
2. Microslides
3. Coverslips no. 1 (Square/ round)
4. Pony hair round Paint Brush
5. Tissue paper/ filter paper
6. Root tip of *Alium cepa* (Onion) / *Allium sativum* (Garlic)

Procedure:

1. Cut the root tip from onion bulbs, about 3-4 mm from the tip (at the base of the meristem) and rinse briefly in distilled water.

2. Place the tip in 10% HCl (v/v) for **8-10 minutes** at room temperature and rinse again in distilled water.
3. Put the tip on aceto-carmine solution for **8-10 minutes** and rinse again in distilled water.
4. Place the stained tip in a drop of water on a micro slide and cover with a coverslip.
5. Gently tap the root tissue with the handle (back side) of paint brush to produce a squash having homogeneous cell suspension. Do not tap hard on the coverslip, it may break the coverslip.
6. Remove the excess liquid from under the coverslip by placing tissue paper/ filter paper over the coverslip and press gently with index finger.
7. Examine under the microscope, various stages of mitosis.

Observations:
Draw your observations below:

Marks Obtained: _____

Signature of Instructor: _____

Review Questions:
1. What is the difference between karyokinesis and cytokinesis?
2. Describe different stages of mitosis.
3. What does S-Phase of cell cycle represent?
4. What is the use of immersion oil in microscopy?
5. What is the difference between magnification, resolution and contrast?

Experiment No. 7

Determination of ABO-Rh blood types

Theory:

The biological uniqueness that each individual attains is frequently noted in the reactions that occur when it receives biological material from other organisms. ABO blood typing is an excellent example of the serological principle of agglutination. Karl Landsteiner determined that there were four different immunological human blood types. This theory was based on the fact that two distinct antigens (agglutinogens), A and B, could be present on the surface of RBCs. Depending on the presence or absence of either or both the antigens, blood types were established as A, B, AB or O. This constitutes the ABO classification system as illustrated in the table given below:

RBC antigen (agglutinogen)	Plasma antibodies (agglutinins)	Blood group
A	Anti-B	A
B	Anti-A	B
A and B	None	AB
None	Anti-A and Anti-B	O

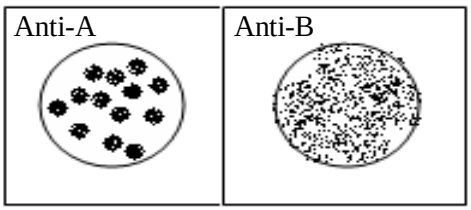
Of medical importance is the fact that the fluid portion of the blood, called as the plasma, may contain antibodies (agglutinins). If present these antibodies are not reactive against the individual's own RBCs. When mixed with the RBC antigens of a different blood type, for example during the course of a blood transfusion, a violent, incompatible agglutination reaction may result. Thus ABO blood typing is a routine prerequisite to blood transfusions.

Rh factor in human beings:

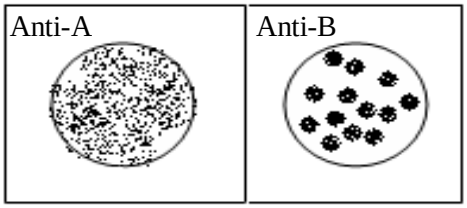
K. Landsteiner and S. Weiener discovered the Rh factor in 1940 from rabbits immunized with the blood of the monkey *Macaca rhesus.* The resulting antibodies were found to agglutinate not only the RBCs of monkey but in the human populations also. Individuals whose blood cells react with Rh antibody are termed as Rh positive and those who do not react are termed Rh negative. The symbol Rh came from the first two letters of the species name of the monkey. A test for Rh incompatibility is accomplished by placing a drop of blood from the subject on a slide and introducing anti-Rh serum. Agglutination of RBCs indicates incompatibility, whereas an even distribution of RBCs indicates no reaction.

The original antigen, now symbolized Rh° is highly antigenic to humans. Thus, cross matching of Rh factor, as well as ABO types of donor and recipient blood is now used to avoid incompatibility agglutination reactions following transfusions. Blood is frequently exchanged between the mother and the fetus during childbirth. Thus, Rh negative mothers may be immunized by blood from Rh positive fetuses (which may result when fathers are Rh positive), to which they gave birth.

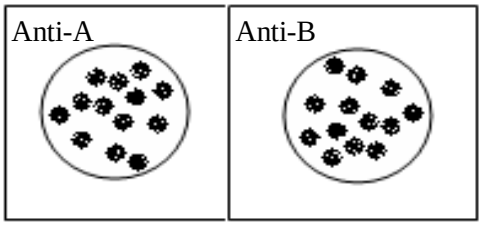
Usually no ill effects are associated with the exposure of the mother to the Rh positive antigen during the first childbirth (unless the mother has been exposed to Rh antigen by transfusion). Subsequently Rh positive children carried by the same mother against the Rh antigen, may develop symptoms of hemolytic jaundice and anemia, a condition referred to as *erythroblastosis fetalis.* The symptoms may be mild or severe, even resulting in the death of the fetus or new born infants if appropriate steps are not taken by the physician. In the experiment to follow, students will perform an ABO typing procedure by separately mixing a drop of their blood with anti-A and anti-B and anti-D sera on glass slides. Determination of the blood type is done by observing for agglutination on the slide preparation as illustrated in the figure given below:



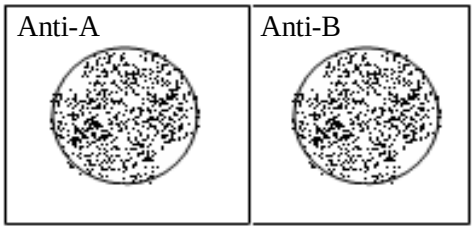
Group A



Group B



Group AB



Group O

Requirements:

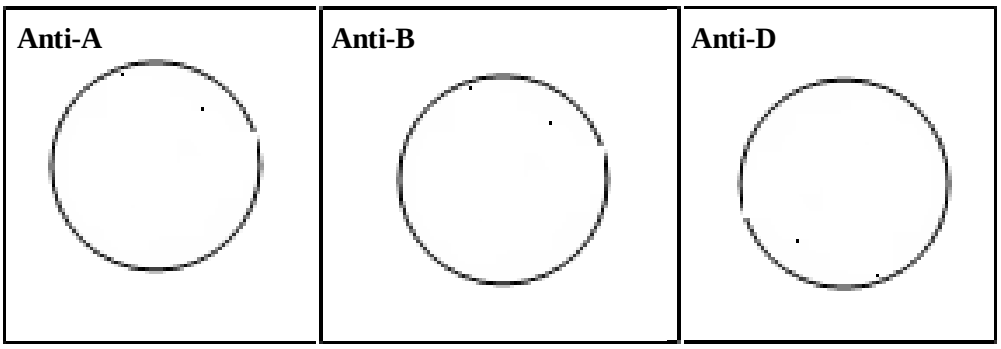
1. Anti-A, Anti-B and Anti-D blood typing sera and 70% alcohol.
2. Microscopic slides, sterile blood lancets, absorbent cotton, wooden applicator sticks and wax pencils.

Procedure:

1. Using wax pencil, label 3 reaction chambers of a porcelain dish as anti-A, anti-B and anti-D.
2. Place one drop of each antiserum on the appropriately labeled section of the slides.
3. Using a piece of absorbent cotton moistened with 70% ethyl alcohol, wipe the tip of the middle finger.
4. Using a sterile bold lancet, prick the disinfected area of the finger.
5. Allow one drop of blood to flow into each of antiserum on the slides.
6. With separate applicator sticks, mix each drop of blood with its respective antiserum.
7. Rock the slide between your fingers in a 'to and fro' motion and observe both mixtures for one minute for clumping (agglutinations).
8. Observe the slides under the microscope.

Observation:

In the following diagram, draw the observed antibody response against three antigens A, B and D.



Results:

1. Determine and indicate the ABO blood type: _____

2. Indicate the agglutinogen present: _____

3. Indicate the agglutinin present: _____

4. Determine and indicate the Rh type: _____

Marks Obtained: _____

Signature of Instructor: _____

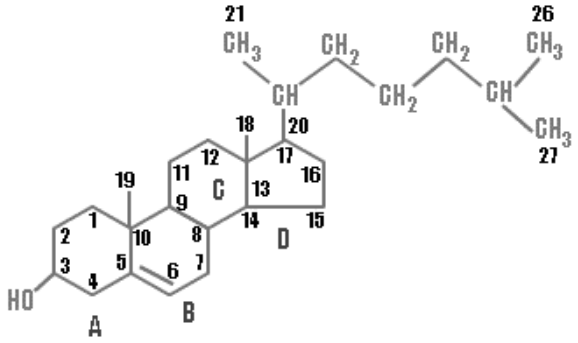
Review Questions:

1. Explain why type B blood cannot be transfused into a person with type blood group A?
2. Why there are no agglutinins present in the plasma of an individual with type AB blood?
3. Can blood typing be performed using only the serum portion of blood?
4. What molecules distinguish antigens A, B and O?
5. What is the cause of erythroblastosis fetalis?

Experiment No. 8
Total cholesterol estimation from blood serum/plasma

Theory:

Cholesterol ($C_{27}H_{46}O$), a major sterol in animal tissues, is amphipathic, with a polar head, containing the hydroxyl group at C-3 and a nonpolar hydrocarbon body containing the steroid nucleus and hydrocarbon side chain at C-17. Virtually all cells and body fluids contain some steroid molecules. Although almost 90% of synthesis takes place in the liver and gut using simple molecules like acetate, a portion of the body's cholesterol is derived from dietary intake. Once synthesized, cholesterol is released into the circulation for transport in combination with specific lipoproteins. Thyroid hormones play a very important role in the synthesis and degradation of cholesterol and triglycerides.



Structure of Cholesterol

Bile acids are polar derivatives of cholesterol that act as detergents in the intestine, emulsifying dietary fats to make them more readily accessible to digestive lipases. Cholesterol is an essential precursor of several hormones and an important component of cell membranes assisting in establishing proper membrane permeability and flexibility. Normal range of cholesterol in a person is 150-250 mg/dl. Though cholesterol is an essential molecule in mammals, its higher levels in the blood can be lethal. Elevation of the total cholesterol values is considered to be a primary risk factor for coronary heart disease. Higher cholesterol concentration in blood can damage arteries, which can ultimately cause heart disease. Elevated levels of serum cholesterol are associated with atherosclerosis, necrosis, diabetes mellitus, obstructive jaundice and myxoedema. However, decreased levels of cholesterol are observed in hyperthyroidism, malabsorption and anaemia.

Assay principle:

Cholesterol esters are hydrolyzed by cholesterol esterase (CE) to give free cholesterol and fatty acids. In subsequent reaction, cholesterol oxidase (CHOD) oxidizes the 3-OH group of free cholesterol to liberate cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidise (POD), hydrogen peroxide couples with 4-aminoantipyrine (4-AAP) and phenol to produce **red** quinoneimine dye. Absorbance of colored dye is measured at 490nm (490-510 nm) and is proportional to amount of total cholesterol concentration in the sample.

Cholesterol esters $\xrightarrow{CE}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{CHOD}$ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + Phenol + 4-AAP $\xrightarrow{POD}$ Quinoneimine dye + H_2O

Requirements:

1. Serum/ plasma (provided)
2. Test tubes
3. Pipettes
4. Dispensers
5. Water batch (37°C)
6. Colorimeter
7. Cholesterol standard (200mg/dL)
8. Cholesterol mono-reagent containing cholesterol esterase, cholesterol oxidase, peroxidase and 4-aminoantipyrine in buffer solution.

Precautions

1. Use clean and dry glassware
2. Use pipette dispensers. Do not pipette the sample/ reagents using mouth.
3. Bring all the reagents to assay temperature before use, because the reaction is sensitive to temperature.

Procedure:

1. Dispense the solutions in the properly labelled tubes as follows:

Pipette into tubes marked	Blank	Standard	Test
Blank (Water)	20 µl	-	-
Cholesterol Standard	-	20 µ1	-
Serum/Plasma	-	-	20 µl
Cholesterol Mono-reagent	2 ml	2 ml	2 ml

2. Mix well and incubate at **Room temperature** for **30 min**.
3. Measure absorbance of the samples at **490 nm.**
4. Calculate results as per given calculation formula

$$\text{Cholesterol concentration (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Observation:

Absorbance of test: _____

Absorbance of Standard: _____

Cholesterol concentration (mg/dL) : _____

Result:

The concentration of serum cholesterol was found to be: _____

Marks Obtained: _____

Signature of Instructor: _____

Review Questions:

1. Why does our body need cholesterol?
2. What are the important dietary sources of cholesterol?
3. How do we define ‘good’ and ‘bad’ cholesterol in our body?
4. What is atherosclerosis and which type of cholesterol is responsible for it?
5. Distinguish saturated and unsaturated fats?

Experiment No. 9

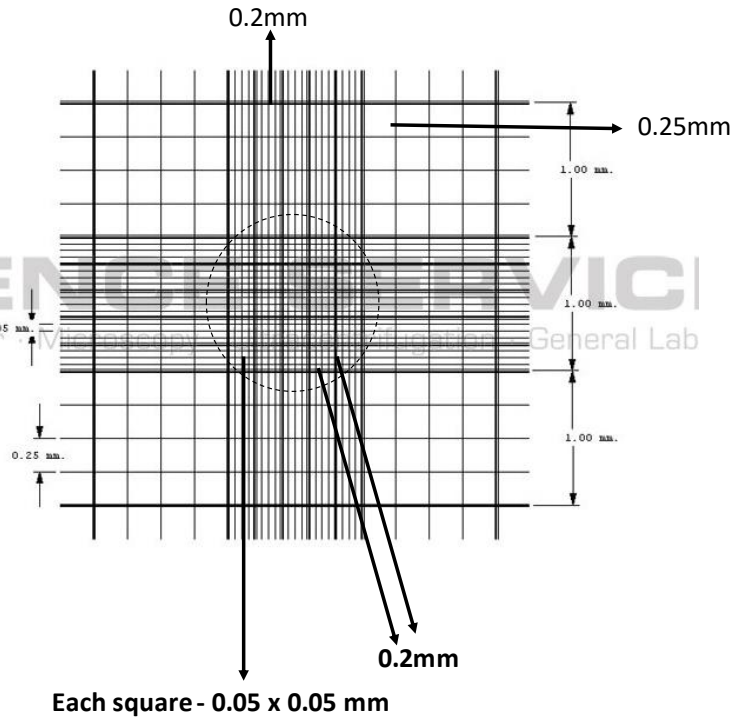
Micrometric measurement of microorganisms

Theory:

It is necessary to determine the concentration of cells for cell culture studies and many of the applications that require use of cell suspensions. The device used for determining the number of cells per unit volume of a suspension is called a Neubauer Chamber or counting chamber. It is the most widely used type of chamber, since it was mainly designed for performing blood cell counts. It is now used to count other types of cells including microorganisms and other microscopic particles as well. The Neubauer Chamber was invented by Louis-Charles Malassez. It is a special type of microscope slide consisting of two chambers, which is divided into nine (1.0mm x 1.0mm) large squares which are separated from one another by triple lines. The area of each square is 1mm². Cover glass is supported over the chambers at a height of 0.1mm. Because of that the entire counting grid lies under the volume of 0.9 mm² on one side. The microbial cell suspensions are introduced into the cover glass. The Neubauer Chamber is placed on the microscope stage and the cell suspension is counted.The glass microscope slide has a rectangular indentation that creates an 'H' shaped chamber at the centre. This chamber is engraved with a laser-etched grid of perpendicular lines. Two counting areas with ruled grids are separated by the horizontal groove of the 'H'. There is also a very flat, reusable cover slip. The glass cover slip is held at 0.1mm above the surface of the counting areas by ground glass ridges on either side of the vertical grooves of the H shape. The device is carefully crafted so that the area bounded by the depth and lines of the chamber is also known. Because the height is constant, the volume of fluid above each square of the grid is known with precision.

Dimensions	Area	Volume at 0.1mm depth
1 x 1 mm	1 mm_2	**100 nl**
0.25 x 0.25 mm (1/16)	0.0625 mm_2	**6.25 nl**
0.25 x 0.20 mm (1/20)	0.05 mm_2	**5 nl**
0.20 x 0.20 mm (1/25)	0.04 mm_2	**4 nl**
0.05 x 0.05 mm (1/400)	**0.0025 mm_2**	**0.25 nl**

400 squares each of 0.05x0.05mm



Each square - 0.05 x 0.05 mm

- The ruled area of the Neubauer Chamber consists of several large 1 x 1 mm (1mm²) squares, which are subdivided in three ways; 0.25 x 0.25 mm (0.0625 mm²), 0.25 x 0.20 mm (0.05 mm²) and 0.20 x 0.20 mm (0.04 mm²). The central, 0.20 x 0.20 mm marked, 1 x 1 mm square is further subdivided into 0.05 x 0.05 mm (0.0025 mm²) squares. Hold the cover slip (0.1 mm) at the raised edges of Neubauer Chamber, which gives each square a defined volume as shown below:

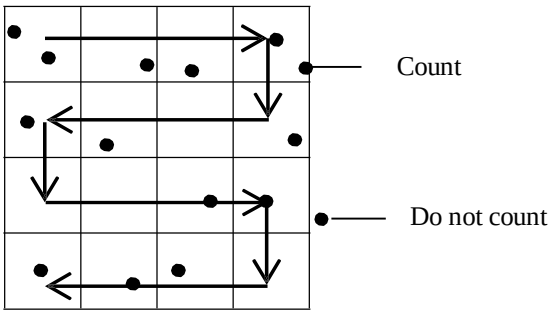
Materials:

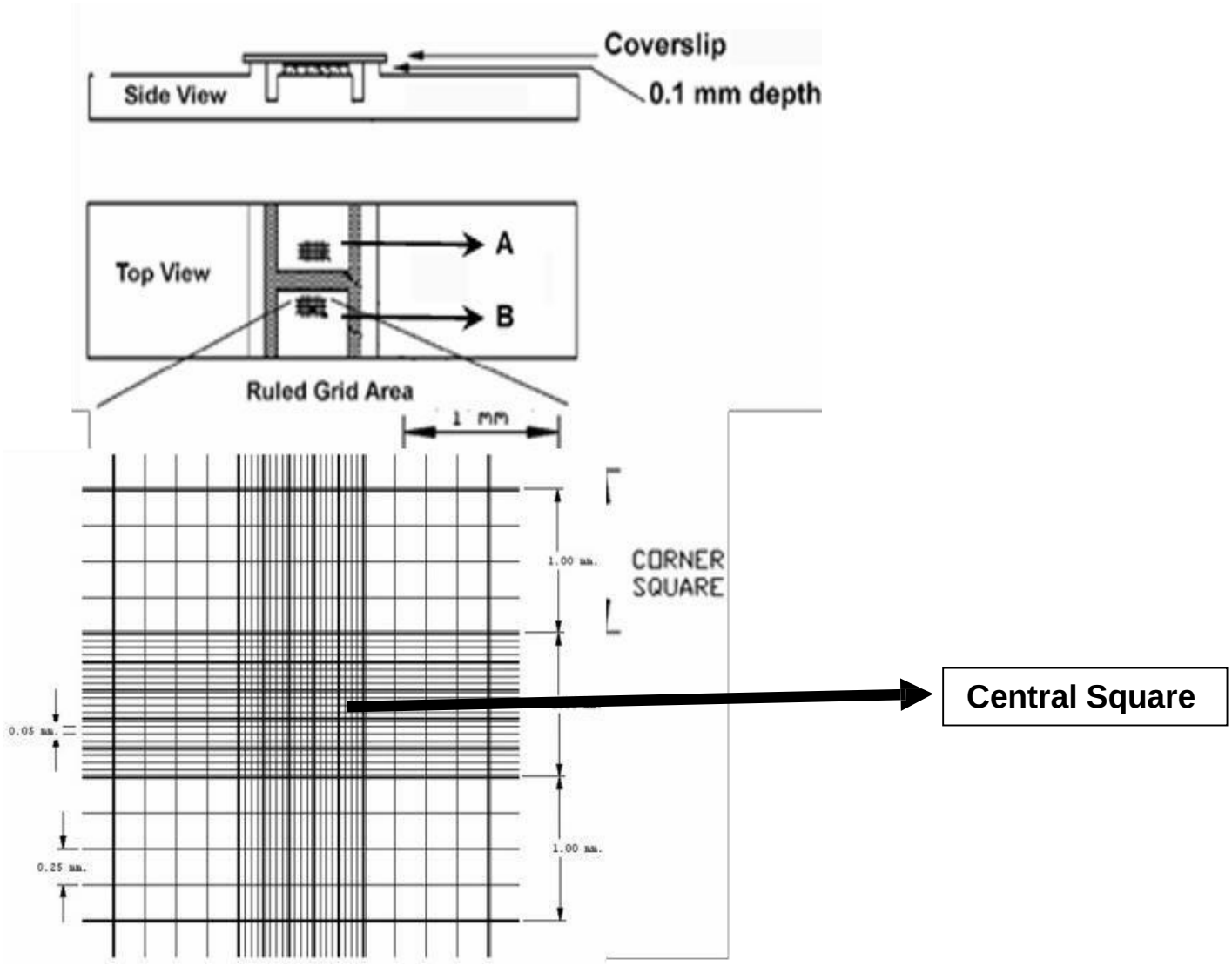
Compound microscope, Neubauer Chamber, micropipettes, 24 hour-bacterial cell culture, distilled water, test tubes.

Procedure:

1. Take 10 ml of the fresh broth culture (24 hour). Centrifuge it at 3000 rpm for 10 minutes. A pellet of bacterial cells will be formed in the base. Use this pellet for serial dilution.
   a. Take the pellet in a test tube and dissolve it in 10ml of distilled water. This is the bacterial suspension. Label this test tube as T1.
   b. In 4 more test tubes labelled as T2, T3, T4 and T5 add 9ml of distilled water.
   c. Shake contents of the T1 and take 1ml bacterial suspension from this and transfer it into T2.
   d. Shake contents of the T2 thoroughly, take out 1ml from this and transfer it into T3. Repeat this procedure for T4 and T5.
   e. T1 is the stock solution or original concentration of bacteria. Dilutions of other tubes are as follows: T2 is 10^{-1} dilution, T3 is 10^{-2}, T4 is 10^{-3} and T5 is 10^{-4}. For rest of the experiment, you can use any of the serial dilutions.
2. Take a Neubauer Chamber and put a cover slip on its central part that has a small grid. From an edge of the cover slip dispense 10µl of diluted bacterial suspension. Let it settle down for 5 minutes.
3. After 5 minutes put the Neubauer Chamber under high power (40X objective lens) and focus to observe the grid formed on the Neubauer Chamber. You have to count the bacterial cells in the central square which is further divided into 25 squares. You have to count the cells in the central square composed of small 25 square and record.

Eye Movement (While observing the slides under 40X)





Neubauer Chamber and ruling

Results:

Average Number of cells in the central square =

Calculations:

The total number of microbial cells is calculated using the following formula:
No. of Cells/ml = Total No. of cells in the Central Square X dilution factor x 10^4

No. of Cells/ml =_____X_____x 10^4

Marks Obtained: _____

Signature of Instructor: _____

Review Questions:

1. What is the other name of Neubauer Chamber?
2. What does an uneven distribution of cells in the Neubauer Chamber means?
3. What are the applications of cell count in different areas of biology?
4. Give any two beneficial role of microorganisms in human body.
5. Why centrifugation of bacterial broth is required in this experiment?

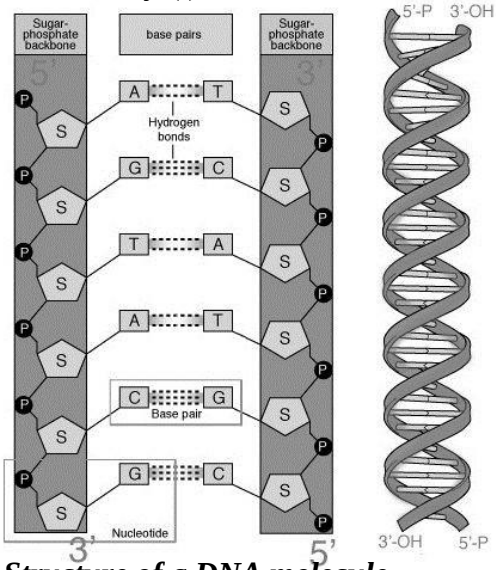
Experiment No. 10

Extraction of DNA from banana

Theory:

DNA provides the blueprint for transmission of genetic information. The strands of DNA inside the nucleus are directions for creating all other components of the cell necessary for living. DNA is present in the nucleus of eukaryotic cells and in the cytoplasm of prokaryotic cells. The length of DNA per cell is about 100,000 times as long as the cell itself. However, DNA only takes up about 10% of the cell's volume. This is because DNA is specially packaged through a series of events to fit easily in the cell's nucleus. The structure of DNA is in the form of a double helix, which is wrapped around proteins, folded back onto itself, and coiled into a compact chromosome. Individual chromosomes can be studied using microscopes, but the double helix of a chromosome can only be detected through procedures such as X-Ray Crystallography and Nuclear Magnetic Resonance spectroscopy. Chromosomal DNA from a single cell is not visible to the naked eye. However, when chromosomal DNA is extracted from multiple cells, the amassed quantity can easily be seen and looks like strands of mucous-like, translucent cotton. DNA is a macromolecule that is one of four that are necessary for life (others are sugars, proteins and fats). DNA is polymer including either one of the four nitrogenous bases, deoxyribose sugar and phosphate. These nucleotides are linked by (i) Covalent bond between phosphates and sugars and (ii) Hydrogen bond between complementary base pairs.

Detergents solubilize and break down the lipids and proteins that form the primary cell membrane and disrupt the bonds that hold the membrane together. The cell contents, including the nucleus, are thus released and become available for further treatment or isolation. Sodium lauryl sulphate (SDS) is an active ingredient in detergents. The final step in the present procedure requires alcohol. The solubilized DNA comes in contact with the alcohol where the two liquid layers interface. The alcohol dehydrates and precipitates the DNA, as DNA is insoluble in the alcohol. If the procedure is done properly, fine, long strands of DNA will form at the interface and can be easily spooled onto a stirring rod.



Structure of a DNA molecule

Principle:

Deoxyribose nucleic acid (DNA) is a long, slender molecule that carries the heritable information in organisms on to future generations. Because of its size, it is impossible to see a single DNA molecule with the naked eye. When subjected to certain conditions, it is possible to collect "large" amounts of DNA to make it visible. This process of collecting DNA is referred to as spooling. During spooling, a solution made from soap/ detergent and salt (NaCl) in a buffer is mixed with the cells. The soap from the buffer solution disrupts the cell membrane's phospholipid bilayer by reacting with the phosphate group of the phospholipid. This releases the cellular components into the buffer. Once the components are released, the sodium ion in thebuffer binds to several of the negatively charged phosphate groups of the DNA's sugar-phosphate "backbone," shielding some of the negative charge of the DNA. Because some of the negative charges are shielded, the DNA molecules can loosely bind together.

In the presence of salt, DNA precipitates from solution containing high percentages of ethanol or isopropanol. Due to its size and abundance, chromosomal DNA forms viscous, clotted masses during alcohol precipitation. A plastic loop is used to mix the two liquids at their interface and collects the DNA as it precipitates from solution at the mixing zone.

Small fragments of DNA and degraded RNA usually contaminate the chromosomal DNA during extraction procedures. They are also precipitated by the alcohol, but have little tendency to spool on the loop because they are too short and form finer, more uniform precipitates. Spooling can be viewed as a method that partially purifies and concentrates high molecular eight DNA.

The purification of chromosomal DNA is frequently the first step in molecular cloning experiments. The precipitate can be collected and re-dissolved in a smaller volume. This is a convenient way to concentrate nucleic acids. Alcohol precipitations also remove small molecules, such as buffer salts, sugars and amino acids from nucleic acid precipitations since they remain in solution.

Materials:

- Sodium chloride
- Sodium bicarbonate
- SDS (Sodium dodecyl sulfate)- Detergent
- Distilled water
- 50 ml beaker
- Ice cold isopropanol
- Banana
- Filter/ Filter Paper
- Test tube
- Plastic loop
- Methylene Blue

Procedure:

1. **Buffer Preparation:**

   Mix the following contents in a clean 100 ml beaker
   a. 60 ml distilled water
   b. 0.75 g sodium chloride
   c. 2.5 g sodium bicarbonate
   d. 0.05 g of SDS

   Chill the buffer in a freezer or on ice for 10-15 minutes.

2. **Extraction of DNA**

   a. Take approximately 10g of banana and smash it on the wax paper.
   b. Place a small amount of pureed banana into a clean 50 ml beaker.
   c. Add 10 ml of chilled buffer solutions. Stir vigorously for at least 2 minutes.
   d. Fit the filter paper in the funnel. Pour the banana/buffer mixture into the filter. Collect the liquid in a test tube.

3. **Spooling of DNA**

   a. Gently (tilt tube and allow alcohol to dribble down the side) add 10 ml of ice cold isopropanol to the mixture in the test tube. Since the DNA/buffer solution is denser than the alcohol, the alcohol will float on top. The boundary between the two is called an interface.
   b. Insert the plastic loop into the test tube. Carefully swirl the loop just below the interface. Wind (spool) the DNA that comes out of solution on to the loop. These are not single DNA molecules, but thousands of molecules. After a minute of spooling, slowly remove the rod from the tube. The DNA, a clear, viscous, clotted mass will adhere to the rod.
   c. Examine and touch the DNA on the loop. Describe the DNA in your observations.

4. **Staining and observing (optional)**

   a. Add a few drops of methylene blue stain to the solution remaining in the test tube. The stain will adhere to any residual DNA that did not spool on the glass rod.
   b. You may observe the spooled DNA with a microscope by carefully applying it to a microscope slide, adding a drop of stain, and covering with a plastic coverslip.

Observations:

Result:

Marks Obtained: _____

Signature of Instructor: _____

Review Questions

1. What does the soap/detergent do to the cells?
2. What causes the DNA to precipitate and spool on the rod?
3. Describe the DNA collected during the spooling process.
4. Is this a single strand of DNA?