

Experiment-1: Qualitative tests of Carbohydrates

Aim: To determine the presence of carbohydrates in the given sample qualitatively

Principle:

Carbohydrates are widely prevalent in the plant kingdom, comprising the mono-, di-, oligo-, and polysaccharides. The common monosaccharides are the glucose, fructose, galactose, ribose etc. the disaccharides, i.e., the combination of two monosaccharides include sucrose, lactose and maltose. Starch and cellulose are polysaccharides consisting of many monosaccharide residues. Cellulose is most abundant organic compound on this planet since it forms part of cell wall in plants.

Aldehydes (-CHO) and ketones (=CO) are active groups in carbohydrates. Carbohydrates contain many hydroxyl groups as well. The number of hydroxyl groups varies with the number of carbon atoms. Monosaccharides contain the free aldehyde group (maltose) and some do not have the free ones (sucrose). The polysaccharides, starch and cellulose, are polymers of monosaccharides linked through the active groups.

The chemical properties of the saccharides vary depending upon the number of hydroxyl groups and the presence or absence of -CHO/ =CO groups. These variations are the basis in the development of color reactions to identify the saccharides.

Chemicals and Reagents:

Reagents

- a) **Iodine Solution:** Add a few crystals of iodine to 2% potassium iodide solution till the color becomes deep yellow.
- b) **Fehling's Reagent A:** Dissolve 34.65 g copper sulphate in distilled water and make up to 500 mL.
- c) **Fehling's Reagent B:** Dissolve 125 g potassium hydroxide and 173 g Rochelle salt (potassium sodium tartrate) in distilled water and make up to 500 mL.
- d) **Benedict's Qualitative Reagent:** Dissolve 173 g sodium citrate and 100 g sodium carbonate in about 800 mL water. Heat to dissolve the salts and filter, if necessary. Dissolve 17.3 g copper sulphate in about 100 mL water and add it to the above solution with stirring and make up to volume to 1L with water.
- e) **Barfoed's Reagent:** Dissolve 24 g copper acetate in 450 mL boiling water. Immediately add 25 mL of 8.5% lactic acid to the hot solution. Mix well. Cool and dilute to 500 mL.
- f) **Seliwanoff's Reagent:** Dissolve 0.05g resorcinol in 100 mL dilutes hydrochloric acid (1:2).

- g) **Bial's Reagent:** Dissolve 1.5 g orcinol in 500 mL of concentrated HCl and add 20 to 30 drops of 10% ferric chloride.

Procedure:

Substantiated by the observations the presence or absence of different carbohydrates needs to be determined.

Table 1.1: Qualitative reactions of Carbohydrates:

	Experiment	Observation	Remarks
1.	<p>Molisch's Test Add two drops of Molisch's Reagent (5% 1-naphthol in alcohol) to about 2mL of test solution and mix well.</p> <p>Incline the tube and add about 1 mL of concentrated sulphuric acid along the sides of the tube.</p> <p>Observe the color at the junction of the two liquids.</p>	A red-cum-violet ring appears at the junction of the two liquids	The color formed is due to the reaction of alpha-naphthol with furfural and/or its derivative formed by the dehydration of sugars by concentrated sulphuric acid. All carbohydrates react positively with this reagent.
2.	<p>Iodine Test Add a few drops of iodine solution to about 1 mL of the test solution</p>	Appearance of deep blue color	<p>This indicates the presence of starch in the solution</p> <p>The blue color is due to formation of starch-iodine complex.</p>
3.	<p>Fehling's Test To 1 mL of Fehling's solution 'A', add 1 mL of Fehling's solution 'B' and a few drops of the test solution. Boil for a few minutes.</p>	Formation of yellow or brownish-red precipitate	The blue alkaline cupric hydroxide present in solution, when heated in the presence of reducing sugars, gets reduced to yellow or red cuprous oxide and it gets precipitated. Hence, formation of the colored precipitate indicates the presence of reducing sugars in the test solution.
4.	<p>Benedict's Test To 2 mL of Benedict's reagent add five drops of the test solution. Boil for five minutes in a water bath. Cool the solution.</p>	Formation of red, yellow or green color/precipitate.	As in Fehling's test, the reducing sugar because of having potentially free aldehyde or keto group reduce cupric hydroxide in alkaline solution to red colored cuprous oxide. Depending on the sugar concentration yellow to

			green color is developed.
5.	Barfoed's Test To 1 mL of the test solution add about 2 mL of Barfoed's reagent. Boil it for one minute and allow to stand for a few minutes.	Formation of brick-red precipitate.	Only monosaccharides answer this test. Since Barfoed's reagent is weakly acidic, it is reduced only by monosaccharides.
6.	Seliwanoff's Test To 2 mL of Seliwanoff's reagent add two drops of test solution and heat the mixture to just boiling.	Appearance of deep red color	In the concentrated HCl, ketones undergo dehydration to yield furfural derivatives more rapidly than do aldoses. These derivatives form complexes with resorcinol to yield deep red color. It is a timed color reaction specific for ketones.
7.	Bial's Test To 5 mL of Bial's reagent add 2-3mL of solution and warm gently. When bubbles rise to the surface cool under the tap	Appearance of green color or precipitate	It is specific for pentoses, they get converted to furfural. In the presence of ferric ion orcinol and furfural condense to yield a coloured product.
8.	Test for non-reducing sugars such as sucrose:		
a)	Do Benedict's test with the test solution	No characteristic color formation	Indicates the absence of reducing sugars in the given solution
b)	Add 5 drops of concentrated HCl to 5mL of test solution in another test tube. Heat for five minutes on a boiling water bath. Add 10% sodium hydroxide solution to give a slightly alkaline solution (test with red litmus paper). Now perform Benedict's test with this hydrolyzed solution	Appearance of red or yellow color	Indicated the formation of reducing sugars from non-reducing sugars after hydrolysis with acid.
9.	Mucic Acid Test Add a few drops of conc. HNO ₃ to the concentrated test solution or substance directly and evaporate it over a boiling water bath till the acid fumes are expelled. Add a few drops of water and leave it overnight	Formation of crystals	The both end carbon groups are oxidized to carboxylic groups. The resultant saccharic acid of galactose is called mucic acid which is insoluble in water.
10	Osazone Test To 0.5g of phenyl hydrazine hydrochloride add 0.1g of sodium acetate and 10 drops of glacial acid. To this mixture add 5mL of test solution and heat on a boiling water	Glucose, fructose and mannose produce needle-shaped yellow osazone crystals. Whereas	The ketoses and aldoses react with phenyl-hydrazine to produce a phenylhydrazone which in turn reacts with another two molecules of

	bath for about half an hour. Allow the tube to cool slowly and examine the crystals under a microscope.	lactosazone in mushroom-shape d. Different osazones show crystals of different shapes. Maltose produces flower-shaped crystals.	phenylhydrazine to form the osazone.
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Notes:

1. For osazone test, the reaction mixture should be between pH 5 and 6. Fructose takes 2min to form the osazone whereas for glucose it is 5min. the disaccharides take a longer time to form osazone. Disaccharides form crystals only on cooling.
2. When a mixture of carbohydrates is present in the test sample, chromatographic methods should be employed to identify the individual sugars.

Result:

Experiment-2: Qualitative determination of Amino acids

Aim: To determine the presence of Proteins in the given sample qualitatively

Principle: Following tests are used to determine the amino acids qualitatively

a) Solubility test

Polar amino acids are more soluble in water than non-polar, due to presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution.

b) Ninhydrin test for alpha L amino acids

Ninhydrin (tri ketohydrindene hydrate) degrades amino acids into aldehydes, ammonia and CO₂ (on pH range 4-8) through a series of reactions. The net result is ninhydrin in a partially reduced form from hydrindantin. Ninhydrin then condenses with ammonia and hydrindantin to produce an intensely blue or purple pigment, sometimes called Ruhemann's purple. The color varies slightly from acid to acid. Proline and hydroxy-proline (amino acids) give yellow color. Many substances other than amino acids, such as amines will yield a blue color with ninhydrin, particularly if reaction is carried out on filter paper.

c) Xanthoproteic test for Aromatic amino acids

Conc. HNO₃ reacts with the aromatic rings that are derivatives of benzene giving the characteristic nitration reaction. Amino acids tyrosine and tryptophan contain activated benzene rings which are easily nitrated to yellow colored compounds. The aromatic ring of phenyl alanine does not react with nitric acid despite it contains a benzene ring, but it is not activated, therefore it will not react.

d) Millon's test for amino acids containing hydroxy phenyl group

The phenol group of tyrosine is first nitrated by nitric acid in the test solution. Then the nitrated tyrosine complexes mercury ion in the solution to form a brick-red solution or precipitate of nitrated tyrosine, in all cases, appearance of red color is positive test.

e) Sakaguchi Test

In alkaline solution, arginine reacts with alpha-naphthol and sodium hypobromite /chlorite as an oxidizing agent, to form red complexes as a positive result.

Materials and Reagents

Hydrochloric acid

Sodium hydroxide

Chloroform

Ninhydrin

Nitric acid

Phenol solution

10% NaOH

α-naphthol in 10% ethanol

5% sodium hypobromate (or sodium hypochlorite)

Test tubes

Water bath

Procedure

Solubility Test

Place 0.5 mL of amino acid sample in 4 test tubes clean, dry containing 4 mL of different solvents (chloroform and hot water), Shake the tubes thoroughly, and then leave the solution for about one minute, Record your result.

Ninhydrin Test

- i. Place 1 mL of each of the solutions in a test tube and add 2 drops of Ninhydrin solution.
- ii. Boil the mixture over a water bath for 2 min.
- iii. Allow to cool and observe the blue color formed
- iv. Complete the below table

Xanthoproteic Test

- i. Label five tubes (1 - 5), then add 0.5 mL of each amino acid solution and phenol solutions to those test tubes each alone.
- ii. Add a few drops of concentrated HNO₃.
- iii. Compare the color with that given by blank using water instead.
Now COOL THOROUGHLY under the tap and CAUTIOUSLY add 5 drops of 10M NaOH to make the solution strongly alkaline.

Millons Test

- i. Label 4 test tubes (1 - 5).
- ii. Add 1 mL of test solutions in separate tubes and the phenol solution in one tube.
- iii. Add to each tube 2 mL Millon's reagent and shake it well.
- iv. Place the test tubes in the boiling bath with care, for 10 min.
- v. Write your observation in the following table.

Sakuguchi Test

- i. Label 3 test tube and put in each one 1 mL of the aminoacid solution.
- ii. Add to each tube 2 mL of NaOH solution. Mix well.
- iii. Add to each tube 2 mL of α -naphthol solution. Mix well.
- iv. Add to each tube 3 drops of sodium hypobromite solution, and record your result.

Table 2.1: Qualitative reactions of Amino acids

S. No	Name of the Test	Observation

Result:

The amino acids in the given sample are qualitatively determined and results were tabulated in Table 2.1

Experiment-3: Determination of Proteins using Biuret Protein Assay

Aim: To determine the presence of Proteins in the given sample using Biuret test

Principle:

Under alkaline conditions substances containing two or more peptide bonds form a purple complex with copper salts in the reagent.

Reagent:

A formula for biuret reagent is (per liter final volume): 9 gm Sodium potassium tartrate, 3 gm Copper sulfate, 5 gm Potassium iodide, dissolved in order in 400 mL 0.2 M NaOH before bringing to final volume.

Procedure:

1. Volumes sample, reagent can be scaled up/down and/or volume ratios varied, as with any assay.
2. Warm up the spectrophotometer 15 min. before use.
3. Prepare standards from bovine serum albumin, preferably calibrated using absorbance at 280 nm and the extinction coefficient. Using 5 mL color reagent to 1 mL sample a recommended range is 0.5 to 20 mg protein.
4. Prepare a reference tube with 1 mL buffer.
5. If possible, dilute unknowns to an estimated 1 to 10 mg/mL with buffer; a range of dilutions should be used if the actual concentration cannot be estimated. Tabulate the values in Table 3.1.
6. Use 1 mL sample per assay tube
7. Add 9 mL Biuret reagent to each tube, vortex immediately, and let stand 20 min.
8. Read at 550 nm.

Analysis

Prepare a standard curve of absorbance versus micrograms protein (or vice versa), and determine amounts from the curve. Determine concentrations of original samples from the amount protein, volume/sample, and dilution factor, if any.

Table 3.1: Table for BSA Standard and Test Samples

S. No	Vol of BSA (mL)	Conc. of BSA (mg/mL)	Vol of distilled water (mL)	Vol of Biuret reagent	Duration of Incubation	OD at 550nm
					20min	
Samples						

Result:

The amount protein present in the given sample was.....

Experiment-4: Titration Methods for Estimation of Amino acids

Aim: Estimation of Amino acids by Titration methods

Principle:

Amino acids react with formalin to form methylene amino acids. But it was found that the reaction was more complex and the main product is monomethylol and di methylol amino acids. Amino acids exist in zwitter ionic form and cannot be titrated directly with alkali. Thus amino groups of amino acids are blocked by reaction with formaldehyde. But formaldehyde does not react with the charged amino groups ($-\text{NH}_3^+$), thus first the amino acid reacts with sodium hydroxide solution to give glycine which condenses with formaldehyde to give a stable anion.

Further formaldehyde solution contains formic acid and also amino acids are not exactly neutral species, thus it is necessary that both the formaldehyde and amino acid solution should have the same pH before mixing and for this purpose each solution is first made just alkaline to phenolphthalein by means of dilute sodium hydroxide solution.

Reagents:

a. Neutral formalin solution

It is prepared by neutralization of formalin solution with alkali to phenolphthalein end point.

b. Standard Glycine solution

An accurately weighed 0.8 grams of Glycine is dissolved in distilled water in a measuring flask 100 mL.

c. Unknown Glycine solution

d. Sodium hydroxide solution 0.1 N

e. Phenolphthalein indicator

Procedure:

1. A standard glycine solution (20 mL) is pipetted out into a conical flask and phenolphthalein (2-3 drops) added.
2. It is titrated with sodium hydroxide solution until a faint pink color appears.
3. Then the neutralized formaldehyde solution (10 mL) is added.
4. The pink color of the solution in the titration flask disappears immediately and the solution becomes acidic.
5. Then the contents of the flasks are titrated with sodium hydroxide solution till the pink color is obtained.
6. Same experiment repeated with unknown glycine solution.

Observations:

Weight of Glycine dissolved in 100 mL = w g

Volume of sodium hydroxide solution used with standard glycine solution = v_1 mL

Volume of sodium hydroxide solution used with unknown glycine solution = v_2 mL

Volume of glycine solution taken = 20 mL

Calculations

V1 mL of NaOH in 20mL standard glycine solution= (w/5) g of glycine

V2mL of Na OH in 20mL of unknown glycine solution= (w/5) x (v2/v1) g of glycine

Thus strength= (w/5) (v2/v1) (1000/20) g/L

$$= (wxv2x10)/v1 \text{ g/L}$$

Result:

Experiment-5: Effect of pH, temperature and substrate concentration on enzyme action

Aim: To determine the effect of pH temperature and substrate concentration on enzyme action

Principle:

Enzyme reaction rates are influenced by several factors such as pH, temperature and the concentration of reactants/substrate concentration. For all enzymes there is an optimum temperature and pH for the formation of enzyme-substrate complexes.

As the temperature increases there is more movement of molecules and therefore more collisions between enzymes and substrates – so the enzyme activity increases. However, there is a limit to which enzyme activity can increase because at a certain temperature an enzyme will denature.

When the pH is too acidic or too basic for an enzyme, its hydrogen bonds begin to break, causing its active site to change its shape. An altered active site can't bind with its substrate so enzyme activity decreases. If the pH is too unfavorable then covalent bonds can break, causing the enzyme to denature.

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum.

Materials:

pH meter, Water baths-2, ice, 500 mL beaker, test tubes, droppers, white spot plates, starch, iodine.

Procedure:

Effect of pH:

- Switch on the water bath and keep it at 40°C.
- Take 3 test tubes (16x125mm) and name them 1 2 3.
- Prepare solutions as per the table 5.1.
- Place test tubes in water bath at 40°C for 30 min.
- Then place 3 drops from each test tube separately on white spot plate and add 2 drops of iodine solution to each. Observe the change in the colour which corresponds to the extent of hydrolysis by colour of test.

Table 5.1: Amylase enzyme activity with different pH buffers

Test tube Number	1% starch Solution (mL)	2mL buffer solution of pH	Amylose or saliva
1	2	2	2
2	2	7	2
3	2	12	2

Effect of Temperature:

1. Switch on the water bath and keep it at 40°C.
2. Take 3 test tubes (13x100mm) and name them 1 2 3. Take 6 mL of your own saliva (which will have salivary amylase) in a 10 mL graduated cylinder.
3. Take 2 mL of saliva in each tube and add 2 mL of 2% starch solution to each tube.
4. Place tube 1 in ice bath (10°C), 2 in water bath with 40°C, and 3 in water bath with 100°C for 30 minutes.
5. Then place 3 drops from each test tube separately on white spot plate and add 2 drops of iodine solution to each. Observe the change in the colour which corresponds to the extent of hydrolysis by colour of test.

Table 5.2: Enzyme activity at different Temperatures

Test tube Number	1% starch Solution (mL)	Amylose or saliva	Temperature
1	2	2	10 °C
2	2	2	40 °C
3	2	2	100 °C

Effect of Substrate:

1. Switch on the water bath and keep it at 40°C.
2. Take 3 test tubes (13x100mm) and name them 1 2 3
3. Prepare solutions as per the table 5.3.
4. Add 1 mL of 3 M HCl to each test tube. Place test tubes in water bath at 40°C. Observe precipitate formation in the test tube. Note the time in seconds and observe which test tube forms the precipitate first. Note down the time for remaining two test tubes also.

Table 5.3: Enzyme activity with different substrates

Test tube Number	2 % Renin Solution (mL)	Distilled Water (mL)	Milk (mL)
1	4	0	3
2	2	2	3
3	1	3	3

Result:

Experiment-6: Paper Chromatography for separation of Amino acids

Aim: To separate Amino acids by paper chromatography.

Principle:

Amino acids are the building blocks of peptides and proteins. They possess two functional groups: the carboxylic acid group gives the acidic character, and the amino group provides the basic character.

The R-represents the side chain that is different for each of the amino acids that are commonly found in proteins. However, all 20 amino acids have a free carboxylic acid group and a free amino (primary amine) group except proline, which has a cyclic side chain and a secondary amino group.

We use the properties provided by these groups to characterize the amino acids. The common carboxylic acid and amino groups provide the acid–base nature of the amino acids. The different side chains, and the solubilities provided by these side chains, can be utilized to identify the different amino acids by their rate of migration in paper chromatography.

In this experiment, we use paper chromatography to identify aspartame, an artificial sweetener, and its hydrolysis products from certain foods.

Materials:

1. 0.1% solutions of aspartic acid, phenylalanine, and leucine
2. 0.5% solution of aspartame (Equal)
3. Diet Coca-Cola
4. 3 M HCl
5. 0.2% ninhydrin spray
6. Butanol: acetic acid: water–solvent mixture
7. Equal sweetener
8. Aluminum foil
9. Whatman no. 1 chromatographic paper: 15x 8 cm; 12x 4 cm
10. Ruler
11. Polyethylene gloves
12. Capillary tubes open on both ends
13. Heat lamp or hair dryer
14. Drying oven, 110°C
15. Wide-mouth jar
16. Iodine crystals
17. Test tubes (100x13 mm)
18. Test tubes (75x10 mm)

Procedure:

1. Dissolve 10 mg of the sweetener Equal in 1 mL of 3 M HCl in a test tube (100 x 13 mm). Heat the solution with a Bunsen burner, using a small flame (or with a micro burner) to a boil for 30 sec. Do not heat the bottom of the test tube, but heat slightly above the surface level of the solution. As the solution boils, do not let the liquid evaporate completely. Set the solution aside to cool; this is the hydrolyzed aspartame.
2. Label six small test tubes (75x10 mm) as follows: (1) phenylalanine, (2) aspartic acid, (3) leucine, (4) aspartame, (5) hydrolyzed aspartame, (6) Diet Coca-Cola. Place about 0.5 mL samples in each test tube.
3. Use plastic gloves throughout in order not to contaminate the paper chromatogram. Take a strip of Whatman No. 1 chromatographic paper, 8x15 cm and 0.016 cm thick. With a pencil, lightly draw a line parallel to the 8-cm edge 1 cm from the edge. Mark the positions of 6 spots, placed equally, where you will spot your samples

Spotting: For each sample, use a separate capillary tube. Then apply a drop of sample to the paper until it spreads to a spot of 1 mm diameter. Further, dry the spot. (If a heat lamp is available, use it for drying.) Do the spotting in the following order: a) Phenylalanine 1 drop; b) Aspartic acid 1 drop; c) Leucine 1 drop; d) Aspartame (in Equal) 1 drop; e) Hydrolyzed aspartame 6 drops; f) Diet Coca-Cola 10 drops.

4. When you do multiple drops of nos. 5 and 6, allow the paper to dry after each drop before applying the next drop (Avoid putting a hole in the paper!). Do not allow the spots to spread to larger than 1 mm in diameter.
5. Pour about 15 mL of solvent mixture (Butanol: acetic acid: water) into a large (1L) beaker. Place a glass rod over the beaker. Using clear tape, affix the chromatographic paper to the rod so that when you lower the rod over the beaker, the paper will dip into the solvent but the pencil marks and the spots will be above the solvent surface.

Cover the rod and beaker with aluminum foil. Place the beaker on a hot plate. Turn to a low setting (e.g., no. 2 out of 10 on the dial) and heat the beaker to about 35°C. Allow the solvent front to advance at least 6 cm (it will take 40–50 min.), but do not allow it to get closer than 1 cm from the top edge. Watch the temperature and make sure it does not rise above 35°C.

While you are waiting for the solvent front to complete its rise, you can do the short chromatography experiment in Part B. Your instructor may choose to make this optional.

6. When the solvent front has advanced at least 6 cm, remove the rod and the paper from the beaker. You must not allow the solvent front to advance up to or beyond the top edge of the paper. Mark immediately with a pencil the position of the solvent front. Under a hood, dry the paper with the aid of a heat lamp or hair dryer. With polyethylene gloves on your hands, spray the dry paper with ninhydrin solution. Be careful not to spray ninhydrin on your hands and do not touch the sprayed areas with bare hands. If the ninhydrin spray touches your skin (which contains amino acids), your fingers will be discolored for a few days. Place the sprayed paper into a drying oven set at 105–110°C for 2–3 min.

7. Remove the paper from the oven. Mark the center of the spots and calculate the Rf values of each spot. Record your observations on the Report Sheet.

8. If the spots on the chromatogram are faded, you can visualize them by exposing the chromatogram to iodine vapor. Place your chromatogram into a wide-mouth jar (or a large beaker, 1-L size) containing a few iodine crystals. Cap the jar (or cover the beaker with aluminum foil) and warm it slightly on a hot plate to enhance the sublimation of iodine. The iodine vapor will interact with the faded pigment spots and make them visible. After a few minutes' exposure to the iodine vapor, remove the chromatogram and mark the spots immediately with a pencil. The spots will fade again with exposure to air. Measure the distance of the center of the spots from the origin and calculate the Rf values.

Calculation

$R_f = \text{Distance traveled by the amino acid} / \text{Distance traveled by the solvent front}$

Table 6.1: Table of Aminoacids and Rf values

S No	Band Colour	Amino acid	Distance (cm)	Rf values
1				
2				
3				
4				

Result:

Experiment-7: Sterilization Techniques

Aim: Effective sterilization techniques are essential for working with isolated cell lines for obvious reasons you don't want bugs from the environment growing in your nice culture medium, and equally, cultures must be sterilised before disposal.

Wet Heat (Autoclaving)

The method of choice for sterilisation in most labs is autoclaving; using pressurised steam to heat the material to be sterilised. This is a very effective method that kills all microbes, spores and viruses, although for some specific bugs, especially high temperatures or incubation times are required. Autoclaving kills microbes by hydrolysis and coagulation of cellular proteins, which is efficiently achieved by intense heat in the presence of water. The intense heat comes from the steam. Pressurised steam has a high latent heat; at 100 °C it holds 7 times more heat than water at the same temperature. This heat is liberated upon contact with the cooler surface of the material to be sterilised, allowing rapid delivery of heat and good penetration of dense materials. At these temperatures, water does a great job of hydrolyzing proteins so that those bugs don't stand a chance.

Dry Heat (Flaming, baking)

Dry heating has one crucial difference from autoclaving. You've guessed it there's no water, so protein hydrolysis can't take place. Instead, dry heat tends to kill microbes by oxidation of cellular components. This requires more energy than protein hydrolysis so higher temperatures are required for efficient sterilization by dry heat. For example sterilization can normally be achieved in 15 minutes by autoclaving at 121°C, whereas dry heating would generally need a temperature of 160 °C to sterilize in a similar amount of time.

Filtration

Filtration is a great way of quickly sterilizing solutions without heating. Filters, of course, work by passing the solution through a filter with a pore diameter that is too small for microbes to pass through. Filters can be scintered glass funnels made from heat-fused glass particles or, more commonly these days, membrane filters made from cellulose esters. For removal of bacteria, filters with an average pore diameter of 0.2µm are normally used. But remember, viruses and phage can pass through these filters so filtration is not a good option if these are a concern.

Solvents

Ethanol is commonly used as a disinfectant, although since isopropanol is a better solvent for fat it is probably a better option. Both work by denaturing proteins through a process that requires water, so they must be diluted to 60-90% in water to be effective. Again, it's important to remember that although ethanol and IPA are good at killing microbial cells, they have no effect on spores.

Radiation

UV, x-rays and γ rays are all types of electromagnetic radiation that have profoundly damaging effects on DNA, so make excellent tools for sterilization. The main difference between them, in terms of their effectiveness, is their penetration. UV has limited penetration in air so sterilisation only occurs in a fairly small area around the lamp. However, it is relatively safe and is quite useful for sterilizing small areas, like laminar flow hoods. X-rays and gamma rays are far more penetrating, which makes them more dangerous but very effective for large scale cold sterilization of plastic items (e.g. syringes) during manufacturing.

Result:

Reference:

- 1) Sadasivam S and Balasubramanian T (1985). Practical Manual (Undergraduate), Tamil Nadu Agriculture University, Coimbatore, p.2.
- 2) Sadasivam S, Manickam A (2008). Biochemical Methods, New Age International Publishers, 3rd Edition, ISBN: 978-81-224-2140-8.