Laboratory instruments and apparatuses

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Intended learning outcomes

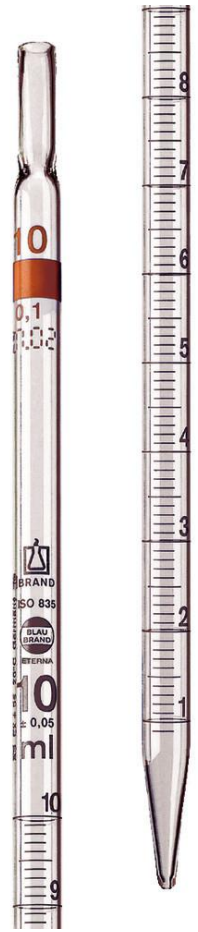
- Identify and categorize the different instruments and apparatuses with their parts and uses in practice
- Identify the photometer with its main parts and uses
- Recognize the principles of photometry and the related laws
- Learn the purpose and proper use of a spectrophotometer
- Prepare stock solutions and perform different dilutions

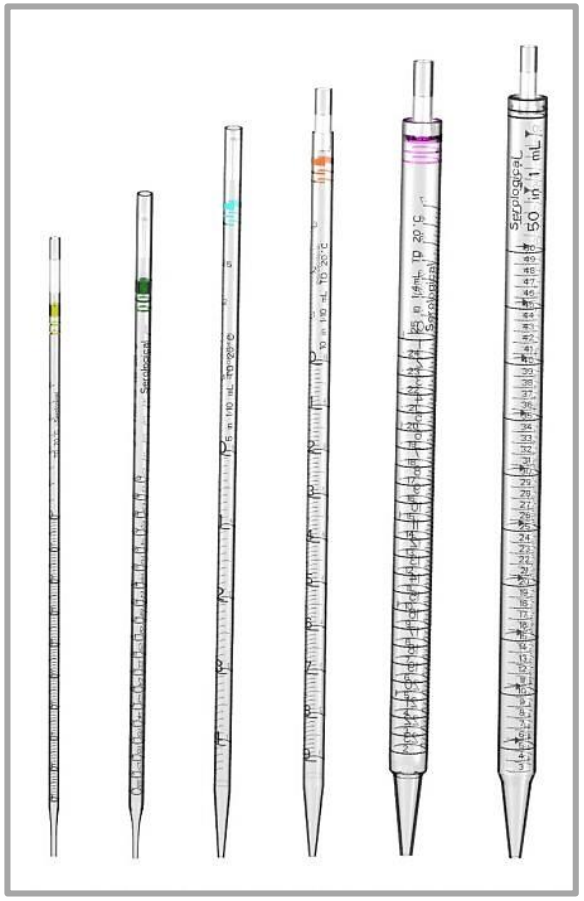
Instrument for liquid volume measurements

1. Pipettes

It measures and delivers exact volumes of liquids

- **Glass graduated pipettes used to transfer small volumes of liquids e.g. (1 ml-10 ml)**





Glass graduated pipette

Pipettes

- **Automatic pipette**

  most accurate of all, used to transfer

  micro volumes of liquids e.g. (1 µl-1000 µl)

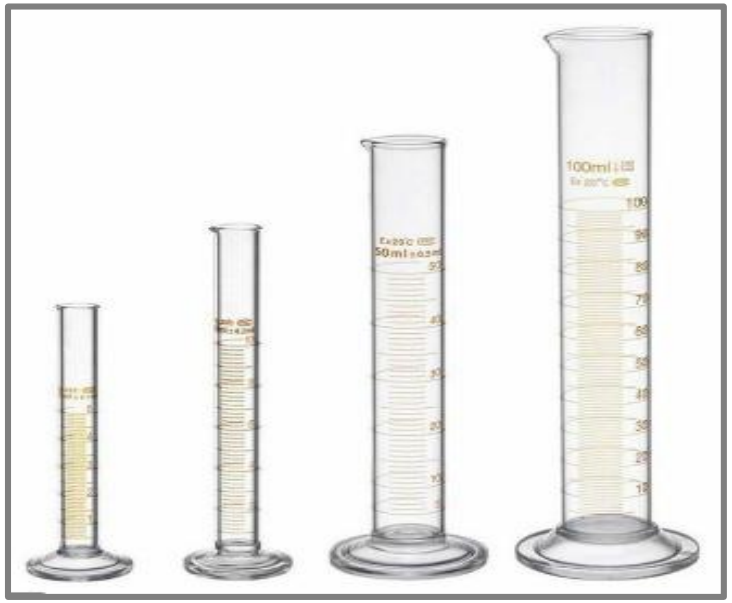
Automatic pipette tips

Automatic pipette

Instrument for liquid volume measurements

2. Graduated cylinders

It is used to measure different volumes of liquids



3. Volumetric flasks

It is used for the preparation of solutions with different concentrations and volumes

Instruments for transfer, mixing and heating of chemicals

1. Test tubes

Used for performing chemical experiments in lab.

For example, holding liquid samples for heating,

dissolution, centrifugation and others

Instruments for transfer, mixing and heating of chemicals

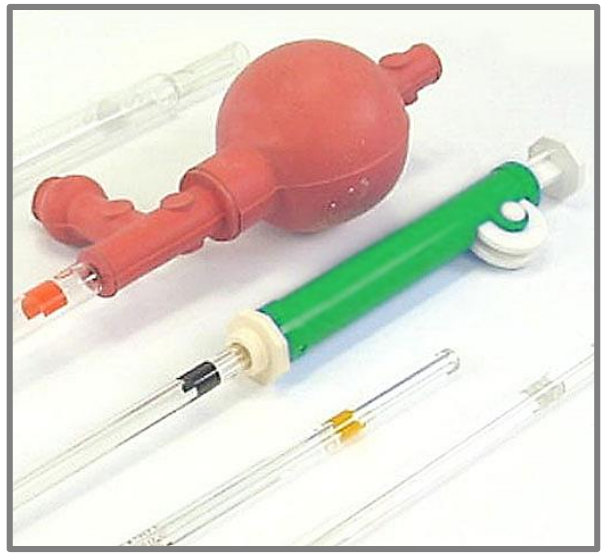
2. Graduated beakers

It hold solids or liquids that will not release gases when reacted or are unlikely to splatter if stirred or heated

Handling of chemicals

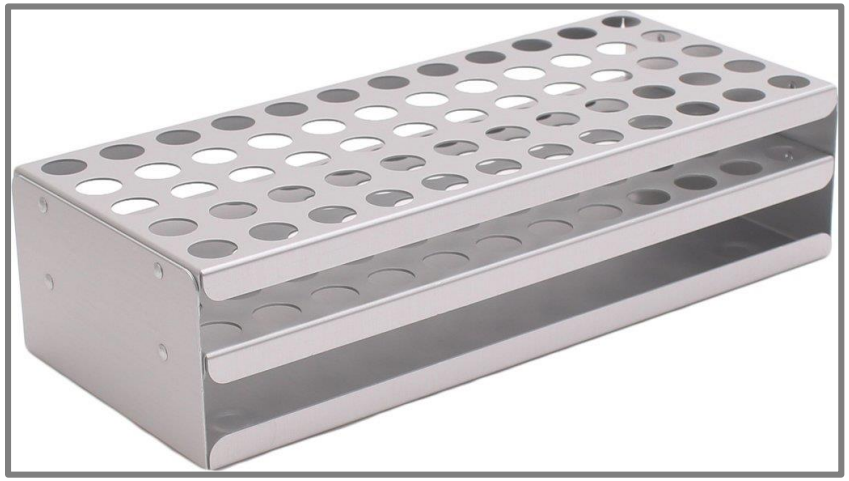
1. **Pipette pump**

It is inserted into the end of the glass pipette to help the delivery of liquid without suction by mouth



Handling of chemicals

2. Test tube Rack



It is used for holding and organizing test tubes on the lab counter. Plastic racks may melt in contact with very hot test tubes

3. Test tube holder

It is useful for holding a test tube which too hot to handle

Laboratory apparatuses

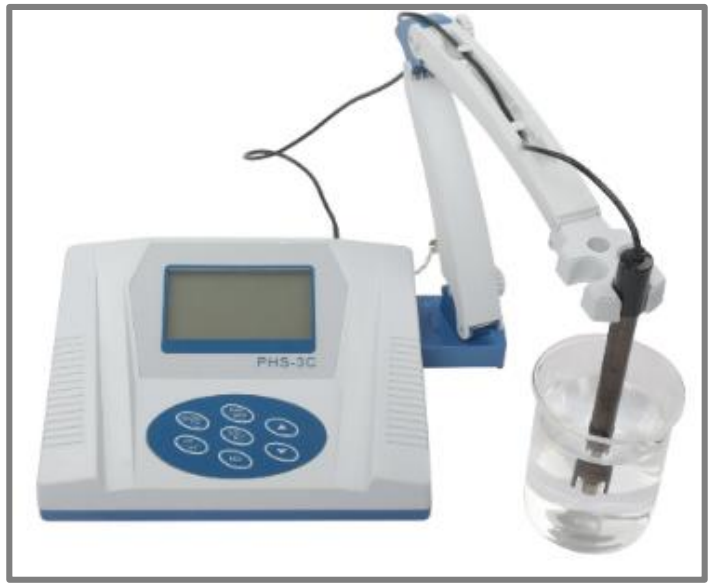
1. Lab water bath

It provide precise temperature for lab reaction control. It available with an analog or digital operating system.

Laboratory apparatuses

2. pH meter

Used to measure the pH of the solution



3. Centrifuge

Spins liquid samples at different high speeds to sediment different fractions.

Laboratory apparatuses

4. Spectrophotometer

Used to measure the absorbance (O.D) of different substances in biological fluid. It used to calculate the corresponding concentrations

Practical analysis using spectrophotometer

A **spectrophotometer** is an instrument which can measure the amount of the light absorbed by the sample at any selected wavelength. It consists of two parts, namely a spectrometer for producing light of any selected color (wavelength) and a photometer for measuring the intensity of the light.

Spectrophotometer

This instrument is used in the clinical laboratory to measure the concentration of chemical substance, depending on their colored solution that can absorb light.

Certain principles and terms should be understood

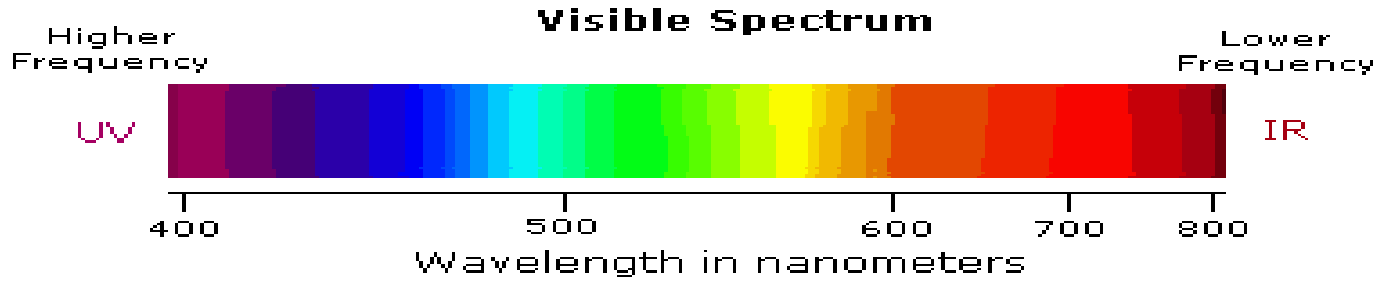
Lights: light consist of photons that propagate and are distributed as waves of different wave lengths measured in nanometre.

Light spectrum: it is the representation of the light distribution.

Certain principles and terms should be understood

Light spectrum is classified according to its wavelength into:

- **Ultraviolet (UV) light** which falls in the region of short wavelengths 200-400 nm. It is invisible
- **Visible light** which falls between the wavelengths of 400 and 700 nm. All of the colors visible by the human eye are found within this range of wavelengths.
- **Infrared (IR) light** which falls in the longer wavelength (700-900). It is invisible



Light absorption and transmission

Any solution, containing a substance which absorbs light in the range of 400-700 nm appears colored to the eye

For example: A red solution will be visualized red, because it transmits light maximally between 220 and 750 (red color), while it absorb other colors.

- **White light:** All colors. It is a Polychromatic light
- **Monochromatic light:** light of one color (light of specific wavelength) passes through a solution

Transmittance, Absorbance, and concentration

- Transmittance is often quoted as % T = [I / I°] x 100
- % transmission is not linearly related to concentration
- For a graph to be useful, a straight line is needed
- ABSORBANCE = log (1/T) = - log (T)

Transmittance, Absorbance, and concentration

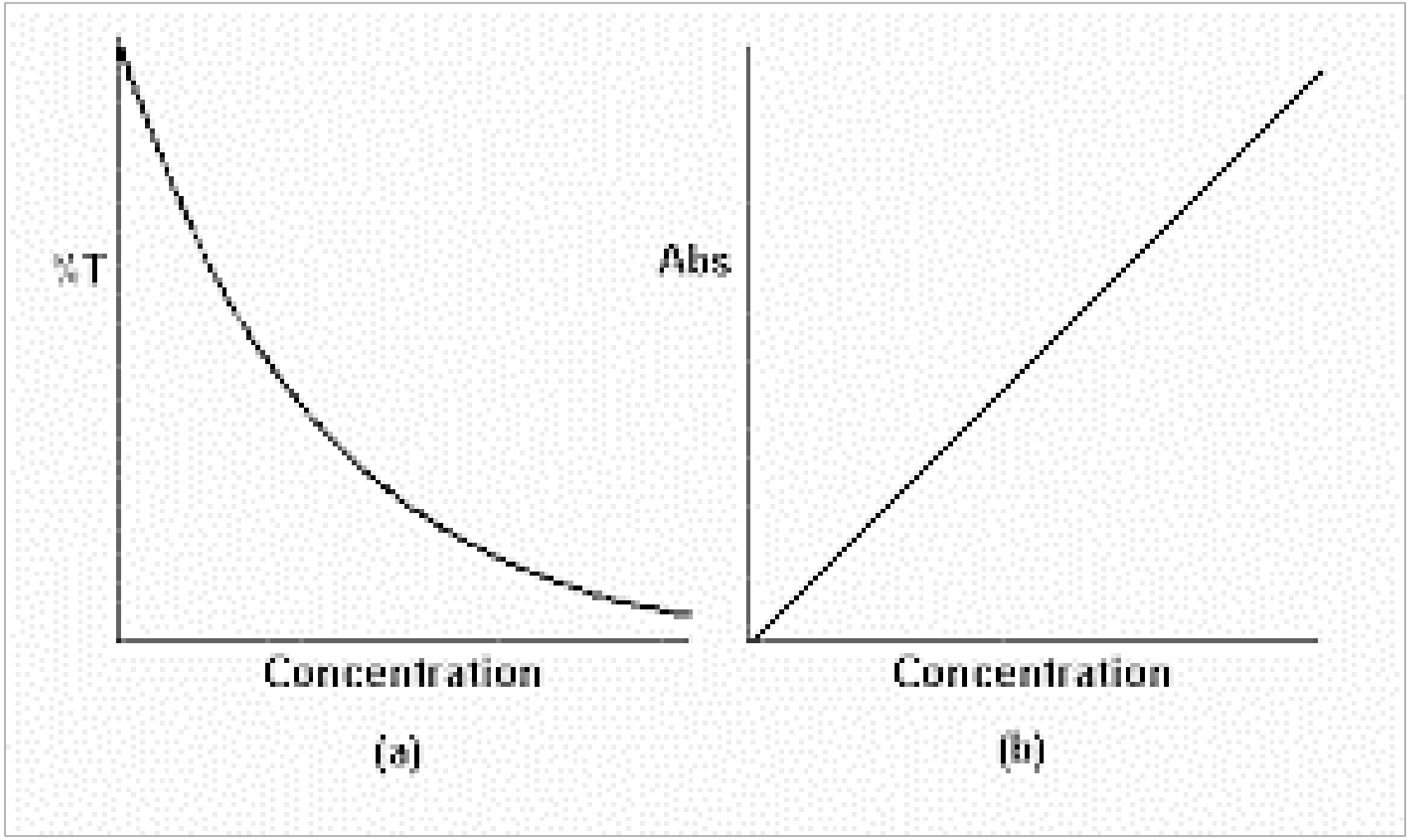
There is usually a quantitative relationship between the solute concentration and the intensity of the transmitted light

- As we increase the concentration of the studied solution, the transmittance varies inversely and logarithmically with concentration

- The graph representing this relation is obviously inaccurate in determining the concentration of unknown solutions if their % T is measured. Fig (a)

- If we draw a graph that represents the relation of absorbance against concentration, it will be linear one Fig (b). this is much more accurate for quantitative analysis.

Transmittance, Absorbance, and concentration



Spectrophotometry Laws

There are two laws that regulate photometric analysis of colored solutions they are Lambert's law and Beer's law:

1. Lambert's Law states that: when monochromatic light passes through a solution, the intensity of light transmitted decreases exponentially with increasing path length

2. Beer's Law states that: the concentration of a substance in a solution is directly proportional to the absorbance (A) of the solution (the amount of light energy absorbed).

Spectrophotometry Laws

The most useful relationship in absorbance arises from the combination of Lambert's law and Beer's law. The combined Beer-Lambert relationship can be expressed as

$$A \alpha c L$$

"L" is the length of light path through the sample

"c" is the concentration of absorbing substance in that path

Absorbance (A), being a logarithm has no units. It is sometimes referred to as extinction or optical density (OD)

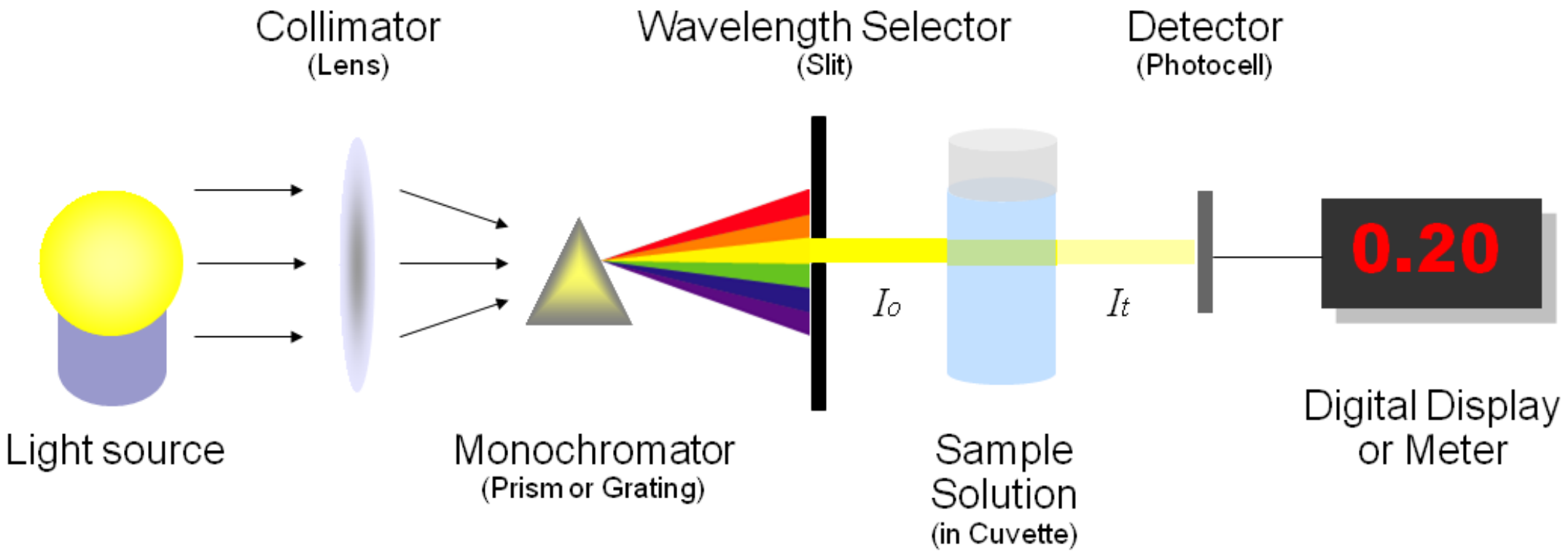
100% T = zero

Construction and properties of spectrophotometers

A **spectrophotometer** consist of

1. Light source
2. Wavelength selector
3. Slit
4. Sample tube or cuvette
5. Light detecting photocells
6. A scale or a meter

Construction and properties of spectrophotometers



Construction and properties of spectrophotometers

1- Light source actually emits a steady amount of light in the range of wavelengths required for the analysis of the sample. Most spectrophotometers have tungsten lamp for analysis in the range of 340 to 900 nm

2- Wavelength selector: each colored solution requires a complementary color to be measured maximally by photometer. This is done by choosing the proper monochromator (filter).

3- Slit is necessary to be able to adjust the intensity of the incident light (I°) by placing a pair of baffles in the light path to form it.

Construction and properties of spectrophotometers

4- **Sample tubes** or cuvettes: these are plastic or glass containers where we put the solution to be measured. They have fixed internal diameter of one cm.

5- **Light- detecting photocells** can detect any light which is not absorbed by the solution in the tube and transmitted to it. The photocell can also convert light energy into electric energy. Finally it is connected to a galvanometer

6- **A scale or a meter**: gives the final records of absorbance of light passing through the solution. It can be digital appearing on a display or a scale with an index

Applications of spectrophotometers

Qualitative analysis : Identify unknown compounds by their absorption spectrum

Absorption spectrum: each solution absorb light maximally at a certain wave length according to its chemical nature

Applications of spectrophotometers

For example

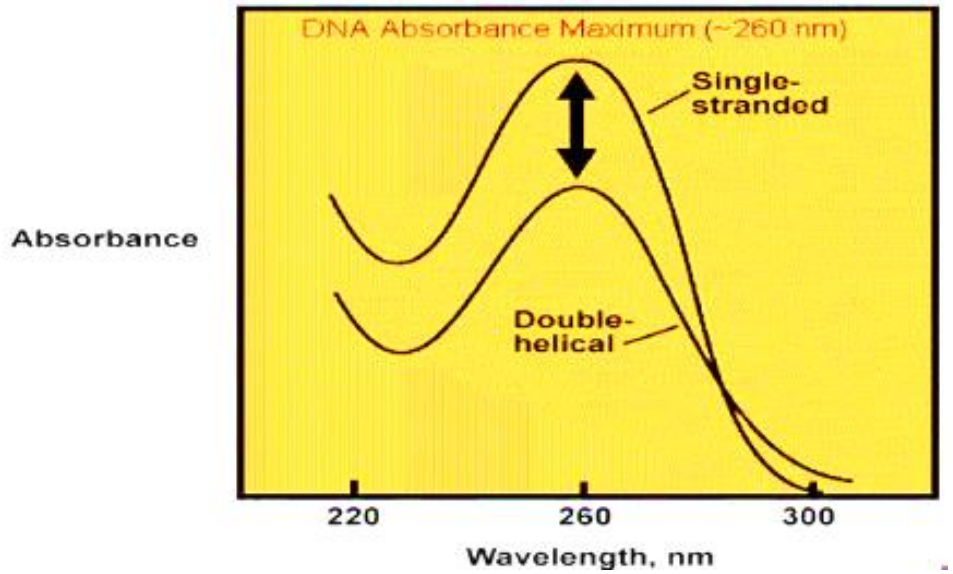
a. **Heme proteins** or **cytochromes** show a maximal absorbance in the range from 500 to 600 nm

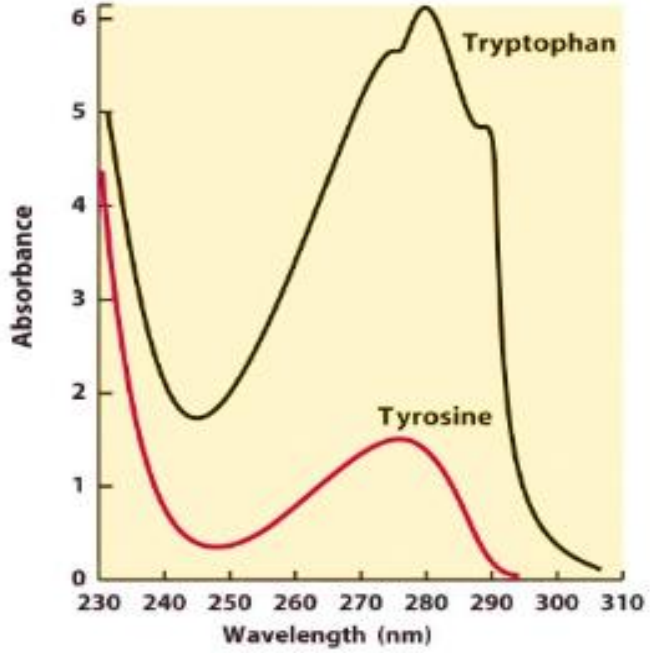
b. The **nitrogenous bases** that comprise **nucleic acids** are known to absorb strongly at 260 nm

c. The **aromatic rings** on **tryptophan** and **tyrosine** are known to absorb strongly at 280 nm

Applications of spectrophotometers

- **So for each colored solution to be measured by photometer we must use the wavelength that is maximally absorbed by this solution**





Applications of spectrophotometers

Quantitative analysis : measurement of the actual concentration of the sample in the solution

Comparative method: it may be sufficient to use a standard solution of known concentration and compare the absorbance of the test solution to that of the standard solution of the same material

Applications of spectrophotometers

Lambert's Law + **Beer's Law** = **Beer-Lambert Law**

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm

Applications of spectrophotometers

$$A_{test} = \varepsilon C_{test} L$$

$$A_{standard} = \varepsilon C_{standard} L$$

$$\frac{A_{test}}{A_{standard}} = \frac{\varepsilon C_{test} L}{\varepsilon C_{standard} L} = \frac{C_{test}}{C_{standard}}$$

$$C_{test} = \frac{A_{test}}{A_{standard}} \times C_{standard}$$

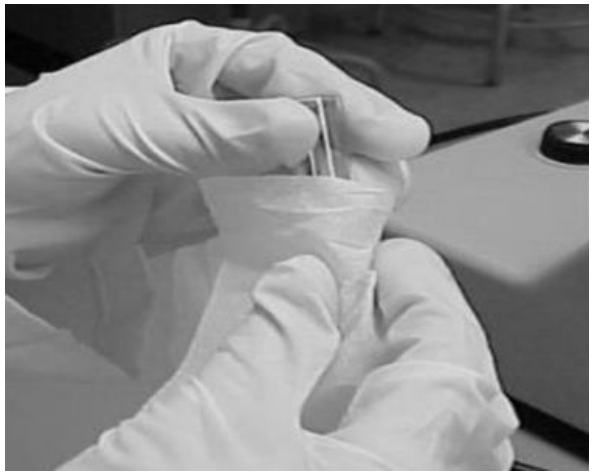
Solution required for photometric measurement

1. **Test or sample**: made from serum or other unknown specimen
2. **Standard**: made from a known concentration of the substance to be measured
3. **Blank**: contains all reagents used in measurement except the substance to be measured, it compensates for non-specific color such as the color of reagents
4. **Control**: contains all reagents used except the active ingredient, it compensates for the unwanted color of serum

Steps in using a spectrophotometric analytical method to measure analytes

1. The instrument must be warmed for at least 15 mins prior to use
2. Use the wavelength knob to set the desired wavelength

Steps in using a spectrophotometric analytical method to measure analytes



3. Pour the reference solution (blank) into the cuvette
4. Wipe the cuvette with a lab wipe. Place the cuvette into the sample holder and close the cover
5. Set the zero absorbance
6. Remove the blank cuvette then repeat step 5 with sample (test) solution

Steps in using a spectrophotometric analytical method to measure analytes

7. Read and record the absorbance

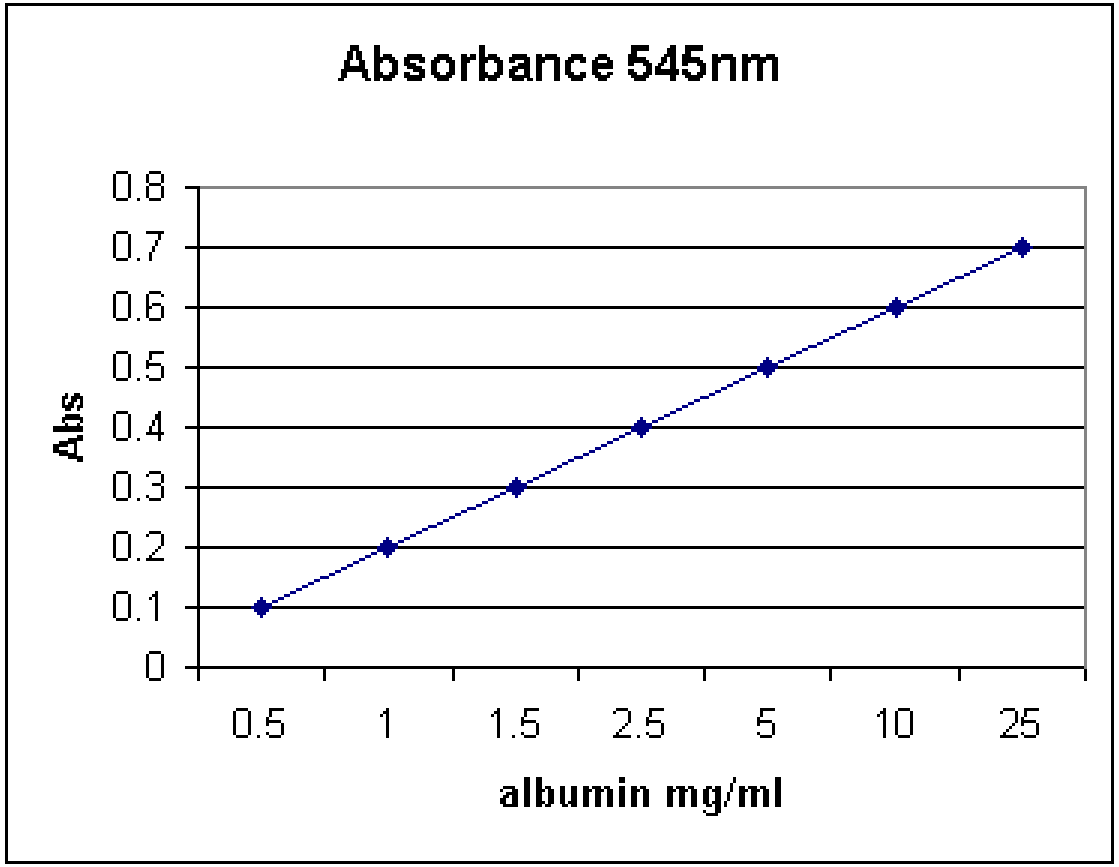
8. Calculate the test concentration by the following equation:

$$C_{test} = \frac{A_{test}}{A_{standard}} \times C_{standard}$$

How would you determine the concentration of an unknown protein sample?

1. Use a set of standard proteins with known concentration
2. Find out their absorbance by using a spectrophotometer
3. Plot absorbance on y-axis vs. protein concentration on x-axis and plot the results
4. Draw a best fit line and use it to determine the concentration of the unknown protein after determining its absorbance using the same spectrophotometer

For example: Albumin standard curve



Standard curve: Absorbance vs. concentration

Expression of concentrations

Solutions

- **Solvent:** materials that dissolve another
- **Solutes:** substance being dissolved

Most common type of solutions involves solid dissolved in liquid

Expression of concentrations

Concentration: the amount of solute in definite volume of solvent or solution

Methods of expression of concentrations

1. Percentage
2. Molarity
3. Normality

Methods of expression of concentrations

1. Percentage (%)

Percent concentration

Describe the amount of solute dissolved in 100 part of solution

The most commonly used two methods are according to the nature of solvent

Methods of expression of concentrations

Solvent

Percent / volume
Solvent is liquid

Percent / mass
Solvent is solid

Methods of expression of concentrations

Percent / volume method is further divided into two types according to the nature of solute

Solute

Volume / volume % (v/v%)	weight / volume % (w/v%)
Solute is liquid	Solute is solid (commonly used)
70% ethyl alcohol (70ml/100ml)	Glucose 5% (5g/100ml)

Problems

1. **An IV solution is prepared by dissolving 25 g glucose in water to make total 500ml solution. What is the (w/v%) of glucose in the IV solution?**

a. **5 %**
b. **20 %**
c. **50 %**

Problems

1. **How much solute is present in 756.1ml of a 14.7% (mass/volume) NaCl solution?**

Mass /volume % = (Mass $_{solute}$ / volume $_{solution}$) * 100

14.7% = (x / 756.1 ml) 100

X = 0.147 (756.1 ml)

X = mass of solute = 111 g

Methods of expression of concentrations

2. Molarity (M)

A concentration that expresses the number of moles of solute in 1 L of solution

Molarity (M) = no. of moles of solute / 1 liter solution

It is abbreviated as a capital M ‘mole/L’

Methods of expression of concentrations

- **Mole:** the basic international unit for matter
- Weight of 1 mole of any substance = its molecular weight (MW) in grams
- **MW :** it is the sum of the atomic weight of all atoms in the formula expressed in gram

eg. One mole of NaH_2PO_4

$23+(2x1)+31+(4x16)= 120$ g = weight of 1 mole

Problems

1. **Prepare 1 molar solution of NaCl (MW of NaCl= 58.5)**

Weighting 58.5g of NaCl and dissolve it in DW to final volume one liter

2. Prepare 0.25 M solution of NaCl

Weighting 14.625 g of NaCl and dissolve it in DW to final volume one liter

Methods of expression of concentrations

3. Normality (N) gm equivalent / L

- The number of gram-equivalents of solute in one liter of solution
- A normal solution contain 1 gram equivalent weight of solute in a liter of solution
- A gram equivalent weight (G.E.W) of an acid or a base is the molecular weight in grams of the solute required to react, or replace one mole of H+ or OH-

1 mole of Na + = 1 N

1 mole of Ca ++ = 2 N

Methods of expression of concentrations

3. Normality (N) gm equivalent / L

Weight of a gram equivalent (G.E.W) of an element = MW of the solute in gram (1 mole) / Valency

Which is the number of replaceable hydrogen or hydroxyl ions

1 N solution = a solution contains 1 G.E.W / L

For example:

Monovalent acids or bases

HCl, NaOH, Nitric **1M = 1N**

Divalent acids

H_2SO_4 **1M = 2N**

Trivalent acids

Orthophosphoric acid **1M = 3N**

Normality (N) = Molarity x Valency

Problems

1. Prepare 1 normal solution of NaCl [MW of Na= 23, of Cl= 35.5]

MW of NaCl= 23+35.5 = 58.5

G.E.W = 58.5

Thus a solution of 1N NaCl contains 58.5g of NaCl / 1L

Methods of expression of concentrations

Dilutions

You dilute a solution whenever you add solvent to a solution. Adding solvent results in a solution of lower concentration

You can calculate the concentration of a solution following a dilution by applying this equation:

$C_i V_i = C_f V_f$

Where:

C= concentration

V= volume

i = initial value

f = final value

Methods of expression of concentrations

Dilution factor

Is the ratio of the final volume to the original volume

If 10 ml of blood are completed to 100 ml with saline, we say that blood is diluted 1 in 10, and the dilution factor is 100 / 10 = 10

The dilution factor = Final volume / original volume

Problems

How many millilitres of 5.5 M NaOH are needed to prepare 300ml of 1.2 M NaOH ?

5.5 M x Vi = 1.2 M x 300 ml

Vi= 65 ml

So to prepare the 1.2 M NaOH solution, you add 65ml of 5.5 M NaOH into your container and add water to get 300 ml final volume

THANK YOU !

ANY QUESTIONS ??

PLEASE ASK