وزارة التعليم العالي والبحث العلمي
الجامعة التقنية الجنوبية
المعهد التقني العمارة
قسم تقنيات المختبرات الطبية

الحقيبة التدريسية لمادة

الأجهزة المختبرية

الصف الاول

.....

تدريسي المادة

م .م رباب نعيم علك

الفصل الدراسي الاول

Laboratory Instrument

جدول مفردات مادة الأجهزة المختبرية

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The purpose of studying laboratory instruments:

1. Understand the working principle of medical devices.
2. Acquire technical skills.
3. Acquire skills in calibration and periodic maintenance of devices.

Target group:

First–year students/ Medical Laboratory Technology.

Educational techniques used:

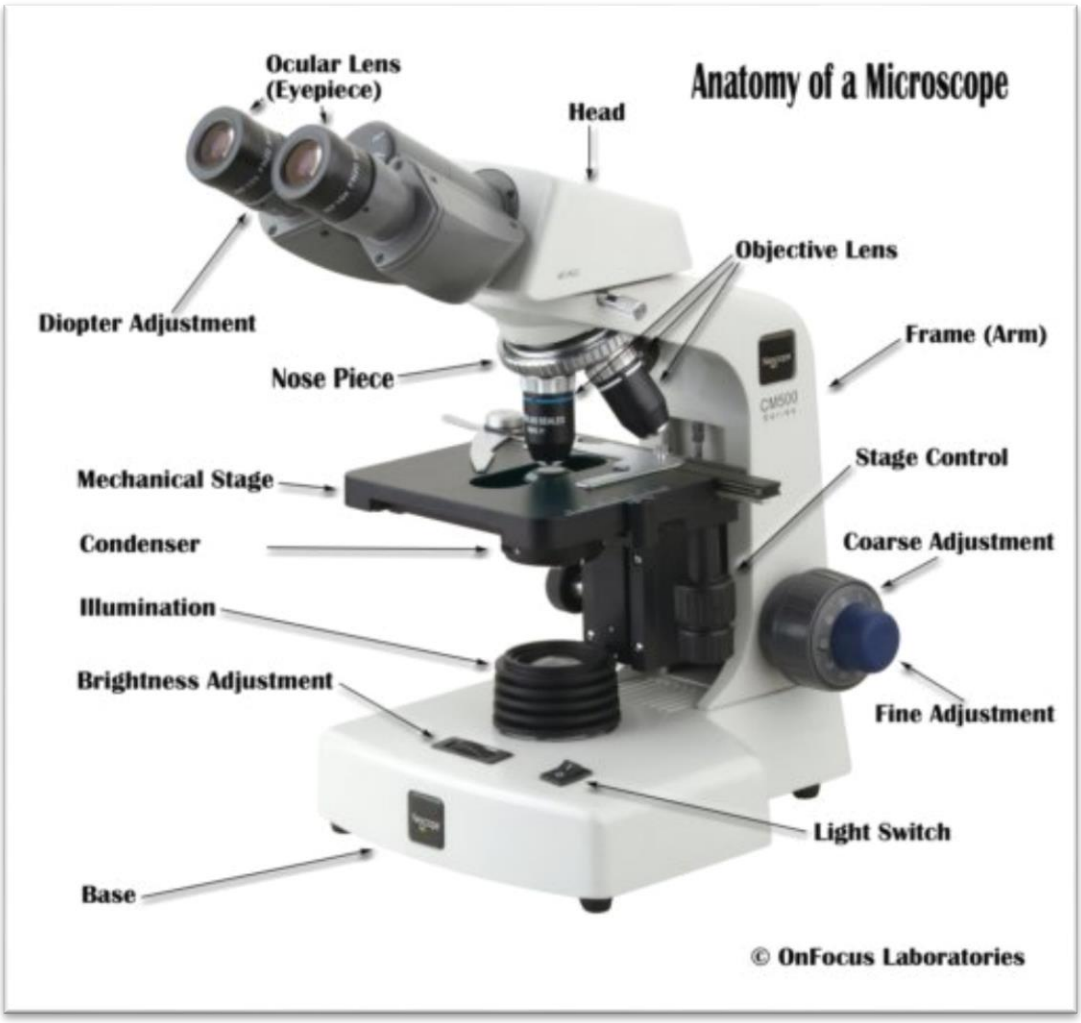
1. Whiteboard and pens.
2. Interactive whiteboard.
3. Data show.
4. Laptop.
5. Instruments required by the curriculum

week 1, 2:

Microscope

Lab. instrument: is a general term for all kinds of instruments and other tools needed for operation in various laboratories.

The Microscope: is a device that enables us to visualize minute objects (animate and inanimate) that cannot be seen by our naked eye.



- **The major part of microscope:**

. Framework of the microscope :This includes:

- An arm (stand): The basic frame of the microscope to which the base, body and stage are attached.
- A stage: the table of the microscope where the slide or specimen is placed.
- A foot, or base: is the rectangular part up on which the whole instruments rest.

B. Focusing system: This encompasses:

- • Coarse adjustment: The coarse focusing adjustment is a pair of large knobs positioned one on each side of the body. Rotations of these knobs move the stage up or down fairly rapidly.
- Fine adjustment: While low power objectives can be focused by the coarse adjustment, high power objectives require a fine adjustment.
- Condenser adjustments: A rotating a knob to one side of the condenser. This moves the condenser up or down.

C. Magnification system: This comprises:

- **Objectives lenses:** - Objectives are components that magnify the image of the specimen to form the primary image. For most routine laboratory work 4x, 10x,40x and 100x (oil immersion) objectives are adequate.
- **Eyepiece:** Eyepiece is the upper optical component that further magnifies the primary image and brings the light rays to a focus at the eye point. It consists of two lenses mounted at the correct distance. It is available in a range of magnifications usually of 4x, 6x, 7x, 10x (most common), 15x and sometimes as high as 20x.

D. Illumination system: include:

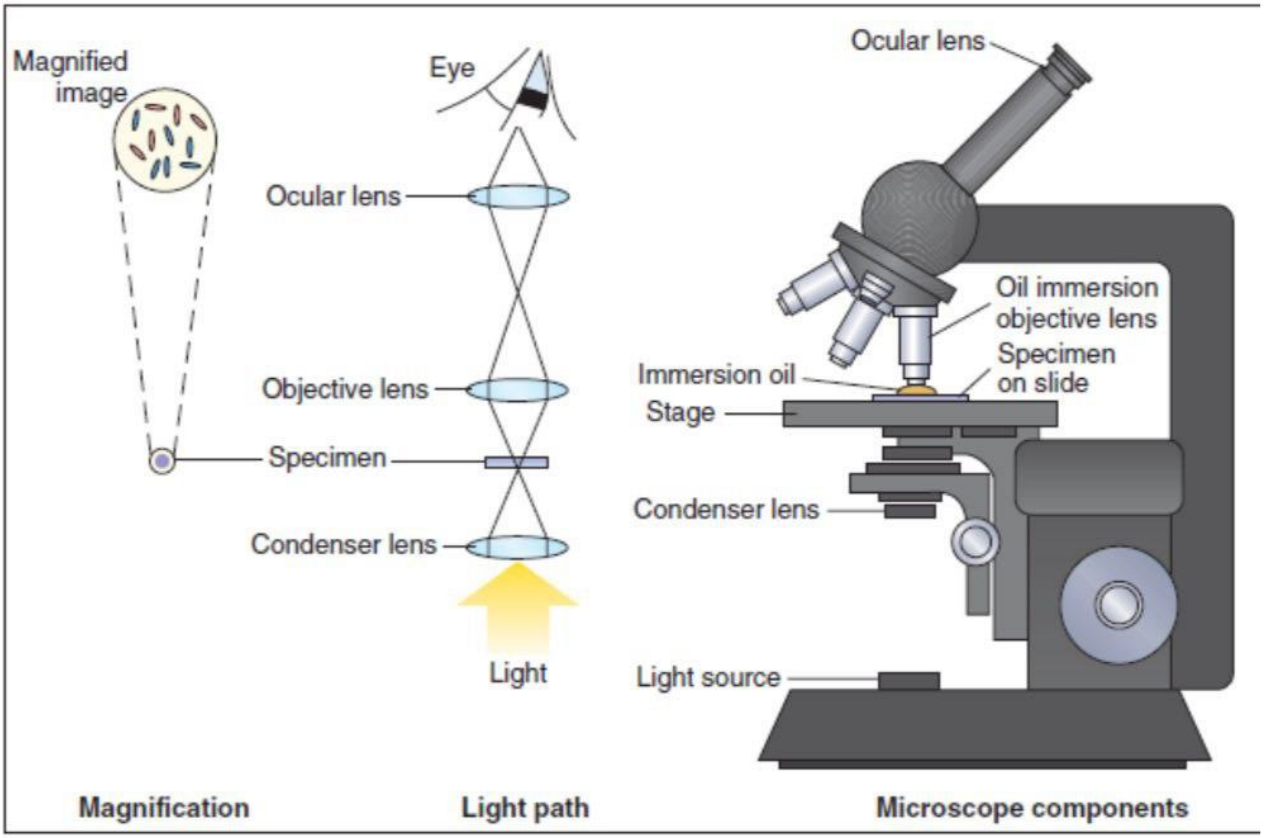
1. **Condenser and iris:** Condenser is a large lens with an iris diaphragm, that receives a beam from the light source and passes it into the objective. The iris is a mechanical device mounted underneath the Condenser and controls

the amount of light entering the condenser. The principal purpose of the condenser is to condense the light required for visualization.

2. **Sources of illumination:** Many microscopes are now provided with correctly aligned built-in sources of illumination, which use tungsten or quartz halogen lamps operating on 6,8or 12 volts through variable transforms.
3. **Filters:** Light filters are used in the microscope to:

- Reduce the intensity of light and increase contrast and resolution.
- Adjust the color balance of the light to give the best visual effect. –
- Provide monochromic light.
- Transmit light of selected wavelength
- Protect the eye from injury caused by ultra-violet.

- **Working principle of the microscope:** For light microscopy, visible light is passed through the specimen and then through a series of lenses that bend the light in a manner that results in magnification of the organisms present in the specimen. The total magnification achieved is the product of the lenses used.



- **Magnification:** In most light microscopes, the objective lens, which is closest to the specimen, magnifies objects 100× (times), and the ocular lens, which is nearest the eye, magnifies10×. Using these two lenses in combination, organisms in the specimen are magnified 1000× their actual size when viewed through the ocular lens.

Objective magnification	Eyepiece magnification	Total magnification
4x	10x	40x
10x	10x	100x
40x	10x	400x
100x	10x	1000x

▪ Resolving power & Resolution of the microscope:

Resolution: defined as the extent to which detail in the magnified object is maintained.

Resolving power is the closest distance between two objects that when magnified still allows the two objects to be distinguished from each other.

The resolving power of most light microscopes allows bacterial cells to be distinguished from one another but usually does not allow bacterial structures, internal or external, to be detected.

The resolving power of an objective is dependent on what is known as the numerical aperture (NA) of the objective.

The numerical aperture is a designation of the amount of light entering the objective from the microscope field. It is dependent on the diameter of the lens and the focal length of the lens.

-The following are the usual numerical apertures of commonly used objectives.

- 10 X objective ---------- NA 0.25
- 40 X objective ---------- NA 0.65
- 100 X objective (immersion oil) ------- NA 1.25.

Role of an Oil Immersion: Objective When a beam of light passes from air into glass it is bent and when it passes back from glass to air it is bent back again to its original direction. This has effect on oil immersion objective and affects the NA of the objective and consequently its resolving power. The oil, which has the same optical properties as glass, enhances resolution by preventing light rays from dispersing and changing wavelength after passing through the specimen.

▪Types of Microscope :

A. Compound (simple) microscope (routinely used in medical laboratories)

B. Phase contrast microscope .

C. Dark field microscope .

D. Fluorescence microscope.

B. Phase contrast microscopy: Phase contrast microscopy utilizes beams of light passing through the specimen that are partially deflected by the different densities or thicknesses (i.e., refractive indices) of the microbial cells or cell structures in the specimen.

C. Dark field microscope:

Principle In dark field microscope, the light enters a special condenser, which has a special ring, so that, is reflected to pass through the outer edge of the condenser lens at a wide angle. The only light entering the eye comes from the microorganisms themselves, with no light entering the eye directly from the light source. In this way, small microorganisms are seen brightly illuminated against a black ground, like stars in a night sky.

D. Fluorescence microscope:

Principle: In Fluorescence microscopy, ultra - violet light, is used to illuminate organisms, cells, or particles, which have been previously stained with fluorescing dyes called florochromes. These dyes are able to transform the invisible ultra - violet light in to longer wavelength visible light. The fluorescent stained organisms, cells, or particles appear glowing (fluorescing) against a dark background.

Practical parts:

▪ Routine Use of The Microscope:

A microscope must always be used with gentleness; care and the following should be noted :

1. Place the microscope on a firm bench so that it does not vibrate.
2. Make sure that it is not be exposed to direct sun light.
3. The user must be seated at the correct height for the convenient use of the microscope.
4. Select the appropriate source of light.
5. Place the specimen on the stage, making sure that the underside of the slide is completely dry.
6. Select the objective to be used.
7. It is better to begin examination with 10x objective.
8. The 10x objective can be used for adjusting the illumination and for searching the specimen before using a high power lens.
9. Bring the objective as close as possible to the slide preparation and while viewing in the eye piece slowly move the objective up ward with the coarse adjustment until the image comes into view and is sharply focused.
10. Adjust the light source until the illumination of image is at its brightest.
11. Focus the condenser. To do this, open fully the iris of the condenser. Using the condenser adjustment knob, focus the condenser on the details of the light source.
12. Adjust the aperture (opening) of the condenser iris according to the specimen being examined.
13. The wider the condenser aperture, the brighter will be the specimen and the smaller will be the details, which can be resolved.
14. Examine the specimen by systematically moving the slide with the mechanical stage.

15. For a higher magnification, swing the 40x objective into place.

16. Focus the 40x objective, using the fine adjustment.

17. If for any reason the image is not visible, lower the objective until it is nearly but not quite touching the specimen.

18. Then looking through the eyepiece, focus up wards with the fine adjustment until the image comes into view.

19. For the highest magnification, add a drop of immersion oil to the specimen and swing the100 x oil immersion objective into place, then open the iris fully to fill the objective with light. Example, stained blood smear, acid-fast stain, etc.

- ## Care, cleaning, and repair of the microscope:

1. Care and cleaning:

A microscope is a delicate instrument both mechanically and optically. Therefore, the following important points should be taken into considerations.

1. Always carry a microscope using both hands.
2. When not in use, a microscope should be protected from dust, moisture, direct sunlight and put in microscope case.
3. Keep it standing in place ready for use, but protected by light cover.
4. In humid climate it is necessary to cover the microscope in a plastic bag with a drying agent (silica gel) over night to avoid molds growing on the lenses.
5. At the end of each day's work, the surface lenses of the objectives, eyepieces, and condenser should be cleaned using lens tissue.
6. Never clean the lens of the objectives and eyepiece with alcohol.

Repair of the microscope:

Except for obvious and simple measures, if a microscope becomes damaged optically or mechanically, it is better to send it or the damaged part to a reliable scientific instrument repairer or preferably to the manufacturer.

Week 3:

Balances

Balances are essential laboratory instruments that are widely used for weighing of various substances (powders, crystals and others) in the laboratory.

The function of balances is to weigh accurately and precisely reagents, stains and culture media.

- ✓ All types of balances used in the laboratory uses the metric system for weighting, where the gram is the unit used.
- ✓ The metric system is easy to use because everything is in tens, hundreds or thousands. Thus, there are a thousand milligram in a gram, and a thousand gram in a kilogram.
- ✓ Milligrams are written (mg), thus, half a gram or 0.5g is the same as 500mg. a tenth of a gram or 0.1g is the same as 100mg.

▪ Types of Balances :

Depending on the accuracy required for weighting, all different types of balances are usually classified into two major groups :

1- **Rough balances** : these include :

A- **Triple (trip) beam balances**, with accuracy to the nearest 0.1g (100mg). all parts of the system work mechanically.

B- **Top pan balances** ,with accuracy to the nearest 0.01g (10mg). most parts of the system work mechanically, with a lamp, lens and light-beam projection system.

2- **Analytical balances :** these involve :

A- Manual analytical balance, with accuracy to the nearest 0.001g (1mg). all parts are mechanical.

B- Automatic analytical balance (analytical projection balance) with accuracy to the nearest 0.00001g (0.01mg). mostly mechanical, with a lamp, lens and light-beam projection system.

▪ Rough Balances

a- Triple (trip) beam balances : This type of balance is less sensitive balance, with accuracy to the nearest 0.1g.

In the triple beam balance, there are on pan and three weighting scale:

- Scales reading from 0 to 10g.
-Scales reading from 0 to 100g
-Scales reading from 0 to 500g.

The three scales (arms) are provided with movable weights. The 0 to 100g and 0 to 500g have a weights that locks into accurately made notches at each calibration to ensure accuracy at each position.

• Basic parts of triple (trip) beam balances :

1- Pan: the pan is used where the object, or weighting vessel holding the substance, in balance.

2- Beams: the beam is a lever supported by a knife plane edge at the center point. The length of the beam is graduated for placement of the sliding weight .

3- Movable weights: these are sliding weights attached to the beam that are moved to bring the balance into equilibrium.

4- Reading scale: this is a scale located on the right hand of balance that shows when the balance in the equilibrium.

5- Poising nut: this is a small piece of screwed weight fixed at the bottom of the pan, that is used for balancing and setting zero the balance.

6- **Extra weights**: Theses weights weigh 500g (one piece) and 1000g (two pieces).

If needed hang at the right side of the beams where they go. The heaviest thing you can weights is thus 2610g .

b- Top pan balance (basic parts):

1- Lighting system: consist of light bulb, lens, reflecting mirror and reading scale.

2- Pan: a single pan is used to weigh the object. It is located on the top of balance.

3- Usually supplied with a spring, located inside the balance. It moves downwards in a way directly proportional to the weight of the object.

4- Extra weights: these weights are inside the balance, and are used by dialing a special knob for this purpose. Extra weights are added on the movable weight inside the balance.

5- Taring knob: present outside the balance and is used to zero balance empty vessel.

Principle of top pan balance:

When used it acts by releasing the spring attached to the movable weights with a force exactly equal to the force applied by the object on the pan .

▪ Analytical balances

Analytical balance is a highly sensitive electronic balances (single pan balances that use an electron magnetic force instead of weights). It may have two pans suspended from a cross beam, inside a glass case. It requires mains electricity or battery (D.C) supplied power.

Basic parts of analytical balance:

1- Glass enclosure : the analytical balance is enclosed in glass to prevent currents of air and collection of dust from disturbing the process of weighting .

2- Leveling screws.

3- Beam : this is a structure from which the pans are suspended.

4- Knife edges : these support the beam during weighting and give sensitivity to the balance, knife edges are vital parts and are constructed of hard metals to give a minimum amount of friction.

5- Pans for weighting :the object to be weight is placed on this pan. The pans are suspended from the ends of beam.

6- Reading scale : this is usually a lighted optical scale giving a high magnification and sharp reading.

Practical part

Procedure of general use :

1- Make sure that all weights are at (0). Make sure that the pan is clean.

2- Place the weighting vessel (if used) on the pan.

3- Register the weight of the empty vessel.

4- Move the movable weights on the scales to the total weights.

5- Gradually add the chemical until rests again to the (0) mark. Remove the weighting vessel and return the movable weights to their zero positions.

6- Wipe out any spilled chemical immediately from the balance pan.

Some important rules for use of these balances :

1- Never weight chemicals directly on the pan, use a container in this case.
2- Don't weights hot object will cause faulty and unstable readings.
3- Position the balance on a firm bench away from vibration, draughts and direct sunlight.
4- Before starting to weigh, zero the balance as directed by the manufacturer. If using a beam balance, check the position of the beam.
5- Don't try to weight objects which may be too heavy to the balance.

6- Don't spill handle any internal component of the balance since finger prints my add weight and cause fault readings.

7- Take care not to spill any chemicals on the pan or floor of the balance.

8- If a level indicator is present, check the balance before weighing .

9- Use small brush to remove any chemical, which may have been spilt on the balance.

Keep the balance clean, being particularly careful not to let dirt accumulate near the pivots and bearings.

Analytical balance

Top pan balance

Triple (trip) beam balances

Week 4:

Photometer & Spectrophotometer:

Visible light spectrum: when a beam of light passes through a colored solution, it interacts with matters in the solution and the result may be refraction, reflection, absorption and transmission among others.

-Refraction: Is defined as sudden change in the direction of the beam when the light passes from one medium to other with a different physical density.

-Reflection: Is a condition where the beam returns. back towards its source such as mirror

-Absorption :Is a situation where some components of .the light (colors) are retained or absorbed.

-Transmission: Refers to the situations where some portions of the light permitted to pass through a given medium.

Radiation is characterized by waves on which basis the electromagnetic radiation spectrum could be divided in many regions including gamma rays, x rays, ultraviolet rays, visible light , infrared, microwaves and radio waves.

visible region is the radiant energy to which the human eye responds and their wavelength varies between 400 and 700 nm.

- Wavelength of about 700 nm are seen by the eyes as red colors while those of progressively shorter wavelengths give in descending order to orange, yellow, green, blue, indigo and finally violet colors which is produced in the short wavelength of 400 nm.

Beer's and Lambert's Law:

Most colorimetric analytical tests are based on the Beer's – a Lambert's law which states that under the correct conditions the absorbance of a solution when measured at the appropriate wavelength is directly proportional to its concentration and the length of the light path through the solution. Using a standard, this law can be applied to measuring the concentration of a substance in : unknown (test) solution by using the formula.

Concentration of test (Ct) = (At)/As & (Cs) .

Absorbance of test (At).

Absorbance of standard (As).

Photometer

It is instrument measure the absorbance optical density. This equipment will measure the transmission and optical density depending on color filter which give the complement color and in this case the reading less accurate than spectrophotometer which is use monochrometer.

Parts of photometer:

1-Light source.

2-Filter : to give approximate wave length according to the color.

3-Sample holder: which can hold the sample.

4-Photocell : it will convert the light to electrical current.

5-Galvanometer : which can measure the current from photocell.

6-Zero adjustment : which can adjust the zero point and reading.

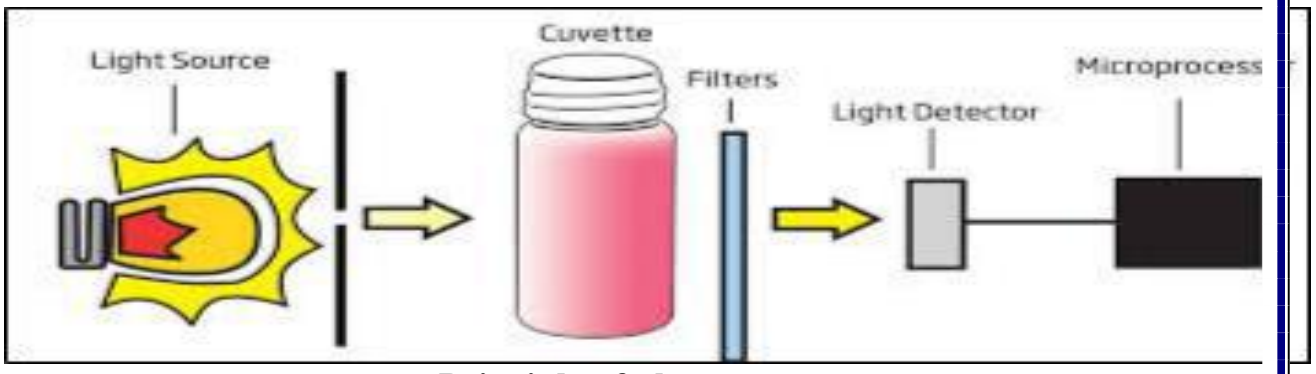
Types of filter:

1-Blue filter : it will pass the wavelength between (400 – 495) nm.

2-Green filter : it will pass the wavelength between(500 – 580) nm.

3-Red filter : it will pass the wavelength between (600 – 800) nm.

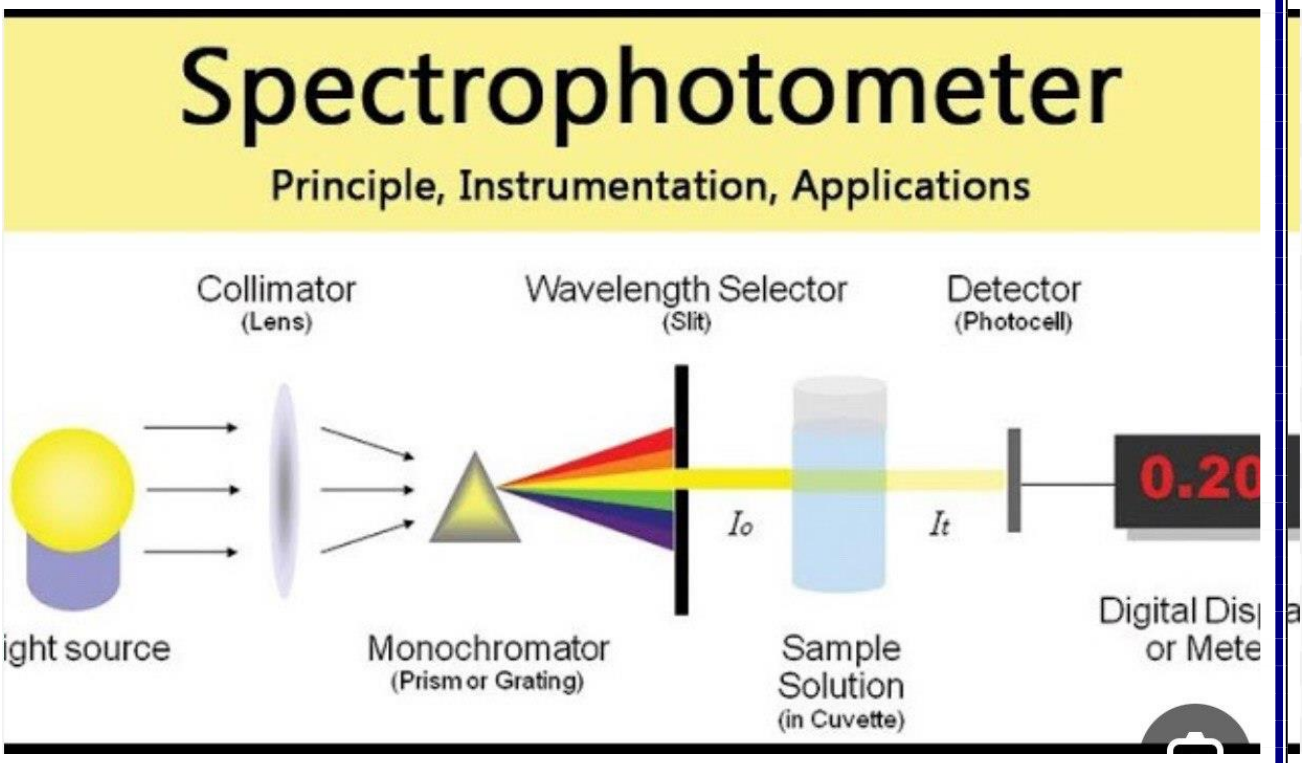
Photometer device



Principle of photometer

Spectrophotometer

It is an instrument that measures the amount of photons (intensity of light) absorbed after it passes through sample solution. In the spectrophotometer, the concentrations of a known chemical substance can be determined by measuring the intensity of light detected depending on the range of wavelength of light source.



Types of Spectrophotometer:

1-UV-visible spectrophotometer: uses light of the ultraviolet range (185- 400 nm) and visible range (400 - 700nm) of electromagnetic radiation spectrum.

2-IR spectrophotometer: uses light of the infrared range (700 – 15000nm) of electromagnetic radiation spectrum.

Parts of spectrophotometer

1- Photocell : convert the light into electrical current.

2- Prism or granting (monochrometer) :it will analyze the light to selected spectra and wavelength.

3- Wavelength (knob) : to control the certain wavelength applied.

4- Cuvatte and sample holder.

5- Galvanometer : it will measure the electrical current from photocell or detector.

6- Zero point adjustment : it is to set up the equipment to the zero point before use.

Practical part:

Proper selection, use and care of cuvettes:

1. Cuvettes are made from plastic, glass, or quartz.

a. Use quartz cuvettes for UV work.

b. Glass, plastic or quartz are acceptable visible work.

2. Cuvettes are expensive and fragile . Use them properly and carefully.

a. Do not scratch cuvettes; do not store them in wire racks or clean with brushes or abrasives.

b. Do not allow samples to sit in a cuvette for a long period of time.

c. Wash cuvettes immediately after use.

Types of photocells

1- Red photocell (600 – 800) nm.

2- Blue photocells (400 – 495) nm.

Source of light:

1-Tungsten filament : The most commonly used source for visible light ranging from (400 – 700) nm

2- Halogen lamp : It will give more bright light with minimum of the red and has a long life in use.

3- Deuterium lamp : It will use in ultraviolet spectra measurement ranging (190 – 400) nm It is use only in spectrophotometer.

Photometer	Spectrophotometer
1-It use filter which give approximate	1-It use prism or grating(monochrometer a wavelength according to the color
2-Lower cost	2-More expensive, but may be necessary
3- Smaller number of calibrations	3- Unlimited number of calibrations
4- Simpler chemistries	4- More complex chemistries

Week 5:

Flame Photometer

Flame photometry is a measure of intensity of emission radiation when the sample molecule are subjected to flame. It is mainly used for the atoms of 1st & 2 nd group elements of periodic table i.e Na, K, Ca, Li etc. It is not applicable to the metals of periodic table other then the 1 st & 2nd group elements. The elements like gold, silver & inert atoms and other atoms are not analysed by flame photometry because they need higher energy to bring about excitation state then the 1st & 2 nd group elements.

The basic components for flame photometer are as follows:

1- Source of flame: A Burner in the flame photometer is the source of flame. It can be maintained in at a constant temperature. The temperature of the flame is one of the critical factors in flame photometry. Ex: Total consumption burner, laminar flow burner.

2- Nebuliser: Nebuliser is used to send homogeneous solution into the flame at a balanced rate.

3- Optical system: The optical system consists of convex mirror and convex lens. The convex mirror transmits the light emitted from the atoms. Convex mirror also helps to focus the emissions to the lens. The lens helps to focus the light on a point or slit.

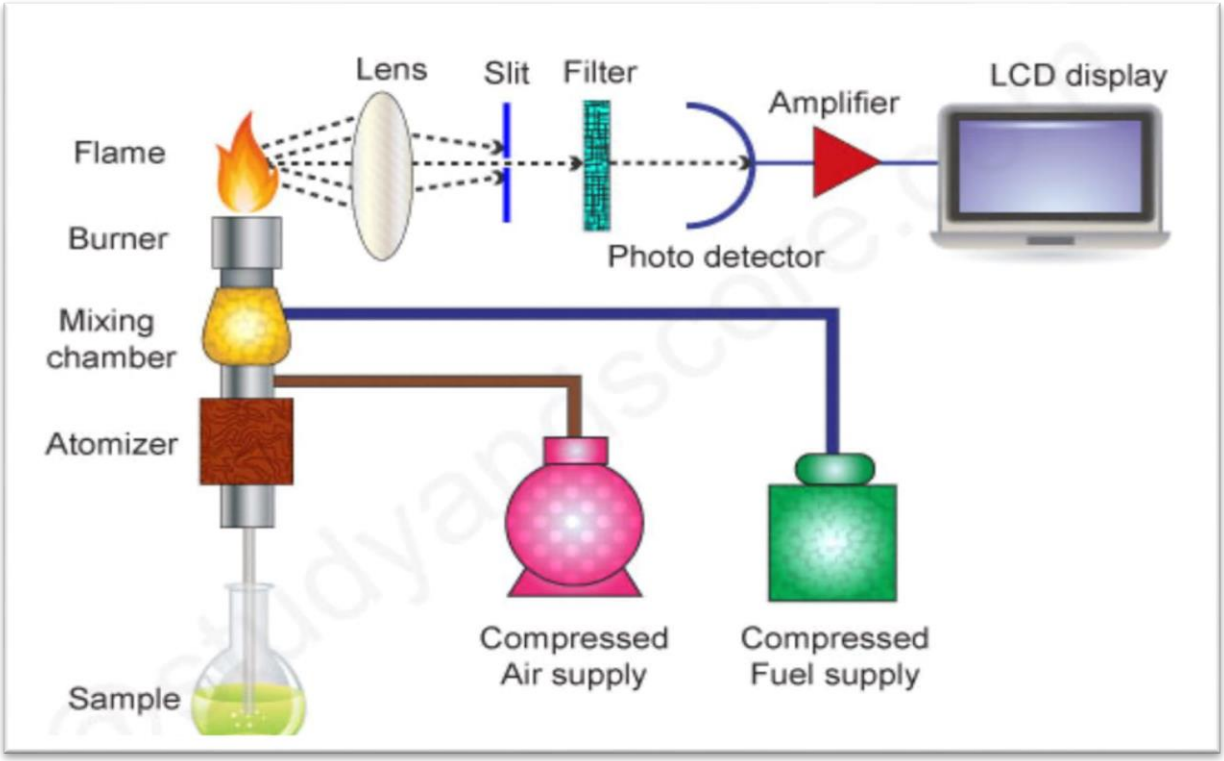
4- Simple colour filters: The reflections from the mirror pass through the slit and reach the filters. Filters will isolate the wavelength to be measured from that of irrelevant emissions. A simple flame photometer contains a filter wheel and when a particular elements has to be analysed the specific filter is selected.

5- Photo-detector: The intensity of radiation emitted by the flame is measured by photo detector. The radiation emitted by the elements is mostly in the visible region. Hence conventional detectors like photo voltaic cell or photo tubes can be used. In a flame spectrophotometer, photomultiplier tube is used as detector. These electrical signals are directly proportional to the intensity of light

- **Principle:**

The principle depend on : The sample in solution is aspirated through an aspirator or nebulizer into the burner which the is flame is usually a propane / air fuel or, even, a purified natural gas/air mixture. The sample matrix evaporates followed by atomization of the sample. Atoms present in the high temperature zone of the flame are excited to higher energy levels by absorbing energy from the flame. As excited atoms return to the ground state they emit radiation in definite wavelength depending on the energy level from which each atom drop. This gives rise to a line spectrum. However, in flame photometry a pre-selected filter is used and it is the intensity of the emission line that is practically measured and is related to the original concentration of the sample in solution

Practical part:



Flame Photometer

- **A brief over view of the process:**

1. First aerosol are formed from sample solution by a jet of compressed gas this process is called nebulization.
2. The liquid sample introduced into flame then the solution is evaporated leaving fine divided solid particles.
3. The solid particles move towards the flame where the gaseous atoms and ions are produced.
4. The ions absorb the energy from the flame and excited to high energy levels.
5. when the atoms return to the ground state, radiation of the characteristic element is emitted energy at characteristic wave length.
6. The measurement of the emitted photon from the element used photomultiplier tube detector.
7. The intensity of emitted light is related to the concentration of the element.

- **Applications (Uses) Of Flame Photometry:**

- Flame photometer can be applied both for quantitative and qualitative analysis of elements. The radiations emitted by the flame photometer are characteristic to particular metal. Hence with the help of Flame photometer we can detect the presence of any specific element in the given sample.
- The presence of some group II elements is critical for soil health. We can determine the presence of various alkali and alkaline earth metals in soil sample by conducting flame test and then the soil can be supplied with specific fertilizer.
- The concentrations of Na+ and K+ ions are very important in the human body for conducting various metabolic functions. Their concentrations can be determined by diluting and aspirating blood serum sample into the flame.
- Soft drinks, fruit juices and alcoholic beverages can also be analysed by using flame photometry to determine the concentrations of various metals and elements.
- Used in determination of calcium and magnesium in cement.
- Used in determination of lead in petrol.

Week 6:

Centrifuges

Centrifuge is equipment that is used to separate solid matter from a liquid suspension by means of centrifugal force. They sediment particles (cells, bacteria, casts, parasites, etc.) suspended in fluid by exerting a force greater than that of gravity. The suspended materials are deposited in the order of their weight.

Working Principle of Centrifuge:

When a body is rotated in circular movement at speed, centrifugal force is created that drives the body away from the center of the circular movement. The greater

the outward pull due to rotation, that is centrifugal force, the more rapid and effective is the sedimentation. As a result, heavier elements are thrown to the bottom of the tube followed by lighter particles. Centrifugal force increases with the speed of rotation that is the revolution of the rotor per minute and the radius of rotation. The actual sedimentation achieved at a given speed depends therefore, on the radius of the centrifuge. Most techniques requiring centrifugation will usually specify the required relative centrifugal force (RCF) expressed in gravity. For example, an RCF of 2000 x G refers to a force 2000 times the force of gravity. Most centrifuge manufacturers specify both the RPM and G.

Where: RCF = relative centrifugal force.

r = radius from the shaft to the tip of the centrifuge tube.

rpm = Revolution per minute. g = Gravitational force.

Parts :

1- Motor

2- Head

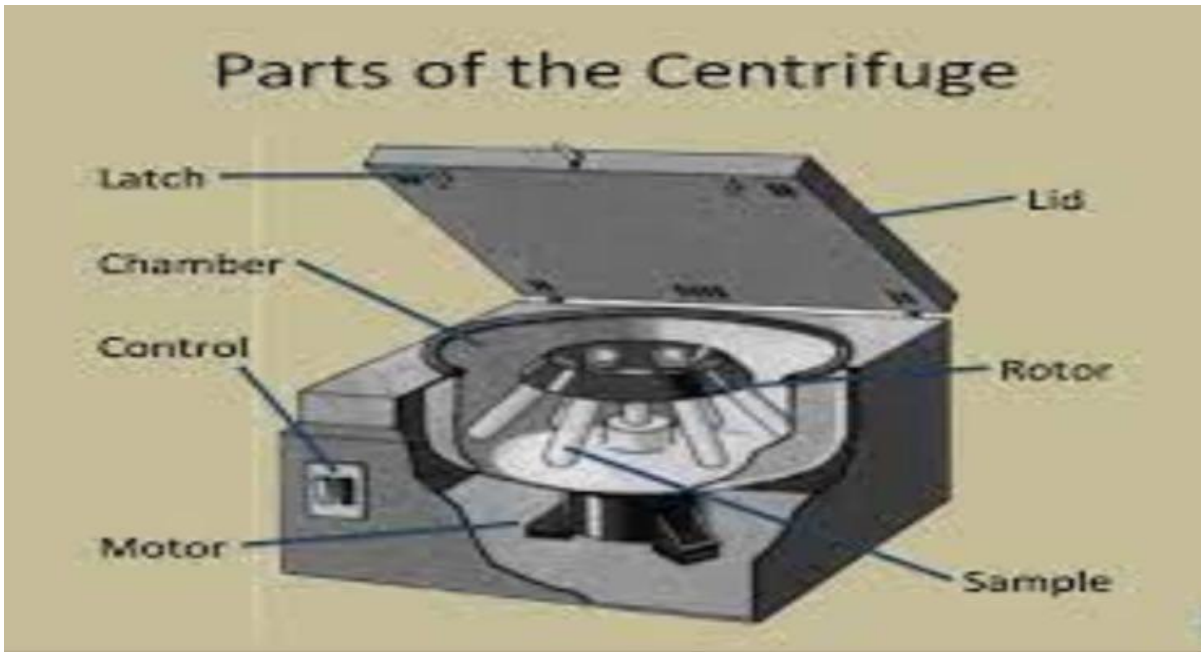
3- Timer

4- Tachometer

5- Brake

6- Temperature controls keys

7- Cover



- **Types of centrifuges:**

1.Laboratory centrifuges :are used in chemistry, biology, and biochemistry for isolating and separating solids from liquids in a suspension. The solids can be insoluble compounds, biomolecules, cell organelles, or whole cells. They vary widely in speed and capacity. They usually comprise a rotor containing two, four, six, or many more numbered wells within which centrifuge tubes may be placed. Like all other centrifuges, laboratory centrifuges work by the sedimentation principle, where the centripetal acceleration is used to separate substances of greater and lesser density.

2. Hematocrit centrifuge(Microcentrifuges) : this is a type of angle-head centrifuge, where by capillary tubes are used containing blood for measuring packed cell volume (PCV) attains a speed of about 10,000 rpm .The microhaematocrit centrifuge used in many haematology laboratories .

3. Ultracentrifuge : Use high centrifugal force for studying properties of biological particles. Ultracentrifuges can isolate much smaller particles, including ribosomes, proteins, and viruses. Ultracentrifuges can also be used in the study of membrane fractionation. This occurs because ultracentrifuges can reach maximum angular velocities in excess of 70,000 rpm.

4. Refrigerated centrifuges : Cooling is important added feature to any laboratory centrifuge, temperature ranges as wide as(-20C – 40C) , making them perfect for DNA, RNA, PCR or antibody analysis. Cold centrifuge can obtain rotational speeds of over 30,000 rpm, and a relative centrifugal force (RCF) of over 65,000x g.

Practical part:

Although most centrifuges are fitted with an imbalance detector, lid interlock, and an automatic braking system, it is important for laboratory workers to know how to use a centrifuge correctly to prevent it from damage and breakages. These include:

1. Reading the manufacturer's instructions. Placing a centrifuge on a firm level bench out of direct sunlight, towards the back of the bench.
2. Placing a centrifuge on a firm level bench out of direct sunlight, towards the back of the bench.
3. Whenever possible using plastic tubes made from polystyrene or autoclavable.
4. Always closing the centrifuge top before turning it on.
5. Always balancing the tubes that are being centrifuged. Tubes of the same weight should be placed directly opposite to each other. Tubes should also be of the same size and should also contain the same amount of liquid.
6. Increasing spinning speed gradually is important.. Five minutes are the usual time required to centrifuge most substances.
7. Never open the centrifuge while it is still spinning. Never try to slow it down with your hand. Most centrifuges have a brake, which will cause the centrifuge to stop faster.

Laboratory centrifuges

Hematocrit centrifuge

Week 7:

Autoclave:

Steam Under Pressure (autoclave) Autoclave is an instrument that operates by creating high temperature under steam pressure. Autoclaving is the most common, effective, reliable and practical method of sterilizing laboratory materials. The principle of autoclave is that steam is kept at a pressure of 15 pound (lb) per square inch to give a temperature of 1210 c, which will kill spores within 15 minutes. At this particular temperature, pressure and time, all forms of lives are destroyed. Steam is more penetrating than hot air, and will give up its latent heat on striking a colder object; there by raising the temperature of the object rapidly It is used to sterilize syringes, needles, glasswares, culture media, etc.

For most purposes, the following cycles will ensure sterilization of correctly loaded autoclaves correctly loaded:

• Three minute holding time at 134Cº.

• Ten minute holding time at 126 Cº.

• Fifteen minute holding at 121 Cº.

• Twenty holding time at 115 Cº.

▪ Types of autoclaves

A- Gravity displacement autoclaves: In gravity displacement autoclave, steam enters the chamber under pressure and displaces the heavier air downwards and through the valve in the chamber drain, fitted with a HEPA filter.

B- Pre- vacuum autoclaves: These autoclave allow the removal of air from the chamber before steam is admitted. The exhaust air is evacuated through a valve fitted with a HEPA filter. At the end of the cycle, the steam is automatically exhausted. These autoclaves can operate at 134C0 and the sterilization cycle can therefore be reduced to 3 minute. They cannot be used to process liquid because of the vacuum.

C- Fuel heated pressure cooker autoclaves: Fuel heated pressure cooker autoclaves should be used if a gravity displacement autoclave is not available. They are loaded from the top and heated by gas or electricity. Steam is generated by heating water in the base of the vessel and air is displaced upwards through a relief vent. When all the air has been removed, the valve on the relief vent is closed and the heat is reduced. The pressure and temperature rise until the safety valve operates at a preset level, which is the start of holding time. At the end of the cycle, the heat is turned off and the temperature allowed to fall to 800C or below before the lid is opened.

Fuel heated pressure cooker autoclaves

Pre- vacuum autoclave

gravity displacement type autoclave

Practical part:

- **Precautions in the use of autoclaves**

1. The following guidelines can help to minimize risks while working with autoclaves

2. Proper use and care of autoclaves.

3. Regular inspection of the chamber, door seals and gauges.

4. The steam should be saturated and free from chemicals that could contaminate the items being sterilized.

5. Materials to be autoclaved should be in containers that allow ready removal of air and permit good heat penetration.

6. The chamber of the autoclave should be loosely packed so that steam will reach the load evenly.

7. Slow exhaust setting should be used when autoclaving liquids, as they may boil over when removed due to superheating.

8. Operator should wear protective gloves for protection when opening the autoclave.

9. Thermocouples should be placed at the center of each load in order to determine proper operating cycles.

10. Ensure that the relief valves of pressure cooker autoclaves do not blocked.

Week 8:

PH meter

A pH meter is an electronic device used for measuring the concentration of hydrogen ions in a solution (acidity or alkalinity of a liquid).

PH meter is very useful than the other pH indicators because it gives accurate reading (e.g., for detection blood pH) and can measure the pH of the liquid as well as a semi-solid of substance.

- **pH Measurement :**
  - The pH rate of a material is directly linked to the degree of the hydrogen ion [H+] and the hydroxyl ion [OH-] concentrations in its aqueous solution.
  - If the H+ density is higher than OH-, the substance is acidic; i.e., the pH amount is less than 7.
  - If the OH- intensity is higher than H+, the substance is basic, including a pH value higher than 7.
  - If identical quantities of H+ and OH- ions are present, the substance is neutral, with a pH of 7.

Application of pH Meter

- In agriculture industries to measure the pH of soil
- It is also used to measure water quality for municipal water supplies, swimming pools.
- In many chemical and pharmaceutical industries, it is used to measure the pH value of solutions .
- pH Meter is additionally employed in the Food industry particularly for dairy products like cheese, curds, yogurts, etc.
- It becomes a vital circumstance in the making of detergents

Advantages of pH Meter :

• pH Calibration is low-priced and robust

• Pocket size pH Meters are user friendly

• Accounts are reliable and specific Disadvantages of pH Meter

• Heat affects the output readings

• pH Calibration utilizing glass electrodes need to be clean as deposition on the electrodes influences the readings

Parts of PH meter:

1. The calomel reference electrode: consists of a glass tube with a potassium chloride (KCl) electrolyte, which is in intimate contact with a mercuric chloride element at the end of a KCl element. It is a fragile construction, joined by a liquid junction tip made of porous ceramic or similar material. This type of electrode is not easily 'poisoned' by heavy metals and sodium
2. The glass electrode: consists of silica glass with a thin glass bulb welded to it. Inside is a known solution of potassium chloride (KCl) buffered at a pH of 7.0. A silver electrode with a silver chloride tip makes contact with the inside solution. To minimize electronic interference, the probe is shielded by a foil shield, often found inside the glass.

3. Thermistor temperature probe: which allows for automatic temperature correction, since pH varies somewhat with temperature.
4. The pH meter converts voltage (potential difference) into a pH reading.

▪ Principle of pH Meter:

A pH meter works by measuring the electrical potential (voltage) difference between two electrodes immersed in a solution, one a reference electrode and the other a measuring electrode. This voltage difference is directly related to the concentration of hydrogen ions in the solution, which determines the pH. The meter then converts this voltage into a pH value, typically on a scale of 0 to 14.

Practical part:

▪ Operating Procedure of pH Meter :

1.Turn on the pH meter.

2.Then wash the electrodes with distilled water.

3. Maintain the sample's temperature at 25 degrees centigrade.

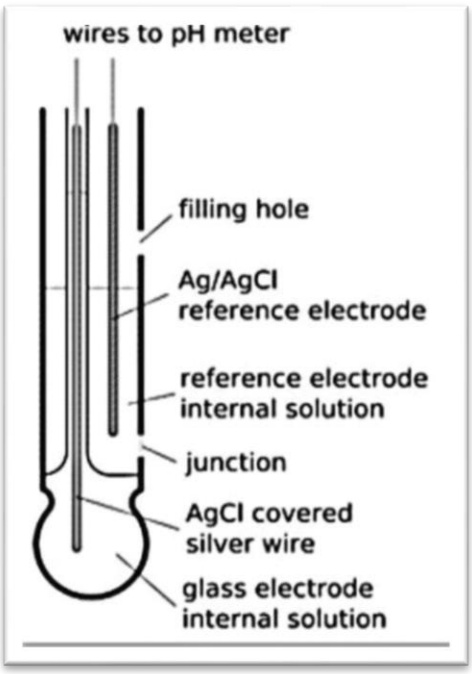
4. Immersed the electrodes within the sample and stir it to create a homogenous sample.

5. Make sure the tip of the electrode is completed dipped into the sample.

6. Wait until the reading becomes stable.

7. Now record the pH.

8. Finally, wash the electrodes with distilled water and store it with the buffer solution.



▪ Three type of pH electrodes:

1. Glass electrode.
2. Reference electrode
3. Combination gel electrode.

▪ Electrode care and maintenance:

When not in use, the glass probe tip must be kept wet at all times to avoid the pH sensing membrane dehydration.

The following general guidelines indicate the care and maintenance required for pH electrodes:

1. To dry the electrode, use clean soft tissues and blot the liquid from the electrode. Immerse in pH 4 buffer for short-term storage.
2. For longer-term storage use the same solution as the reference electrolyte of the electrode.
3. Never store your electrode in water.
4. Always rinse thoroughly with deionized water after use.
5. Gentle use of abrasive paper can sometimes remove the precipitate.

6. Ensure that the electrode is used and stored within its specified temperature range.
7. Extreme changes in temperature between samples will affect response time, and electrodes used above their temperature range will age rapidly.
8. Handled carefully – the normal lifetime of glass electrodes is approximately two years.
9. Ensure that air bubbles are not trapped at the bottom of the electrode. If present, bubbles should be removed by holding the electrode vertically and gently tapping the electrode body.

❖ **Electrode cleaning:**

The solution used to clean pH electrodes depends on the presence of possible contaminants. Mechanically intact electrodes may show slow response due to coating.

❖ **Electrode storage:**

As a general rule, store the pH electrode in the same solution as the reference electrolyte of the electrode. Never store your electrode in water as this will cause ions to leach out of the glass membrane, leading to a sluggish response.

Week 9:

Microtome

Microtome is the instrument which is used to cut thin section of tissue. Microtome is a sectioning instrument that allows for the cutting of extremely thin slices of material, known as sections. Microtome are an important device in microscopy preparation.

Types of Microtome

There are several types of microtome each designed for a specific purpose although many have a functional role:

1- Rotary microtome.

2- Sledge microtome (Sliding microtome).

3- Rocking microtome.

4- Freezing microtome (Cryomicrotome).

5- Ultra microtome.

The most common applications of microtomes are:

1-**Traditional Histology Technique:** Tissues are hardened by replacing water with paraffin. The tissue is then cut in the microtome at thicknesses varying from 2 to 50 µm.

2- **Cryosectioning Technique:**

Water-rich tissues are hardened by freezing and cut in the frozen state with a freezing microtome or microtome-cryostat; sections are stained and examined with a light microscope. This technique is much faster than traditional histology (15minutes vs 16 hours) and is used in conjunction with medical procedures to achieve a quick diagnosis.

3-Electron Microscopy Technique:

After embedding tissues in epoxy resin, a microtome equipped with a glass or gem grade diamond knife is used to cut very thin sections (typically 60 to 100 nanometer). Sections are stained with an aqueous solution of an appropriate heavy metal salt and examined with a transmission electron microscope (TEM). This instrument is often called an ultramicrotome.

4- Botanical Microtomy Technique:

Hard materials like wood, bone and leather require a sledge microtome. These microtomes have heavier blades and cannot cut as thin as a regular microtome.

▪ Rotary Microtome:

It is most commonly used microtome. This device operates with a staged rotary action such that the actual cutting is part of the rotary motion. In a rotary microtome, the knife is typically fixed in a horizontal position. A rotary action of the hand wheel actuate the cutting movement. Here the advantage over the rocking type is that it is heavier and there by more stable. Hard tissues can be cut without vibration. Serial sections or ribbons of sections can easily be obtained. The block holder or block (depends upon the type of cassette) is mounted on the steel carriage that moves up and down and is advanced by a micrometer screw. Auto-cut microtome has built in motor drive with foot and hand control. With suitable accessories the machine can cut thin sections of paraffin wax blocks and 0.5 to 2.0 micrometer thin resin sections.

▪ Advantages

1. The machine is heavy, so it is stable and does not vibrate during cutting.
2. Serial sections can be obtained.
3. Cutting angle and knife angle can be adjusted.
4. It may also be used for cutting celloidin embedded sections with the help of special holder to set the knife.

The flywheel in microtomes can be operated by hand. This has the advantage that a clean cut can be made, as the relatively large mass of the flywheel prevents the sample from being stopped during the sample cut. The flywheel in newer models is often integrated inside the microtome casing. The typical cut thickness for a rotary microtome is between 1 and 60 µm. For hard materials, such as a sample embedded in a synthetic resin, this design of microtome can allow for good "Semi-thin" sections with a thickness of as low as 0.5 µm.

Main Parts:

- Block holder
- Knife and its holder.
- Base.

• Section thickness adjustment knob.

• Handle wheel.

• Internal and external lock.

- **Microtome knives:** It is the important instrument used to cut uniform thin serial sections of the tissue. Various types of knives are used with different microtomes. Hardness of knife is essential to obtain good tissue sections.

1-Steel blades

2-Glass knives

3-Industrial grade diamond knives.

Sharpening of microtome knife - To achieve good sections knife should be very sharp. The knife is put in the knife back to sharpen. Knife can be sharpened manually or by the use of automatic machine.

Honing - This is done to remove nicks and irregularity from the knife edge. Coarse and fine honing is done using different abrasives.

Stropping - The purpose of stropping is to remove the "burr" formed during honing and to polish cutting edge.

Other types of knives are diamond and glass knives. These knives are very expensive and used for ultramicrotomy.

Practical part:

Operation :

1. Fix the paraffin block on the block holder .
2. Fixed the knife by screws.
3 Adjustment the block.
4. Fixed the thickness gauge at (20) micron.
5. Operating the handle to leveling the surface.
6. Change the thickness gauge to (8) micron.
7. Operating the handle.

Care of the Microtome Knife

- Store the knife in its box, when not in use.
- The knife should be cleaned with xylene before and after use.
- When knife is being stored for a long time, it should be smeared with grease or good grade of light oil.
- Knife edge should not be touched.
- Knife edge should never be come badly nicked.
- It is advisable to use separate knife for cutting hard issue like bone.
- The above points are important if re-usable knife is being used.

Points to remember

1- For routine histopathology rotary microtome is used.
2- Ultramicrotome is used to cut semi-thin sections or ultrathin sections.
3- Traditional type of knife requires honing and stropping to smoothen the cutting edge.
4- Disposable knives are expensive but do not need honing or stropping.
5- Knife edge is spoiled if properly decalcified tissue is not used.

- **Maintenance:**

1- Keep it clean from paraffin .

2- The sharpening of the knife is the main maintenance of the microtome.

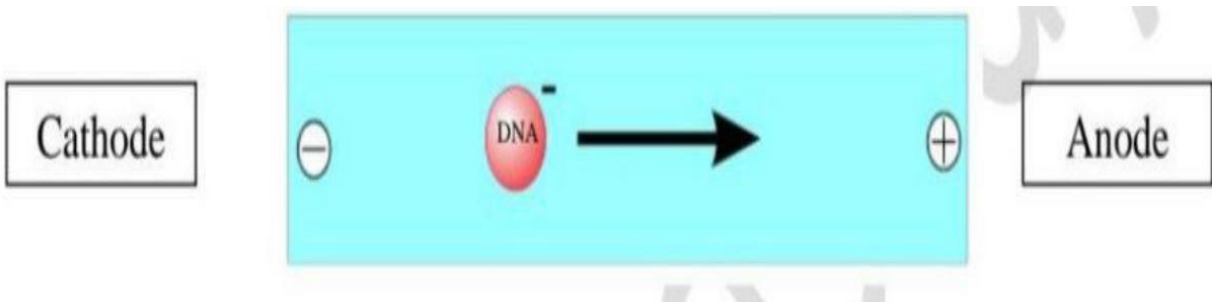
3-The exposed ends of the knife or blade must at all time be protected by magnetic or clip-on knife guards.

Week 10:

Electrophoresis

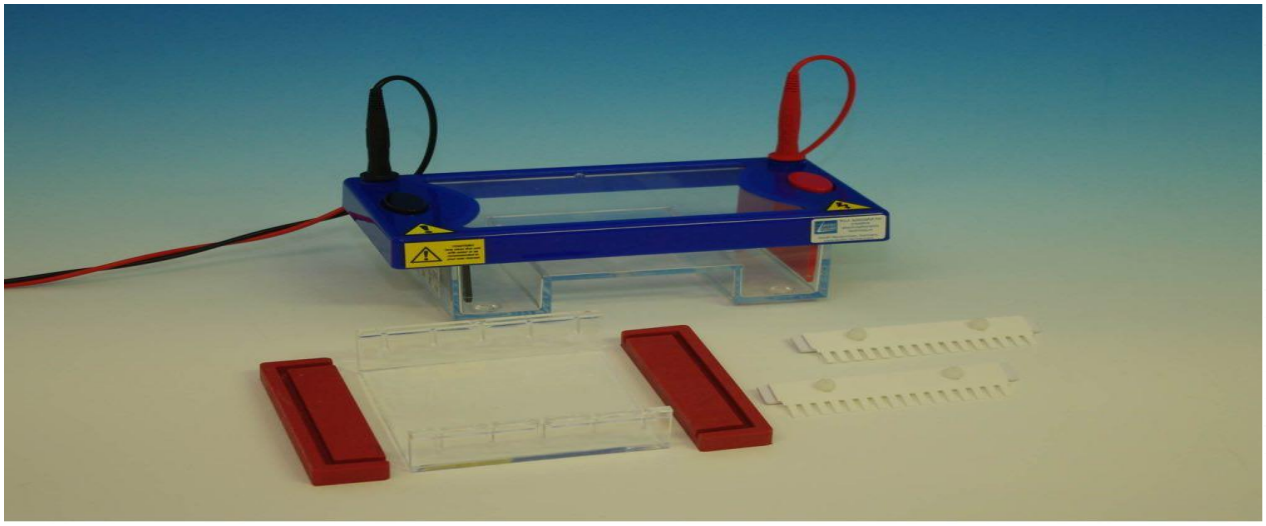
Electrophoresis is a separation device of the charged molecules in solution; through migrate in response to an electrical field.

Many important biological molecules such as amino acids, peptides, proteins, nucleotides, nucleic acids possess ionisable groups and, therefore, under the charge of an electric field, these charged particles will migrate either to cathode or to anode, depending on the nature of their net charge.



In many electrophoresis, the gel is medium between two buffer chambers containing separate electrodes, so that the only electrical connection between the two chambers is through the gel.

Rate of migration depends on the strength of the field, on the net charge, size, and shape of the molecules, and on the ionic strength, viscosity, and temperature of the medium in which the molecules are moving.

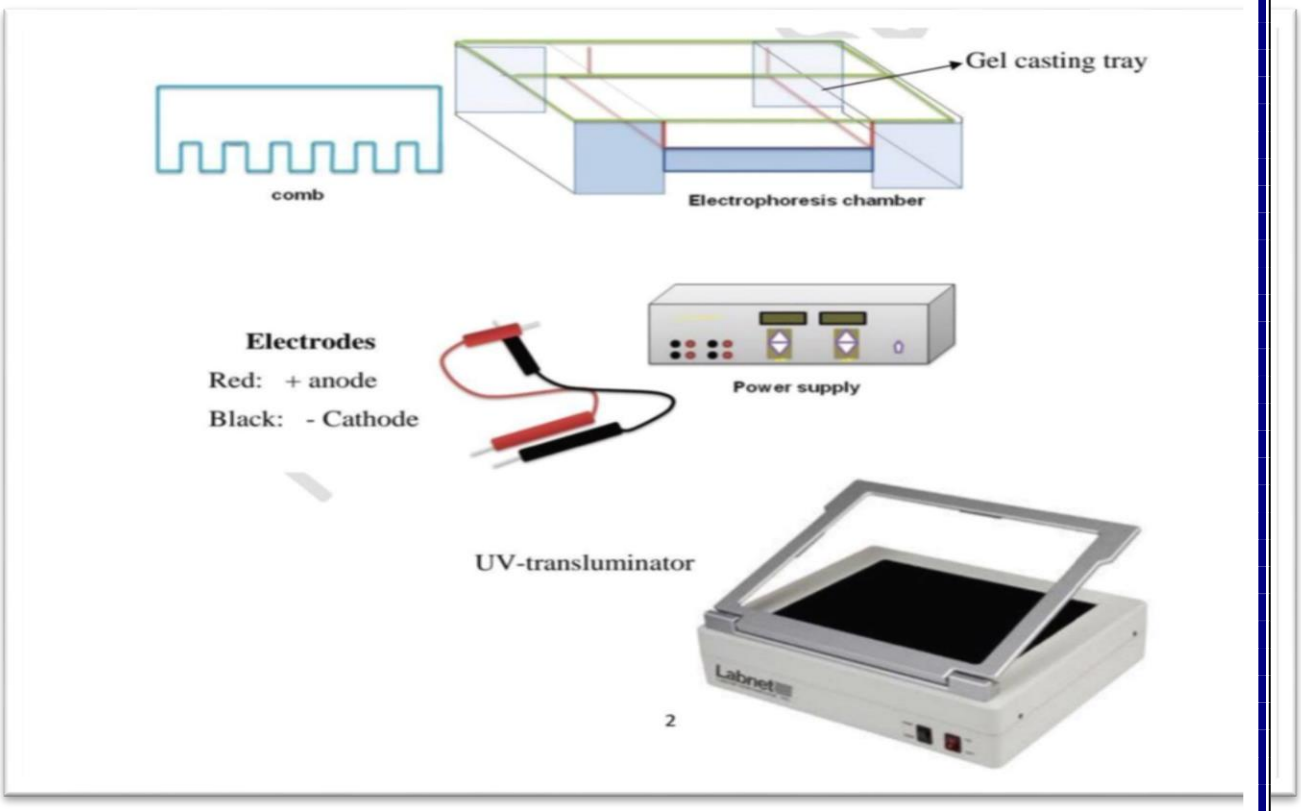


- **Types of electrophoresis:**
  1. Agarose gel electrophoresis.
  2. -Poly-acryl amide gel electrophoresis.
  3. -Capillary gel electrophoresis.
  4. Disc electrophoresis for Protein.
- Application of electrophoresis:

1. Separation of DNA and RNA.
2. Estimation of the size of DNA molecule.
3. Analysis of PCR product.
4. In forensic science.
5. In determining molecular weight of protein.
6. In molecular biology, genetic, microbiology & biochemistry lab.

- **Part of electrophoresis**:

1. Power supply.
2. Electrophoresis chamber.
3. Electrodes: Red + anode & Black: - Cathode.
4. Gel casting tray: Made of transparent plastic that allows UV penetration, the vessel must be closed with special rubber seals before pouring the gel.
5. Comb: is a tool used to working drill for place the sample (DNA) in the gel.
6. UV-transluminator: for reading the result, by to give a fluorescent glow under ultraviolet ligh.



Practical part:

- **The basic steps to performing gel electrophoresis are** :

1. Pouring the gel.
2. Preparing samples.
3. Add samples to the gel.

4. Running the gel (exposing it to an electric field).
5. Staining the gel.

- **The maintenance:**
    1. Cleaning the buffer chamber of a gel electrophoresis system is essential for maintaining its performance.
    2. Start by emptying the buffer solution from the chamber, and ensuring the power supply is disconnected.
    3. Rinse the chamber with distilled water to remove any remaining buffer solution .

Week 11:

Heating instruments (Water bath , Oven & Incubator)

Oven:

Hot - air ovens are instruments that are used for drying of chemicals and glasswares. They are also used for the sterilization of various glasswares and metal instruments. They consist of double walls that are made of copper or steel.

- **Operation Principle:** Heat is generated through sets of electrical resistors transferring this thermal energy to the chamber. These resistors are located in the lower part of the oven and heat is transferred and distributed by natural or forced convection (in oven with internal ventilators). They are heated by circulation of hot air from gas burners between the metal walls or by electrical mains. There is a thermometer on the top of the ovens and generally an automatic device (thermostat) is fitted to regulate the temperature.

- **Parts of Oven: Oven is consist of two parts, they are:**

1. Mechanical part

a- The coat (cover).

b- Fiber glass.

c- The external form.

d- The container.

e- The shelved.

2. Electrical part.

a-The power supply.

b- The heater.

c- Thermostat

d- Temperature indicator.

e- Resistors.

f- Timer.

g- Fuses.

h- Control panel.

Notification: - Avoid using flammable or explosive materials in the oven, spills of acid solutions or corrosive vapors inside the oven to prevent corrosion of the surfaces and interior shelves and use personal protection elements (insulated gloves, Safety glasses and tongs for placing or removing substances or materials inside the drying oven).

Oven

Water Bath:

A water bath is an instrument where water is heated and the set temperature is maintained at a constant level. It is used to incubate liquid substances. When only a few samples in tubes require incubating, it is more convenient and less expensive to use a dry heat block (dry bath incubator). Chemical tests react best at a specific temperature. Many tests react at room temperature (18 to 22 ºC) and others require a specific temperature as body temperature (35 to 37 ºC). Such procedural requirements are met by using water bath. When the reactants in tubes are placed in a water bath, the water surrounding the tubes warms the substances inside the tube and it takes the same temperature as the water.

- **The main parts of water bath:**

1-Container or tank bath

2-Heater.

3-Thermometer.

4-Thermostat or regulator.

- **Types of water bath:**

1-Circulating water bath.

2-Non-circulating water bath.

3-Shaking water bath.

- **Principle of water bath:**

principle of water bath This device depends on the heat applied to the sample using the heater.

- **Use and Care of a Water bath:**

1. Read the manufacturer's instructions carefully.

2. Fill the bath and maintain its level with distilled water if

a. unavailable with boiled water, preferably boiled and filtered rainwater. This is necessary to minimize salts depositing on the heater.

b. To minimize the growth of microorganisms in the water, add a bactericidal agent such as merthiolate at a dilution of 1 in 1000 to the water.

3. Before incubating samples check that the temperature of the water is correct using thermometer.

4. Ensure that the level of the water is above the level of whatever is being incubated.

5. Use the lid to prevent loss of heat from the bath and to minimize particles from entering the water. When removing the lid after incubation, take care to avoid any water entering uncapped tubes. Whenever possible, use capped tubes.

6. Clean the water bath regularly, taking care not to damage the heating unit. If there is a buildup of scale on heater and sides of the bath, this can be removed by using lemon juice.

7. Unplug the bath from the wall socket when not using it, when there is an electric storm, and when cleaning the bath and carrying out any maintenance work.

8. Every three to six months, check the bath for correction.

Note: If you are using a boiling water bath and ovens, be sure you use heat resistant glass or plastic wares.

Incubator:

in microbiology, is an insulated and enclosed device that provides an optimal condition of temperature, humidity, and other environmental conditions required for the growth of organisms. An incubator is a piece of vital laboratory equipment necessary for the cultivation of microorganisms under artificial conditions. An incubator can be used for the cultivation of both unicellular and multicellular organisms. Principle/ Working of Incubator An incubator is based on the principle that organisms require a particular set of parameters for their growth and development with the optimal condition (under artificial conditions) of temperature, humidity, oxygen, and CO2 levels.

- **Principle:**

Working of Incubator: An incubator is based on the principle that microorganisms require a particular set of parameters for their growth and development. All incubators are based on the concept that when organisms are provided with the optimal condition of temperature, humidity, oxygen, and carbon dioxide levels, they grow and divide to form more organisms.

▪ Components of laboratory incubators :

A laboratory incubator is made up of various units, some of which are:

1- Cabinet: The cabinet is the main body of the incubator consisting of the double-walled. The outer wall is made up of stainless steel sheets while the inner wall is made up of aluminum. The space between the two walls is filled with glass wool to provide insulation to the incubator. The insulation prevents heat loss and in turn, reduces the electric consumption, thereby ensuring the smooth working of the device.

2- Door: A door is present in all incubators to close the insulated cabinet. The door also has insulation of its own. It is also provided with a glass that enables the visualization of the interior of the incubator during incubation without disturbing the interior environment. A handle is present on the outside of the door Control Panel. On the outer wall of the incubator is a control panel with all the switches and indicators that allows the parameters of the incubator to be controlled.

3- Thermostat: A thermostat is used to set the desired temperature of the incubator. After the desired temperature is reached, the thermostat automatically maintains the incubator at that temperature until the temperature is changed again.

4- Perforated shelves: Bound to the inner wall are some perforated shelves onto which the plates with the culture media are placed. The perforations on the shelves allow the movement of hot air throughout the inside of the incubator. In some incubators, the shelves are removable, which allows the shelves to be cleaned properly.

5- L-shaped thermometer: A thermometer is placed on the top part of the outer wall of the incubator. One end of the thermometer provided with gradations remains outside of the incubator so that temperature can be read easily. The next end with the mercury bulb is protruded slightly into the chamber of the incubator.

6- HEPA filters: Some advanced incubators are also provided with HEPA filters to lower the possible contamination created due to airflow.

7- Humidity and gas control: The CO2 incubators are provided with a reservoir underneath the chamber that contains water.The water is vaporized to maintain the relative humidity inside the chamber. Similarly, these incubators are also provided with gas chambers to give the desired concentration of CO2 inside the incubator.

Once the cultures of organisms are created, the culture plates are to be placed inside an incubator at the desired temperature and required period of time. In most clinical laboratories, the usual temperature to be maintained is 35–37°C for bacteria.

▪ Types of incubators :

1. **Benchtop incubators**: These incubators are the basic types of incubators with temperature control and insulation.
2. **CO2 incubators** : CO2 incubators are provided with automatic control of CO2 and humidity. It's used for the growth of the cultivation of different bacteria requiring 10% of CO2 concentration.
3. **Cooled incubators**: Incubators are fitted with modified refrigeration systems with heating and cooling controls.
4. **Shaker incubator**: A thermostatically controlled used to cultivate microorganisms (broth or liquid culture media).
5. **Portable incubator:** Portable incubators are smaller in size and are used with environmental microbiology and water examination.

▪ Applications of lab incubators:

Some of the uses of incubators are given below:

1. Incubators are used to grow microbial culture or cell cultures.

2. Incubators can also be used to maintain the culture of organisms to be used later.

3. Some incubators are used to increase the growth rate of organisms, having a prolonged growth rate in the natural environment.

4. Specific incubators are used for the reproduction of microbial colonies and subsequent determination of biochemical oxygen demand.

5. These are also used for breeding of insects and hatching of eggs in zoology.

6. Incubators also provide a controlled condition for sample storage before they can be processed in the laboratories.

- **Use and Care of Incubator**

1. Read carefully the manufacturer's instruction.
2. Make sure the incubator is positioned on a level surface and that none of the ventilation openings are blocked.
3. If the incubator does not have a temperature display, insert a thermometer in the vent hole through the roof of the incubator. Adjust the thermostat dial until the thermometer shows the correct reading, i.e., 35 - 37Oc for the routine incubation of bacteriological cultures.
4. Before incubating cultures and tests, check the temperature of the incubator.
5. Clean the incubator regularly; making sure it is disconnected from its power supply. 6. Every three to six months check the condition of the incubator.
6. At the time of purchase, it is advisable to buy a spare thermostat and thermometer if these are of special type and are not available locally.

Incubator

Week 12:

Water Purification (Distilator& Deionizers)

The quality of water used in the laboratory is very crucial. Its use in reagent and solution preparation, reconstitution of lyophilized materials and dilution of samples demands specific requirements for its purity. All water used in medical laboratory should be free from substances that could interfere with the tests being performed.

Water distilling apparatus is an instrument that is used to purify impure water by a process known as distillation.

Distillation is a process by which impure water is boiled and the steam produced is condensed on a cold surface (condenser) to give chemically pure distilled water that is water from which non-volatile organic and inorganic materials are removed.

1. Operation Principle of Distillation:

Distillation works by exploiting the different boiling temperatures of liquids. To separate two or more liquids by distillation, you first heat them in a flask. The more volatile liquid (the liquid with the lower boiling point) will typically evaporate first and the vapor will pass into a condensing column, where it can revert into a liquid (condense) on the cool glass where it trickles into a collection flask. Heating further will cause the less volatile liquids to evaporate and distill at higher temperatures.

The two main kinds of distillation are **simple distillation** and **fractional distillation**, and both are used widely.

2. Distillation of a Pure Liquid:

A pure liquid has a constant boiling point as long as liquid and vapour are in equilibrium. In a simple distillation of a pure substance, as the temperature rises, the vapour pressure increases. As the vapour expands, it passes out of the heated portion of the apparatus until it comes into contact with the cold surface of the water-cooled condenser. When the vapour is cooled, it condenses and passes down the condenser into the receiver.

3. Parts of a Water Distiller:

1. Vapour generator (boiling tank): the container where the water to be distilled is stored.

2.Water level: Device which allows the quantity of water to be regulated inside the vapour generator.

3. Control valve: Mechanical or electromechanical device which allows the flow of water towards the vapour generator tank to be regulated.

4. Hydraulic connection: Network which supplies water in liquid phase to the vapour generator tank.

5. Water in liquid phase: Water inside the vapour generator tank. It receives thermal energy from the immersion resistors and it is converted to vapour when the required temperature and pressure conditions are met.

6. Immersion resistors: Devices generating heat when an electrical current circulates through them.

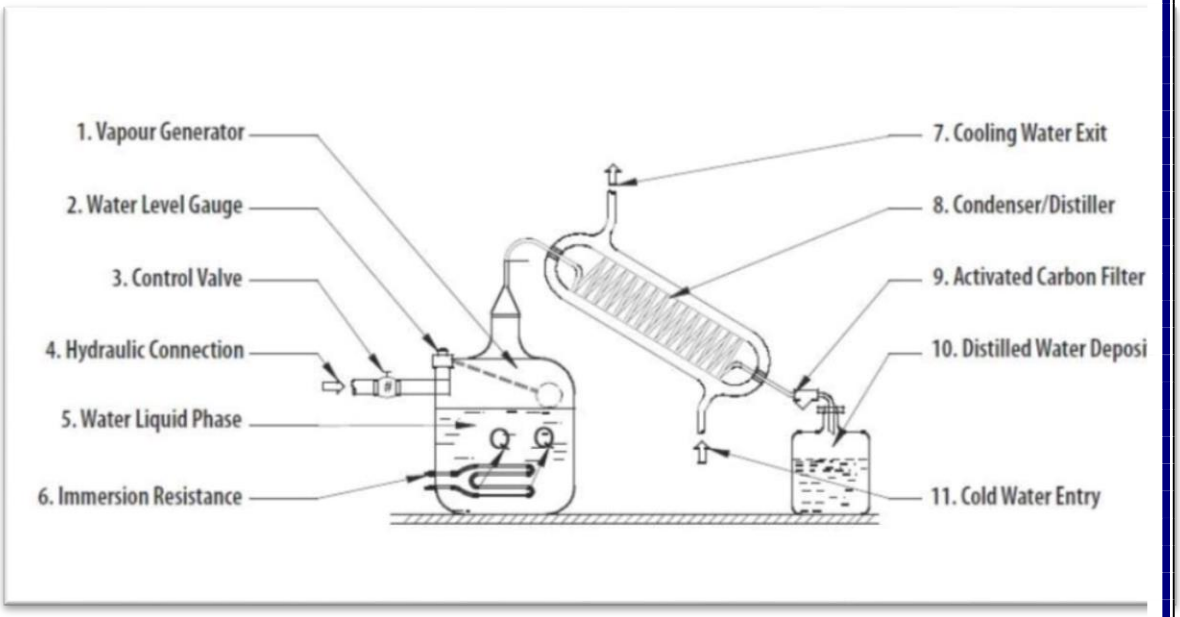


Diagram of Water Distiller

7. Refrigeration water outlet: Line carrying the water used for condensing the water vapour thus removing the thermal energy from it (cooling).

8. Condenser: Device in which the vapour loses thermal energy, cools and returns to its liquid phase.

9. Filter: Distillers have activated carbon filters located at the exit of the condenser or collector. These eliminate flavours or particles which may be present in the vapour being condensed.

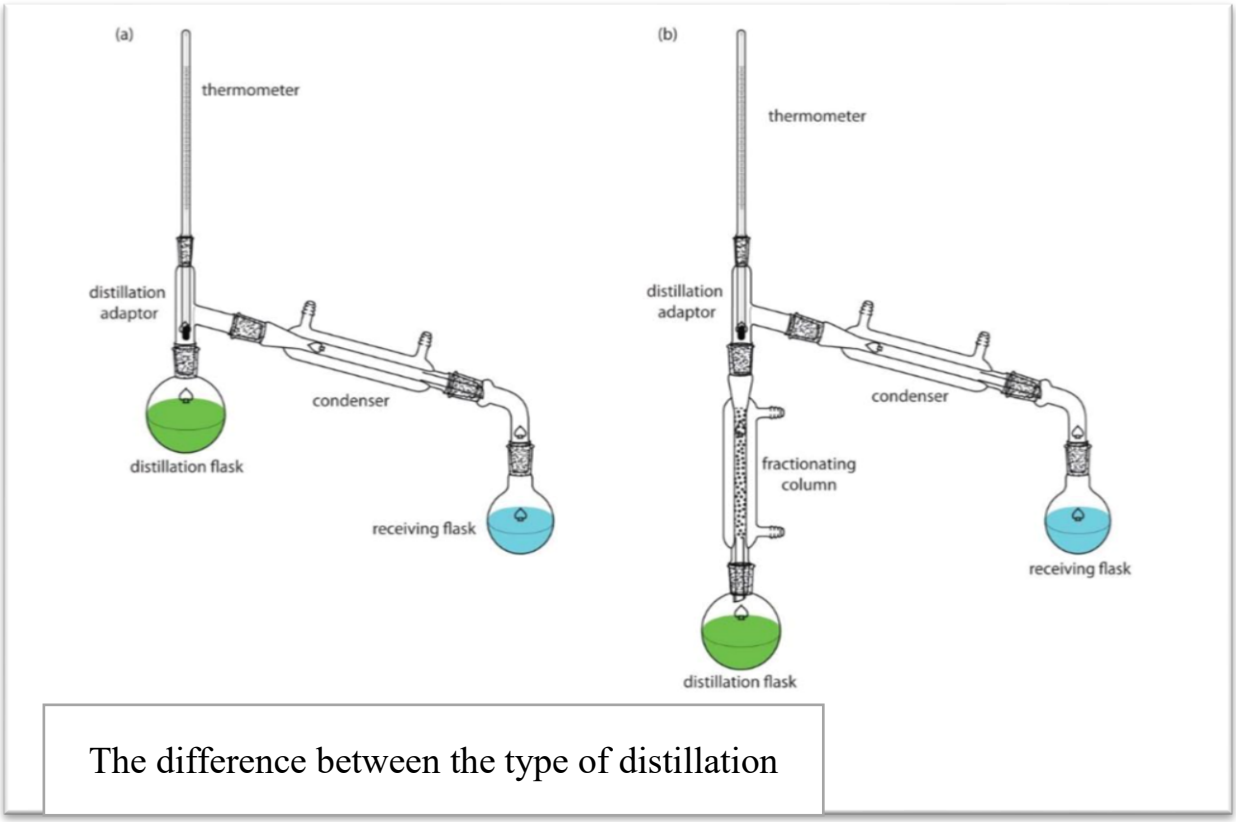
10. Distilled water container: Device in which the fluid completing the distillation process is collected. Distilled water must be stored in special plastic containers to avoid ionic contamination.

4.Types of Distillation: There are two types of distillation:

1. Simple Distillation: A simple distillation apparatus consists of a boiling flask (round-bottom flask) attached to an adapter holding a thermometer (to

determine the boiling temperature of the liquid). The adapter connects to a condenser into which cold water is constantly passed through. The condenser leads into a collection flask for the purified liquid.

2. Fractional Distillation: Fractional distillation is essentially the same as simple distillation except that a fractionating column is placed between the boiling flask and the condenser. The fractionating column is usually filled with glass or plastic beads. These beads improve the separation between the liquids being -distilled. The reason that fractional distillation gives better separation between the liquids is because the glass beads in the fractionating column provide "theoretical plates" on which the refluxing liquid can condense, re evaporate, and condense again, essentially distilling the compound over and over. The more volatile liquids will tend to push towards the top of the fractionating column, while more boiling liquids will stay towards the bottom, giving a better separation between the liquids. Of course, the more theoretica plates that you add to a column (the more surfaces or beads), the longer the distillation will take (typically), and the more energy required to keep re-evaporating liquid in the fractionating column (this is more of a concern in industrial distillations than in an academic lab where energy cost is not a major cause for worry)



The difference between the type of distillation

Comparison between simple and fractional distillation:

	Simple distillation	Fractional distillation
Advantages	❖ Simpler setup than fractional. ❖ Faster distillation time. ❖ Consume less energy.	❖ Much better separation between liquids than simple distillation. ❖ Can more readily purify complex mixtures than simple distillation.
Disadvantages	❖ Requires the liquids to have large boiling point differences (>70°C). ❖ Gives poorer separation than fractional distillation. ❖ Only works well with relatively pure.	❖ More complicated setup than simple distillation. ❖ Takes longer for liquids to distill. ❖ Consumes more energy than simple distillation.
Best used for	Separating relatively pure liquids with large boiling differences or liquids with solid impurities.	Separating complex mixtures of liquids with smaller boiling point separations.

Deionizer:

Deionizer is an apparatus used to produce ion free water. Deionization is a process in which chemically impure water is passed through anion and cation exchange resins to produce ion free water. Deionized water has low electrical conductivity, near neutral pH and is free from water-soluble salts but is not sterile. Cations, which may be present in the water such as calcium, magnesium and sodium, are exchanged by the cation resin, which in turn releases hydrogen ions. Anion impurities such as sulfate, bicarbonate, silicate, nitrate and chloride are exchanged by the anion resin, which in turn releases hydroxyl ions. Finally, the hydrogen ions combine with the hydroxyl ions to give ion - free water.

Deionizer resin can cause irritation if it is allowed to enter the eye or skin. It is therefore, advisable to wear plastic gloves and protective eye goggles when filling the plastic tube.

Week 13:

Autoanalyzers

Auto analysis: The word automation is inspired by word automatic. Automatic means exercising control without interference. So automation means getting work done by machines which can run on their own without our continuous monitoring. Automation refers to machines with intelligence and adaptability which reduces our workload and need for nonstop supervision. Auto Analyzers were used mainly for routine repetitive medical laboratory analyses, they determine levels of albumin, alkaline phosphatase blood urea nitrogen, bilirubin, calcium, cholesterol, creatinine, glucose, inorganic phosphorus, proteins, and uric acid in blood serum or other bodily samples.

▪ Types of auto analyzer:

a- Continuous flow analysers.

b- Centrifugal analysers.

c- Discrete auto analysers.

d- Dry chemical analysers**.**

▪ Advantages to automating procedures:

- Increase the number of tests performed by one individual in a given time period (short turn around time)….speeds up the result

- Human factor is decreased during the mechanical and repetitive part of an assay as labor is an expensive commodity in Medical laboratories.
- To minimize the variation in results from one individual to another (for accuracy, coefficient of variation is reduced hence the reproducibility increases).
- The quality of patients test results is monitored continuously for improvement of testing process.
- Automation eliminates the potential errors of manual analyses such as volumetric pipetting steps, calculation of results, and transcription of results (human error is reduced).
- Instruments can use very small amounts of samples and reagents subsequently allowing less blood to be drawn from each patient. In addition, the use of small amounts of reagents decreases the cost of consumable.

▪ The principle of a continuous flow analyzer (CFA):

Is made of different modules, such as: Sampler, pump, mixing coil, Heater/incubator, Sample treatment chamber (dialysis, distillation etc), Signal detector, Read out device (data generator). This provides man analysis per analyte for one sample at a time. The main principle of Continuous flow processing is the flowing carrier solution passes through small tubes continuously.

The disadvantage of CFA:

1. Even when there is no test to be done, reagents are drawn to maintain the flow. This adds to the cost per test
2. Maintenance of instrument is required more frequently
3. The probe and internal tubing must be free of clogs. When there is no sample the probe must be dipped in distilled water to avoid blockage or precipitation
4. The machine itself occupies large space.

Practical part:

- **The procedure technique of CFA:**

1. Sample collection from the patient.
2. sample is injected into a flowing carrier solution then mixes with diluents and reagent and it is sent through the tubing and mixing coils.
3. The machine prevents carry over effect between different samples by injecting bubbles of air, which create separate space for different reactions to take place inside the tubing and mixing coils.
4. The tubing passes the samples from one apparatus to the other. There are different apparatus for different functions, such as ion exchange, heating, incubation, and finally recording of the signal.
5. The flow conditions are regulated. When reaction is taking place, the optical density of the color formed is read and results are obtained. So we do not have to wait till the reaction ends, For example, for better understanding. In a nephrotic syndrome patient, you want to analyze total protein, albumin and creatinine.

Chemical Autoanalyzer