2.3.2

ICT ENABLED TEACHING SUPPORTIVE DOCUMENT

LAB MANUAL

DEPT OF BIOTECHNOLOGY (UG)

Name of the Test	Application
Molish's test	General test for carbohydrates
Iodine's test	Test for starch and glycogen
Barfoed's test	Distinguishing test between monosaccharide and Disaccharide
Saliwannoff's test	For aldoses and ketoses
Fehling's test	For reducing sugar
Benedict's test	For reducing sugar
Picric acid test	For reducing sugar
Mucic acid test	For glucose
Bial's test	For pentose
Osazone' test	Confirmation test for monosaccharide To study about different crystal for mation in carbohydrates.

Qualitative Test for Carbohydrates

Aim:

To study the different reaction of carbohydrates and Identification of the given carbohydrates in given sample.

Introduction:

Carbohydrates are hydrophilic Substations with a potential or free aldehydic or keto group and a number of Hydroxyl groups. Monosaccharides are simple sugars in the series and have single carbon chain.

Glucose and (aldohexose) and fructose (a ketohexose) are the most common monosaccharides. Disaccharides are the most common carbohydrates, which on hydrolysis form two manosaccharides units. Maltose, lactose and sucrose are the common disaccharides. The former two are reducing disaccharides, sucrose is non-reducing.

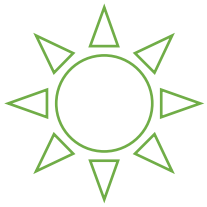
The most commonly available polysaccharide is starch which is a mixture of amylase and amylopectin. The individual glucose units in amylase are linked by (1-4) glycosidic linkage. Amylopectin has the branching points contributed by (1-6) glycosidic bonds, starch is insoluble in cold water but forms a colloidal solution in hot water. Starch has no detectable reducing activity.

Sample 1:

Test	Observation	Inference
Molish's test: 2-3 drops of α-napthol+2ml of sample solution+Conc.H_2SO_4 along the sides of the test tube.	Reddish- violet ring appears at the junction of two layers	The given sample contain Carbohydrates.
Iodine test: 1ml of sample + 1 drop of iodine solution	No colour Change	The given sample doesn't contain starch or glycogen
Barford's test: Take 2ml of Barford's reagent in attest tube + 1ml of sample. Keep the testtube in boiling water bath	Appearance of brick red colour within 8=10 mins	The given sample is a Monosaccharide.
Saliwannoff's test: Take 1ml of sample + 2ml of saliwannoff's reagent keep testtube in boiling water bath.	No appearance of red colour.	The given sample contains aldose sugar
Fehling's test: Take 1ml of Fehling's reagent in a testtube + 1ml of test solution' keep the testtube in boiling water bath.	Appearance of red colour.	The given sample contains reducing sugar.
Benedict's test: Take 0.5ml or 1ml of test solution + 2ml of benedict's reagent. Keep the testtube in boiling water bath.	Appearance of the red colour	The given sample contains reducing sugar.
Picric acid test: Take 1ml of picric acid + 2ml of sample +0.5ml of Na2CO3.Keep the test tube in boiling water bath.	Appearance of red colour red colour	The given sample contains reducing sugar.
Osazone test: 1ml of sugar + pinch of pheniyl Hydrozin + few drops of acetic acid. keep the testtube in Boiling water bath for 1hr. Cool the solution and observe microscopically.		The given sample is glucose

Result: The given sample is a Carbohydrate, monosaccharide, aldose sugar, reducing sugar So, the sample may be _____.

Sample 2:

Test	**Observation**	**Inference**
Molish's test: 2-3 drops of α-napthol+2ml of solution+Conc.H_2SO_4 along the sides of the test tube.	Reddish- violet ring appears at the junction of two layers	The given sample contain Carbohydrates.
Iodine test: 1ml of sample + 1 drop of iodine solution	No colour Change	The given sample doesn't contain starch or glycogen
Barford's test: Take 2ml of Barford's reagent in attest tube + 1ml of sample. Keep the testtube in boiling water bath	Appearance of brick red colour within 8-10 mins	The given sample is a Monosaccharide.
Saliwannoff's test: Take 1ml of sample + 2ml of saliwannoff's reagent keep testtube in boiling water bath.	Appearance of red colour.	The given sample contains ketose sugar
Fehling's test: Take 1ml of Fehling's reagent in a testtube + 1ml of test solution' keep the testtube in boiling water bath.	Appearance of red colour.	The given sample contains reducing sugar.
Benedict's test: Take 0.5ml or 1ml of test solution + 2ml of benedict's reagent. Keep the testtube in boiling water bath.	Appearance of the red prepicipitate	The given sample contains reducing sugar.
Picric acid test: Take 1ml of picric acid + 2ml of sample +0.5ml of Na2CO3.Keep the test tube in boiling water bath.	Appearance of red colour red precipitate	The given sample contains reducing sugar.
Osazone test: 1ml of sugar + pinch of pheniyl Hydrozin + few drops of acetic acid. keep the testtube in Boiling water bath for 1hr. Cool the solution and observe microscopically.	Significant formation of crystals are not formed	

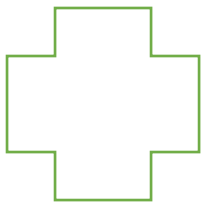
Result: The given sample is a Carbohydrate, monosaccharide, ketose sugar, reducing sugar So, the sample may be Fructose

Sample 3:

Test	**Observation**	**Inference**
Molish's test: 2-3 drops of α-napthol+2ml of solution+Conc.H_2SO_4 along the sides of the test tube.	Reddish- violet ring appears at the junction of two layers	The given sample contain Carbohydrates.
Iodine test: 1ml of sample + 1 drop of iodine solution	No colour Change	The given sample doesn't contain starch or glycogen
Barford's test: Take 2ml of Barford's reagent in attest tube + 1ml of sample. Keep the testtube in boiling water bath	Brick red colour doesn't appear.	The given sample is a disaccharides.
Saliwannoff's test: Take 1ml of sample + 2ml of saliwannoff's reagent keep testtube in boiling water bath.	No appearance of red colour.	The given sample contains aldose sugar
Fehling's test: Take 1ml of Fehling's reagent in a testtube + 1ml of test solution' keep the testtube in boiling water bath.	Appearance of red prepicipitete.	The given sample contains reducing sugar.
Benedict's test: Take 0.5ml or 1ml of test solution + 2ml of benedict's reagent. Keep the testtube in boiling water bath.	Appearance of the red colour	The given sample contains reducing sugar.
Picric acid test: Take 1ml of picric acid + 2ml of sample +0.5ml of Na2CO3.Keep the test tube in boiling water bath.	Appearance of red colour red colour	The given sample contains reducing sugar.
Osazone test: 1ml of sugar + pinch of pheniyl Hydrozin + few drops of acetic acid. keep the testtube in Boiling water bath for 1hr. Cool the solution and observe microscopically.		The given sample is Lactose.

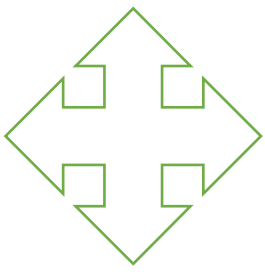
Result: The given sample is a Carbohydrate, disaccharides, aldose sugar, reducing sugar So, the sample may be Lactose.

Sample 4:

Test	**Observation**	**Inference**
Molish's test: 2-3 drops of α-napthol+2ml of solution+Conc.H_2SO_4 along the sides of the test tube.	Reddish- violet ring appears at the junction of two layers	The given sample contain Carbohydrates.
Iodine test: 1ml of sample + 1 drop of iodine solution	No colour Change	The given sample doesn't contain starch or glycogen
Barford's test: Take 2ml of Barford's reagent in attest tube + 1ml of sample. Keep the testtube in boiling water bath	Brick red colour doesn't appear.	The given sample is a disaccharides.
Saliwannoff's test: Take 1ml of sample + 2ml of saliwannoff's reagent keep testtube in boiling water bath.	Appearance of red colour.	The given sample contains ketose sugar
Fehling's test: Take 1ml of Fehling's reagent in a testtube + 1ml of test solution' keep the testtube in boiling water bath.	No coloured precipitate is formed.	The given sample contains reducing sugar.
Benedict's test: Take 0.5ml or 1ml of test solution + 2ml of benedict's reagent. Keep the testtube in boiling water bath.	No coloured precipitate is formed.	The given sample contains reducing sugar.
Picric acid test: Take 1ml of picric acid + 2ml of sample +0.5ml of Na2CO3.Keep the test tube in boiling water bath.	No coloured precipitate is formed.	The given sample contains reducing sugar.
Osazone test: 1ml of sugar + pinch of pheniyl Hydrozin + few drops of acetic acid. keep the testtube in Boiling water bath for 1hr. Cool the solution and observe microscopically.	Significant formation of crystals are not formed.	

Result: The given sample is a Carbohydrate, disaccharides, ketose sugar, non-reducing sugar So, the sample may be Sucrose.

Sample 5:

Test	**Observation**	**Inference**
Molisch's test: 2-3 drops of α-napthol+2ml of solution+Conc.H_2SO_4 along the sides of the test tube.	Reddish- violet ring appears at the junction of two layers	The given sample contain Carbohydrates.
Iodine test: 1ml of sample + 1 drop of iodine solution	No colour Change	The given sample doesn't contain starch or glycogen
Barford's test: Take 2ml of Barford's reagent in attest tube + 1ml of sample. Keep the testtube in boiling water bath	Brick red colour doesn't appear.	The given sample is a disaccharides.
Saliwannoff's test: Take 1ml of sample + 2ml of saliwannoff's reagent keep testtube in boiling water bath.	No Appearance of red colour.	The given sample contains aldose sugar
Fehling's test: Take 1ml of Fehling's reagent in a testtube + 1ml of test solution' keep the testtube in boiling water bath.	Appearance of red Precipitate.	The given sample contains reducing sugar.
Benedict's test: Take 0.5ml or 1ml of test solution + 2ml of benedict's reagent. Keep the testtube in boiling water bath.	Appearance of the red Precipitate.	The given sample contains reducing sugar.
Picric acid test: Take 1ml of picric acid + 2ml of sample +0.5ml of Na2CO3.Keep the test tube in boiling water bath.	Appearance of red colour red Precipitate.	The given sample contains reducing sugar.
Osazone test: 1ml of sugar + pinch of pheniyl Hydrozin + few drops of acetic acid. keep the testtube in Boiling water bath for 1hr. Cool the solution and observe microscopically.		The given sample is maltose.

Result: The given sample is a Carbohydrate, disaccharide, aldose sugar,

Name of the Test	Colour Appearance

reducing sugar So, the sample may be Maltose.

Sample 6:

Test	Observation	Inference
Molish's test: 2-3 drops of α-napthol+2ml of solution+Conc.H_2SO_4 along the sides of the test tube.	Reddish- violet ring appears at the junction of two layers	The given sample contain Carbohydrates.
Iodine test: 1ml of sample + 1 drop of iodine solution	Formation of violet colour.	The given sample doesn't contain starch or glycogen

Result: The given sample is a Carbohydrate, polysaccharide, Starch.

Ninhydrin test	Purple colour appears.
Xanthoprotic test	Orange colour appears.
Millon’s test	Brick red colour appears.
Biuret test	Purple colour appears
Ammonium sulphate precipitate test	Precipitate appears
Heat Coagulation test	Coagulation appears

Qualitative Analysis of proteins

Aim:

To detect the presence of protein in given sample by colour reaction.

Introduction:

Protein are made up of amino acid resides. Joined by peptide bonds. Because of the presence of peptide bond and difference amino acid reside, protein react with the variety of reagent to form coloured reaction of proteins.

Several of these reaction are of importance in qualitative detection and qualitative of protein and their constituent amino acids.

Test	Observation	Inference
Ninhydrine Test: Take 1ml of sample in test tube + add few drops of ninhydrin reagent & vortex + heat in boiling waterbath for 5 mins and cool.	Appearance of purple-blue colour.	The given sample in protein.
Xanthoprotic Test: 1ml of sample + few drops of connitric acid & vortex + boil the content and cool + few drops of alkali.	Appearance of yellow colour in acidic medium & orange in alkaline medium.	The given sample in protein.
Millon's Test: Take 1ml of sample + few drops of millon's reagent vortex + boil for 3-5 minutes and cool.	Formation of red colour solution.	The given sample is protein
Biuret test: Take 2ml of sample + add few drops of alkali + vortex +	Formation of Violet purple colour	The given sample is protein.
Ammonium sulphate Precipitation test: Take protein sample in test tube + equal volume of saturated ammonium sulphate. Centrifyge 2500-3000rpm to separate the ppt format.decent the saturate this supernatant By adding solid ammonium sulphate with constant strring.	The precipitate is observed	The given sample is protein.
Heat Coagulation test: Take 2ml sample + acidity by adding acetic acid + heat the solution for few minutes.	Appearance of coagulum	The given sample is proteins.

Result: It given sample is Proteins.

Observation and Calculation:

Concentration of standard= 100mg / 100ml.

Serial No.	Index	Volume of Standard (ml)	Concentration of Standard (llg)	Volume of water (ml)	Volume of reagent		OD 540 nm
1	Blank	0	0	2.5		Keep The tubes in boiling water bath for 10ml of water to all list tubes	
2	1	0.2	200	2.3	2ml of DNS reagent		
3	2	0.4	400	2.1			
4	3	0.6	600	1.9			
5	4	0.8	800	1.7			
6	5	1	1000	1.5			
7	6	Test		1.5			

Quantitative Estimation of Reducing Sugar by DNS Method

Aim:

To Estimate the amount of reducing sugar in the given sample.

Principle:

Reducing sugar in alkaline medium convert DNS from yellow colour to red. The Intensity of the red colour developed can be measured at 540nm.

Reagent Required:

- Standard : Glucose- 100mg/100ml.
- DNS reagent:

Procedure:

- Take clean and dry testtube and mark them as blank 0.2, 0.4, 0.6, 0.8, 1ml and test.
- Prepare a blank with Distilled water and pipette out the sample as marked.
- Make up the volume 1.0ml by adding distilled water.
- To each of the testtube add 2ml of DNS reagent.
- Mix the Contests well and keep it in boiling water bath for 10 mins.
- Cool the testtube and add 10ml of distilled water to each of the testtube.
- 7.Mix the content in the testtube well and read the OD at 540nm.
- Plot a Graph of Concentration various OD.

Result:

The concentration of reducing sugar in the given unknown sample is _____.

Observation and Calculation:

Concentration of standard= 500mg / 100ml.

Tubes	Volume of Standard (ml)	Concentration of Standard (llg)	Volume of Distilled water	Volume of biuret reagent		OD 540 nm
Blank	0		3	Add 5ml of b Biweet reagent to all the testtube.	Keep it aside for 30 min in room Temport ature	
1	0.2	1000	2.8			
2	0.4	2000	2.6			
3	0.6	3000	2.4			
4	0.8	400	2.2			
5	1	5000	2			
6	Test					

Quantitative Estimation of Protien by Biuret method

Aim:

To estimate the quantity of protein in the given sample.

Principle:

The CONH group (peptide Bond) of protein forms a purple colour with copper ion in an alkaline solution. The intensity of the purple coloured complex, is measured at 540nm. This method is commonly used to estimate the protein in the range from 0.5 to 5llg.

Requirement:

- Standard protein: 500mg of bovine serum albumin is dissolved in 100ml of distilled water.
- Biuret reagent.

Procedure:

- Take a clean testtube and label them. Pipette out the standard solution(0.2,0.4,0.6,0.8) ml into the testtube.
- Prepare blank with distilled water and the pipette out the test sample.
- Make up the volume to 3ml by adding distilled water.
- Add 5ml of Biuret reagent. Mix the contents of the testtube well and keep for 30minsat 37^0c . Read the OD at 540nm.
- Plot the graph taking conc along x axis and OD along y axis.

Result:

The Concentration of the given unknown sample is 1800mg/ml.

Observation and Calculation:

Concentration of standard= 100mg / 100ml.

Tubes	Volume of Standard (ml)	Concentration of Standard (llg)	Volume of Distilled water	Volume of biweet reagent		OD 720 nm
Blank	0		1.5	Add 5ml of Copper reagent to all keep for 10 mins.	Add 0.5 ml fc reagent and keep in dark for 30 mins.	
1	0.2	200	1.3			
2	0.4	400	1.1			
3	0.6	600	0.9			
4	0.8	800	0.7			
5	1	1000	0.5			
6	Test					

Quantitave Estimation of Protein by Lowry's method

Aim :

To estimate the concentration of protein using lowry's method.

Principle:

Protein react with folin's ciocaletee reagent to give coloured complex. The colour formed is due to the reaction of alkaline copper reagent with protein and the reduction of phospholdate in the folin's reagent by amino acid tysone and tryptoplasm present in the given protein sample.The intensity of the colour depends on amount of aromatic amino acids present and thease varies for different proteins.

Requirements:

- Standard protein: 100mg of protein in 100ml of distilled water.
- Copper reagent
- Folin's ciocaltere reagent.

Procedure:

- Take clean dry test tube and table them
- Pipette standard solution 0.2, 0.4, 0.6, 0.8, 1 into the test tube.
- Prepare blank with distilled water and pipette out the test sample.
- Make up the volume 1.5ml by adding water .
- Add 5ml of copper reagent to the above mixture.
- Mix the contents of the test tube well and keep side for 10 minutes.
- Now add dilute fc reagent 0.5ml mix and keep the test tube in dark about 30 minutes.
- Read the OD at 720nm.
- Plot the graph by taking concentration along x-axis and OD along the y-axis.

Result:

The Concentration of the given unknown sample is 510uglml.

Observation and Calculation:

Concentration of standard= 100mg / 100ml.

Tubes	Volume of Standard (ml)	Concentration of Standard (µg)	Volume of Distilled water	Volume of DPA reagent		OD 595 nm
Blank	0		1.5	Add 5ml to all the test tubes.	Keep into the test tubes in boiling water bath for 10 minutes.	
1	0.2		1.3			
2	0.4		1.1			
3	0.6		0.9			
4	0.8		0.7			
5	1		0.5			
6	T(ml)					

Quantitave Estimation of DNA by Diphynyl Amine method

Aim:

To estimate the concentration of DNA by diphenyl amine method.

Principle:

Deoxyribose in DNA in presence of acide found β-vydroxy levulinylealdenyde this aldenyde condenses in acidic medium with diphenyl amine produce de blue coloured condensed product the develop colour can be red at 595 nm.

Requirements:

- DNA standard – 100mg of DNA is desolved in 100ml of saline.
- Diphenyl amine reagent.

Procedure:

- Take clean and dry test tube and table them.
- Pipette out the standard solution 0.2, 0.4, 0.6, 0.8, and 1ml into the test tudes.
- Prepare blank with distilled water and test sample.
- Make up the test tubes by adding 3ml of water.
- Add 5ml of DPA reagent to the above mixture.
- Mix the content of the test tube well
- And keep in boiling water for 10 minutes.
- Read the OD at 595nm.
- Plot the graph by taking concentration along x-axis and OD at y-axis.

Result:

The given unknown solution is 690µg/ml.

Observation and Calculation:

Concentration of standard= 100mg / 100ml.

Tubes	Volume of Standard (ml)	Concentration of Standard (µg)	Volume of Distilled water	Volume of orcinal reagent		OD 595 nm
Blank	0		2	Add 3ml of orcinal reagent all the test tubes.	Mix the contents and keep the test tubes in boiling water bath for 15 minutes.	
1	0.2		1.8			
2	0.4		1.6			
3	0.6		1.4			
4	0.8		1.2			
5	1		1			
6	T(ml)		Iml			

Quantitave Estimation of RNA by Orcinal method

Result:

To estimate the estimation of in given sample by orcinal reagent method.

Principle:

The rebose sugar present in RNA 1's converted into furfural under acidic condition. This furfural in presence of ferric chloride reacts with orcinal to give a given coloured comnplex with orcinal to give absorption maxima at 665 nm.

Requirement:

- Standard RNA solution dissolve 100mg of RNA in 100ml of centrate buffer
- Orcinal reagent.

Procedure:

- Take clean dry test tube and table them.
- Pipette out the standard solution into the test tubes (0.2, 0.4, 0.6, 0.8 and 1)
- Prepare blank with distilled water and pipette blank with distilled water and pipette out the test sample.
- Make up the volume to 2ml by adding water.
- Add 3ml of orcinal reagent to all the test tubes.
- Mix the contents of the test tubes well and keep the in boiling water both for 15 minutes.
- Read the OD at 665 nm.
- Plot the graph taking concentration along x-axis and OD along y-axis.

Result:

The Concentration of RNA in given unknown sample is 500 µg/ml.

Observation and Calculation:

Concentration of standard= 100mg / 100ml.

Tubes	Volume of Standard (ml)	Concentration of Standard (µg)	Volume of Distilled water	Volume of orcinal reagent		OD 595 nm
Blank	0		2	Add 3ml of orcinal reagent all the test tubes.	Mix the contents and keep the test tubes in boiling water bath for 15 minutes.	
1	0.2		1.8			
2	0.4		1.6			
3	0.6		1.4			
4	0.8		1.2			
5	1		1			
6	T(ml)		Iml			

Assay of Salivary Amylase Enzyme

Aim:

To determine the activity of salivary amylase enzyme.

Principle:

The enzyme salivary amylase acts on the polysaccharides such as starch and cellulose.

A(1,4) and β(1,6) Glyosidic linkage and monosaccharides are form which reacts with indicator to give blue colour.

Requirements:

Starch solution- dissolve one gram of starch in 100ml of distilled water boil till clear liquid is form.

Iodine indicator- dissolve 1g of iodine and 1g of potassium iodine in water and make up the volume upto 500ml.

Amylase enzyme- take 1ml of water in mouth collect in a beaker and allow to stand for 10 min for the supernatant into another beaker.

Procedure:

- Take test tube and table them as blank, test, boil.
- Take one ml of enzyme to the test and boil test tube.
- Prepare a blank with 1ml of distilled water.
- Boil the content of the test tube labelled as boil.
- Add the 1ml of starch substrate to all the test tubes
- Mix the contents and allowed to stand for 15min.
- After inqubation add indicator to each test tube drop wise till the blue colour develop.
- Observe the result.

Result:

Test: Colourless.

Blank: Deep blue.

Boiled: Deep blue.

Activity of Salivary Amylase:

Tubes	Substrate	Enzyme	Distilled Water	Indicator or Iodine	Result
Test	1ml	1ml	-	1ml	
Blank	1ml	-	1ml	1ml	
Boiled	1ml	1ml(boil)	-	1ml	

Assay of Salivary Urea's Enzyme

Aim:

To determine the activity of urea's enzyme.

Principle:

Urease enzyme is present in large amount in biologically active soils since micro- organisms degrades are hydrolyse Urea enzymatically.

Assay of urease activity gives information the rate of degradation of nitrogen containing compounds especially proteins and the activity of different fertile soil with vegetation contains more urease than that without vegetation.

H2N-CO-NH2:H2O ---urease---> CO2+2NH8

The ammonia liberated reacts with nesslerise reagent to give brown colour.

Requirements:

Enzyme-obtae octant from norsegram

Substrate- 1% urea

Nesslers reagent.

Procedure:

- Take three test tubes and lable them as blank, test and boil.
- Add one ml of enzyme to the test and boil tube.
- Prepare the blank with 1ml distilled water .
- Boil the content of the test tube table as boil.
- Add 1ml of usedsubstrate to all the test tubes.
- Mix the content and allow to stand for 15 minutes.
- After inqubation add nessler's reagent in drop wise manner till brown colour develop.
- The developed brown colour indicate the positive reaction.

Result:

Test- Brown.

Boil- Olive green.

Blank- colourless.

Tubes	Enzyme	Distilled Water	Substrate (urea)	Reagent	Result
Test	-	1ml	1ml	1ml	
Blank	1ml	-	1ml	1ml	
Boiled	1ml(boiled)	-	1ml	1ml	

Wave Length	OD of potassium permanganate	OD of methyl orange	OD of malachite green
400			
420			
470			
500			
580			
620			
660			
700			

Determination of Absorption Maxima of the Solution

Aim:

To determination to the absorption maxima of the solution.

Principle:

The coloured compound have there own characteristicsabsorption spectra and careful selection of solution where the maximum absorption is found mixture of two coloured substance to amylase.

Requirements:

- Potassium permanganate
- Methyl orange
- Malachite green.

Procedure:

- The coloured dye is taken in the tube.
- The blank is prepared with didtilled water.
- The colourimeter is set to 420 nm.
- The colourmeter is set to 0 absorbent with a blank.
- The absorbent is noted at the wavelength 420 nm.
- The procedure is repeated for different wavelength.
- The same procedure is repeated for another dye.
- The standard graph is plotted by taking absorbent of each dye along x-axis and OD along y-axis.
- The maximum absorption of each dye is recorded.

Result:

The wavelength that gives maximum absorption for

Potassium permanganate- 520 nm

Methyl orange- 500 nm

Malachite green- 620 nm

Observation and calculation :

1. **Glycine:**

$R_{fG}=\frac{solute\ front}{solvent\ front}=\frac{1}{3}$=0.3 nm

2. **Tryptophan :**

$R_{f\,T}=\frac{solute\ front}{solvent\ front}=\frac{1.8}{3}$=0.6 cm

3. **Arginine:**

$R_{f\,A}=\frac{solute\ front}{solvent\ front}=\frac{1.3}{3}$=0.43 cm

4. **Unknown:**

$R_{f\,U}=\frac{solute\ front}{solvent\ front}=\frac{1.3}{3}$=0.43 cm

Radial Paper Chromatography for Amino acids

Aim:

To separate acid identity amino acid in a given sample.

Principle:

The separation of solutes (amino acids) is based on liquid – liquid, liquid – hyper liquid portion of amino acid in paper chromatography.

The position is taken place between the water molecule (static phase) absorb to the paper and the organic molecules (mobile phase).

Requirements:

- Solvent system.
- Standard solution of amino acid.
- Reagent.
- Petridish (glass)
- Whatmann filler paper.
- Small twist of filter which acts as wickes.

Procedure:

- Take a clean dry petridish place about 10-15ml of solvent in it, and cover it with a lid take a circular whatmann filter paper slitly bigger in diameter than the petridish.
- Draw a 1cm circle from the centre of the filter paper.
- Draw one more circle of radius of 4cm from the center of circular filter paper.
- Mark four equidistance point on the inner circle.
- Mark a small hole in its centre through the spot such that wick (made through) another filter paper passes through it.
- Mix now load three different known amino acid and an unknown amino acid on the respective spot marked on the inner circle of the filter paper.
- Reattached the wick to the filter paper and dip the wick into the solvent in the petridish such that the chromatography rest on the rim of the dish.
- Cover the petridish and allow the solvent to move horizontally along the paper.
- When the solvent reaches near the outer and of the filter paper take it out and dry the paper.
- Spray the visualising agent.
- Mark the spots, measure the distance travelled by the solvent, calculate the R_f value of each amino acid and determine the unknown amino acid.

Result:

The given mixture content arginine.