COLORIMETRY

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colorimetry

- It is the most common analytical technique used in biochemical estimation in clinical laboratory.
- It involves the quantitative estimation of color.
- A substance to be estimated colorimetrically, must be colored or it should be capable of forming chromogens (colored complexes) through the addition of reagents.

- Colored substance absorb light in relation to their color intensity.
- The color intensity will be proportional to the conc. Of colored substance.
- The instruments used in this method are colorimeter or photometer or absorptiometers.

principle

- Colored solutions have the property of absorbing certain wavelength of light when a monochromatic light is passed through them.
- The amount of light absorbed or transmitted by a colored solution is in accordance with two laws:
- Beer's law
- Lambert's law

Beer's law :

- When a monochromatic light passes through a colored solution, amount of light transmitted decreases exponentially with increase in concentration of colored substance.
- i.e. the amount of light absorbed by a colored solution is directly proportion to the conc. Of substance in the colored solution.

Lambert's law :

- The amount of light transmitted decreases exponentially with increase in pathlength (diameter) of the cuvette or thickness of colored solution through which light passes.
- i.e. the amount of light absorbed by a colored solution depends on pathlength of cuvette or thickness or dept of the colored solution.

- Combined *beer's- lambert's law* is thus expressed as amount of light transmitted through a colored solution decreases exponentially with increases in conc. Of colored solution & increase in conc. of colored solution & increase in the pathlength of cuvette or thickness of the colored solution

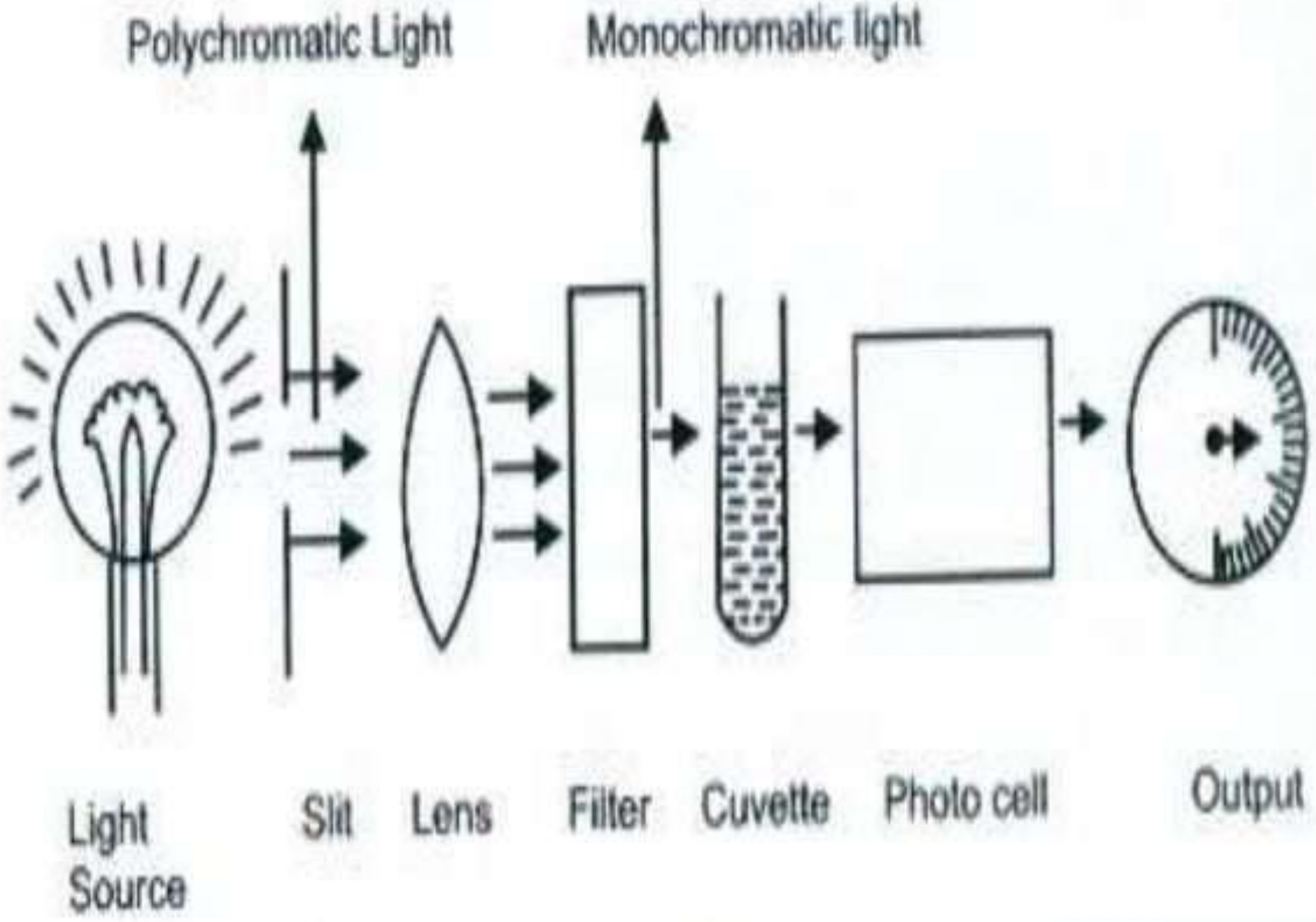

Fig. 27.1: Parts of the colorimeter

Parts of the colorimeter

Light source : tungsten filament lamp

Slit : it is adjustable which allows only a beam of light to pass through. it prevents unwanted or stray light

Condensing lenses: light after passing through slit falls on condenser lense which gives a parllel beam of light.

Filter :

- made of colored glass. Filters are used for selecting light of narrow wavelength.
- filters will absorb light of unwanted wavelength and allow only monochromatic light to pass through.

For ex: a green filter absorbs all color, except green light which is allowed to pass through.light transmitted through a grenn filter has a wavelength from 500-560 nm.

- Filter used is always complimentary in color to the color of solution.

Table 27.1: Filters used in a colorimeter

Color of Solution	*Filter*	*Wavelength Range (nm)*	*Peak*
Yellow/green	Violet	390-490	430
Yellow	Blue	460-540	480
Orange/Purple	Green	500-590	540
Blue/Green	Orange	580-650	600
Blue	Red	650-700	670

Cuvette(sample holder) : the monochromatic light from the filter passes through the colored solution placed in a cuvette.

- it is made up of special glass/plastic/quartz material.
- it may be square/rectangular/round shape with fixed diameter (usually 1 cm)& having uniform surface.the colored solution in the cuvette absorbs part of light & remaining is allowed to fall on detector.
- For ex : a solution of red color transmits red light & absorbs the complimentary color green.

Detector (photocell):

- Detector are photosensitive elements which converts light energy into electrical energy.
- The electrical signal generated is directly proportional to intensity of light falling on the detector.

Output : the electrical signal generated in photocell is measured by galvanometer, which displays percent transmission & optical density.

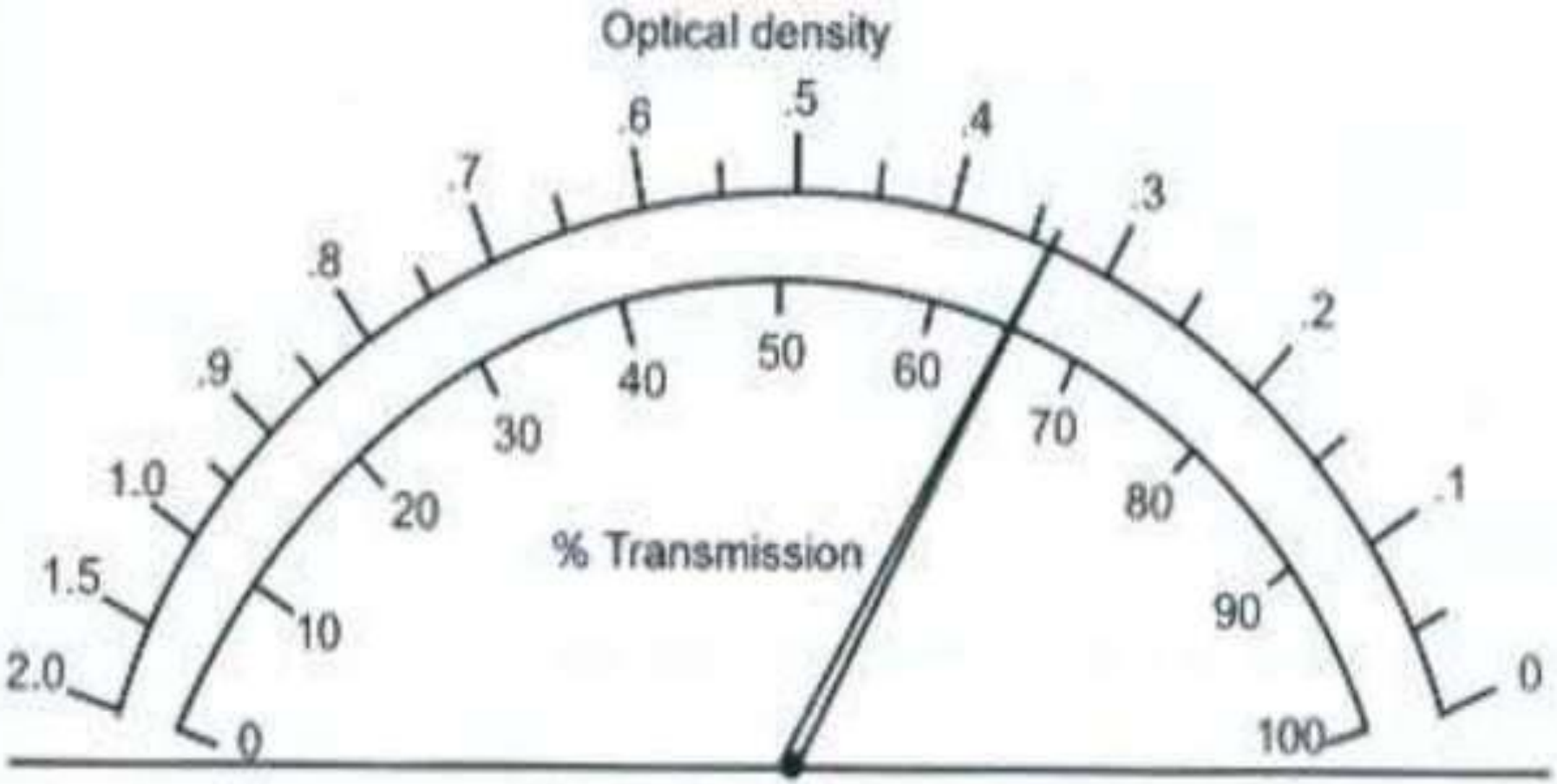

Fig. 27.2: Galvanometer

Use of Test (T), Standard (S) and Blank (B)

In colorimetric estimations, it is necessary to prepare a blank (B), a standard (S) and test (T).

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Test : this solution is prepared by treating a specific volume of specimen (blood,urine, CSF…etc) with reagents.

- **Standard** : prepared by treating a solution of the pure substance of unknown conc. With reagents.
- **Primary standard** : the same substance is used as standard one which is to be estimated.

For ex : pure glucose is taken as standard in estimation of blood glucose.

- **Secondary standard** :

Here the substance taken as standard is different from the substance to be estimated.

This substance taken as standard should match the color of final product.

For ex : methyl red is taken as standardin estimation of serum bilirubin.

- **Blank** : prepared for rule out color produced by reagents alone.
- Two types of blank :

A) Distilled water as blank

B) reagent blank (reagent used in the estimation is taken as blank)

Calculation :

- conc. Of substance in mg /100mg or gm/100ml of sample.
- $\frac{\text{OD of test- OD of blank}}{\text{OD of standard – OD of blank}} \times \frac{\text{conc. of standard}}{\text{vol. of test sample}} \times 100$

Application of colorimetric assay:

Used in determination of amount of many substances in blood, urine, saliva, CSF & other specimens.

Ex for common colorimetric assay are : determination of blood glucose, blood urea, serum creatinine, serum proteins, serum cholesterol, serum inorganic phosphate, urine creatinine & glucose in CSF, etc.

THANK YOU