

NEPHELOMETRY AND TURBIDIMETRY

By

M Asif Shaheen

NEPHELOMETRY AND TURBIDIMETRY

INTRODUCTION:

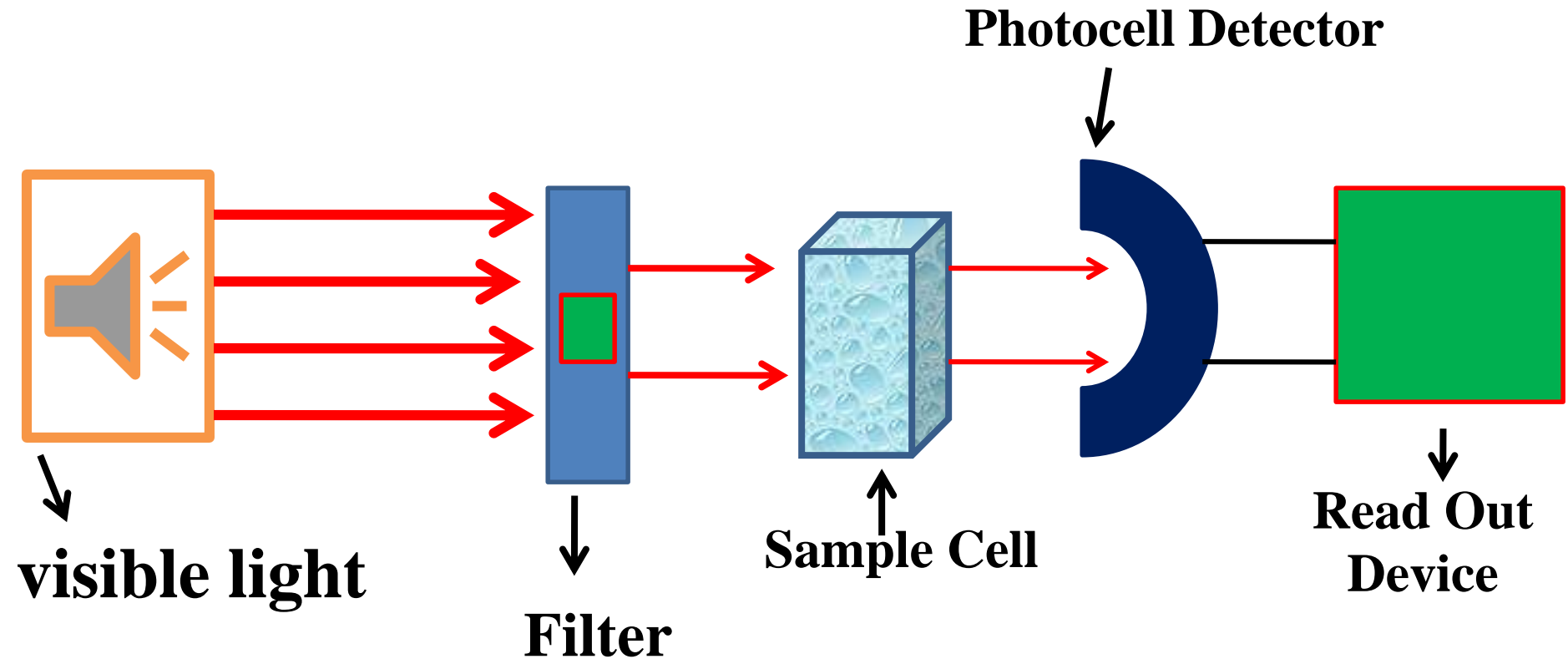
When electromagnetic radiation (light) strikes on a particle in solution, some of the light will be absorbed by the particle, some will be transmitted through the solution and some of the light will be scattered or reflected. The amount of light scattered is proportional to the concentration of insoluble particle.

NEPHELOMETRY AND TURBIDIMETRY

INTRODUCTION:

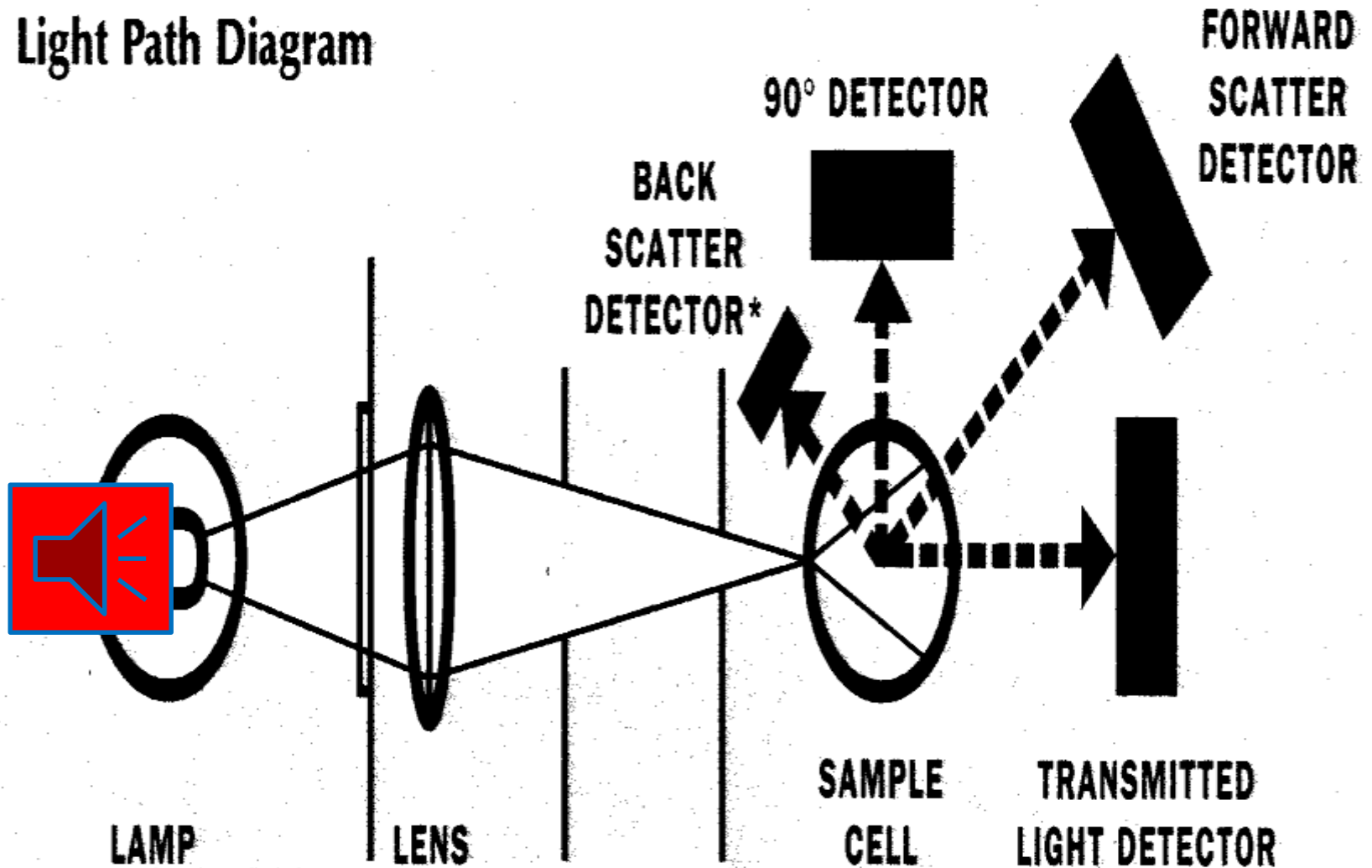
- The amount of light scattered is proportional to the concentration of insoluble particle. We will focus on the concept of light scatter
- Scattered light may be measured by
 - Turbidimetry
 - Nephelometr

Turbidimeter

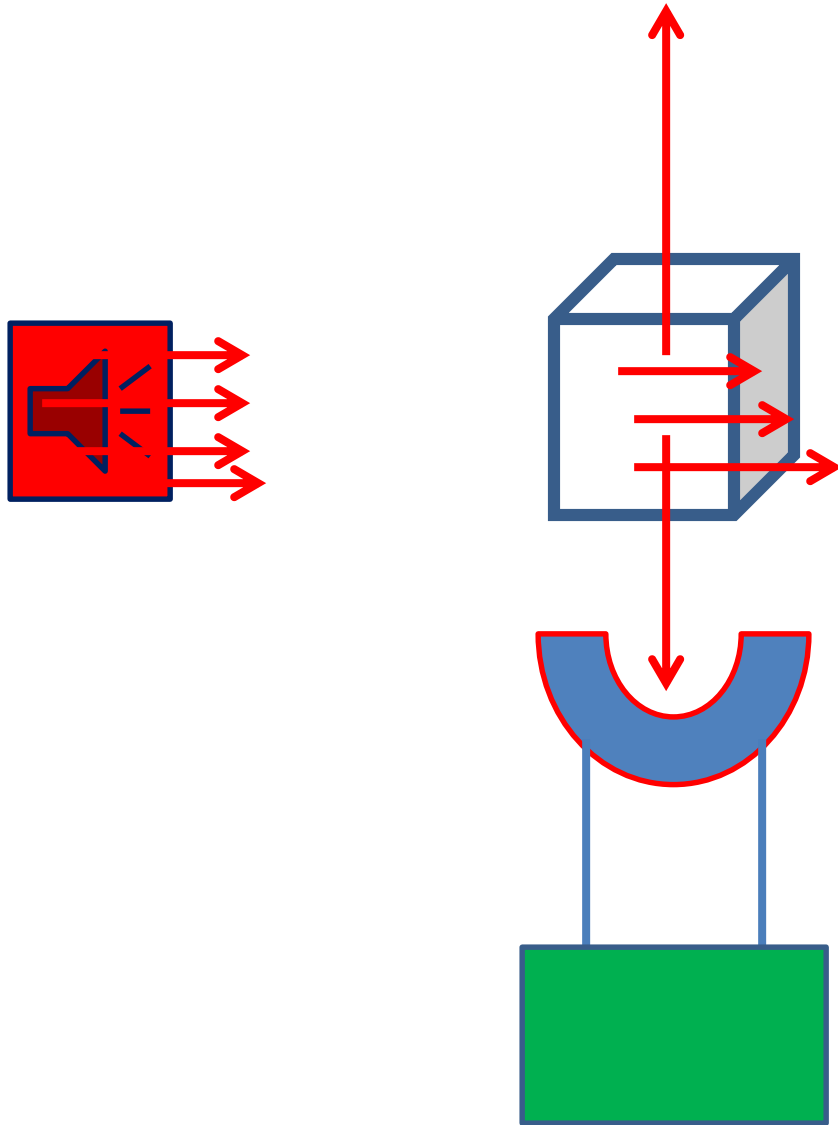


NEPHELOMETER

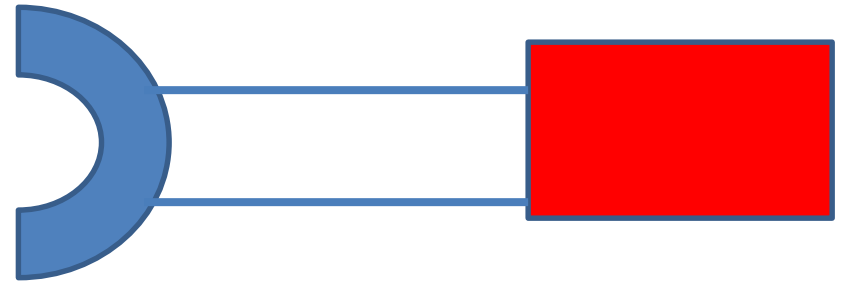
Light Path Diagram



- Light Scattering Phenomenon:
- The **blue color** of the sky and the **red color** of the sun at sunset result from scattering of light of small dust particles, H₂O molecules and other gases in the atmosphere.
- The efficiency with which light is scattered depends on its wavelength, λ .
- *The sky is blue because violet and blue light are scattered to a greater extent than other longer wavelengths.*



Nephelometry



Turbidimetry

THEORY

- Turbidimetry deals with measurement of Intensity of **transmitted light** .
- Nephelometry deals with measurement of Intensity of **scattered light**.
- Turbidometric measurements are made at 180° from the incident light beam.
- In Nephelometry, the intensity of the scattered light is measured, usually at *right angles to the incident light beam*.

Factors affecting on scattering of light:

- **Concentration of particles**
- **Particle size**
- **Wavelength**
- **Distance of observation,**
- **MW of particles**

Concentration of particles :Turbidimetry

- **Concentration of particles:** At low concentration of particles for scattering of light Beers Lamberts law is applicable.

$$S = \text{Log}_{10} \frac{I_0}{I_t}$$

$$S = KtC = -\log T$$

- **Turbidance is directly proportional to concentration**
- **i.e. $S \propto C$**
- *Where ;S = Turbidance*
- *IO= Intensity of incident light*
- *It=Intensity of transmitted radiation*
- *T=Turbidance*
- *C=Concentration of solution*
- *K=constant depend on linearity of light*

Concentration of particles :Nephelometry

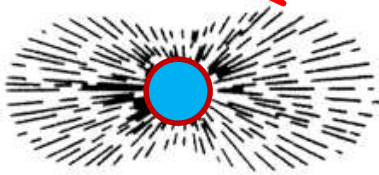
- In Nephelometry an equation that describe the relation between the intensity of scattered radiation , intensity of incident radiation , and concentration of particles
$$I_s = K_s \times I_0 \times C$$
- *Where ;*
- *I_0 = Intensity of incident light*
- *I_s = Intensity of scattered radiation*
- *K_s = It is constant which depend on suspended particle and suspension medium.*
- *C = Concentration of solution*

Particle Size

- The fraction of light scattered at any angle depends upon size and shape of particles.
- The amount of scattering (**S**) \propto proportional to square of effective radius of the particle.
- To control the particle size and shape, sample solutions and standards must be prepared under identical conditions.
- *Following care must be taken:*
 - i) *Concentrations of two ions forming ppt.*
 - ii) *Ratio of concentration of the solutions.*
 - iii) *Order of mixing of ppt.*
 - iv) *Temperature at which suspension is prepared.*

(A) Small Particles

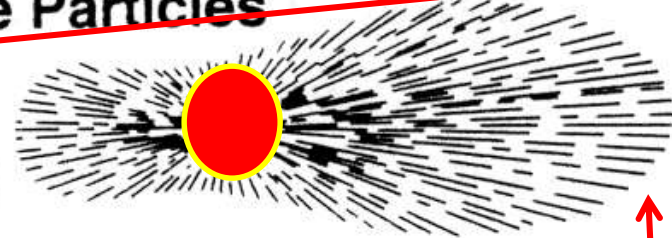
→
Incident Beam



Size: Smaller Than $\frac{1}{10}$ the Wavelength of Light
Description: Symmetric

(B) Large Particles

→
Incident Beam

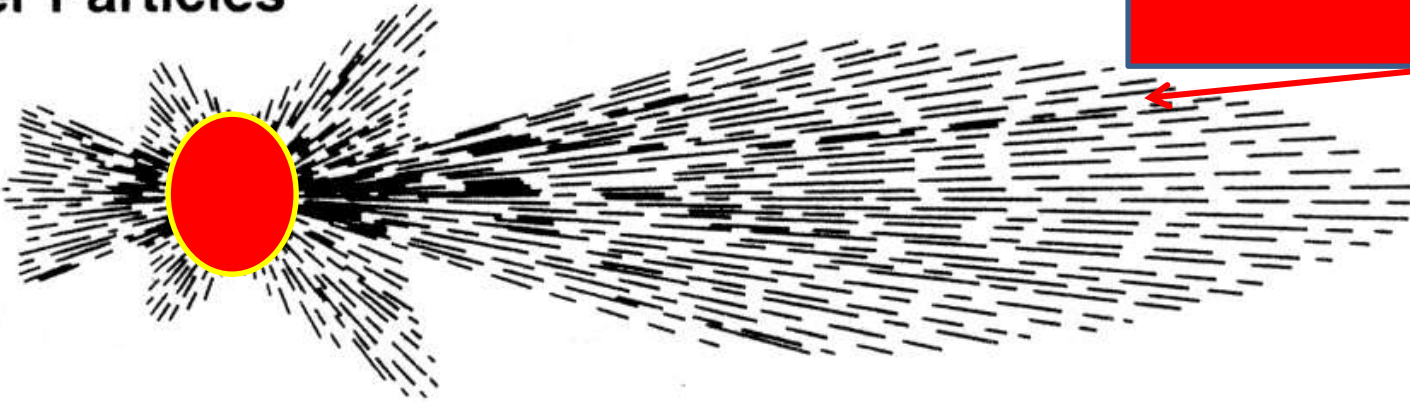


Size: Approximately $\frac{1}{4}$ the Wavelength of Light
Description: Scattering Concentrated in Forward Direction

Nephelometry

(C) Larger Particles

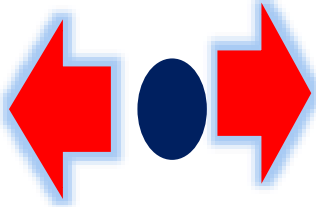
→
Incident Beam



Size: Larger Than the Wavelength of Light
Description: Extreme Concentration of Scattering in Forward Direction; Development of Maxima and Minima of Scattering Intensity at Wider Angles

Turbidimetry

- **Smaller particle (Symmetrically scattering)**



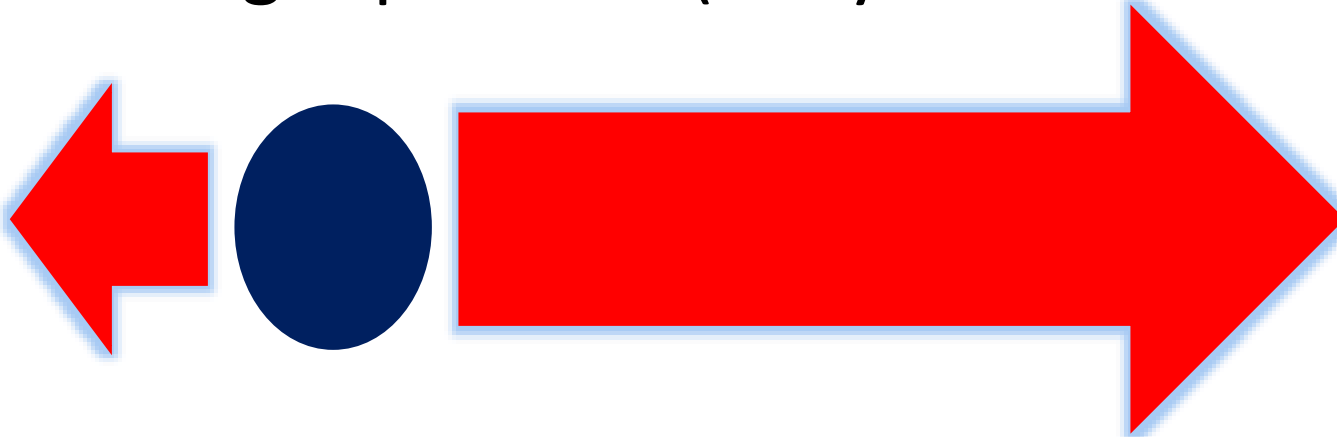
Particle size smaller $1/10$ of incident light

- Large particles (Unsymmetrical scattering)



Particle size large $1/4$ of incident light

- Larger particles (Unsymmetrical scattering)



Wavelength

- The intensity of scattered radiation depends upon wavelength of the incident light.
- Shorter wavelength are scattered to greater extent than the longer one.
- Wavelength of light is chosen in such a way that analyte solution does not absorb strongly.
- *Turbimetric & Nephelometric measurements are carried using white light.*

Molecular Weight

- Direct relationship exist

Distance of Observation

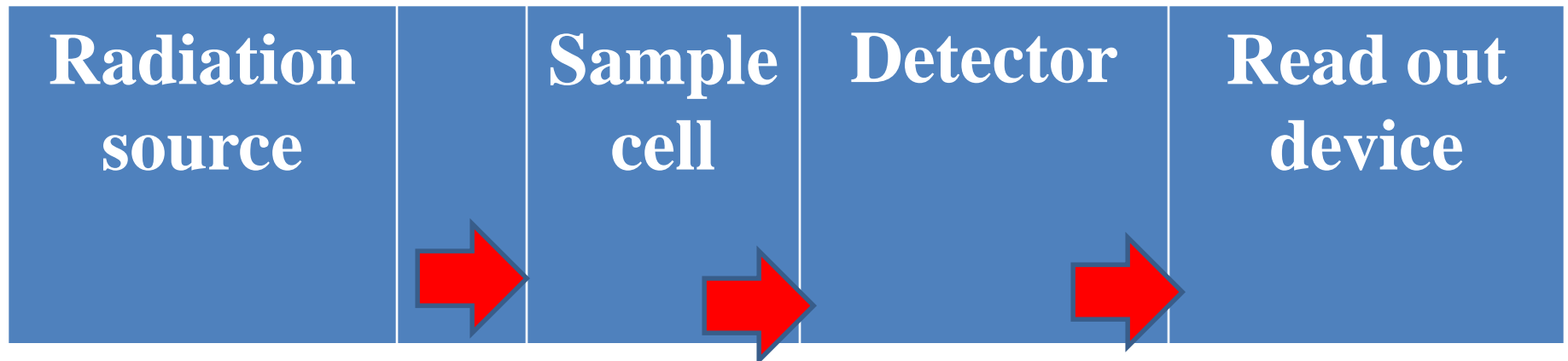
- Light scattering decrease by the distance $(r)^2$ from the light scattering particles to the detector

$$S \propto 1/r^2$$

Instrumentation:

The instrument called as Turbidimeter and Nephelometer.

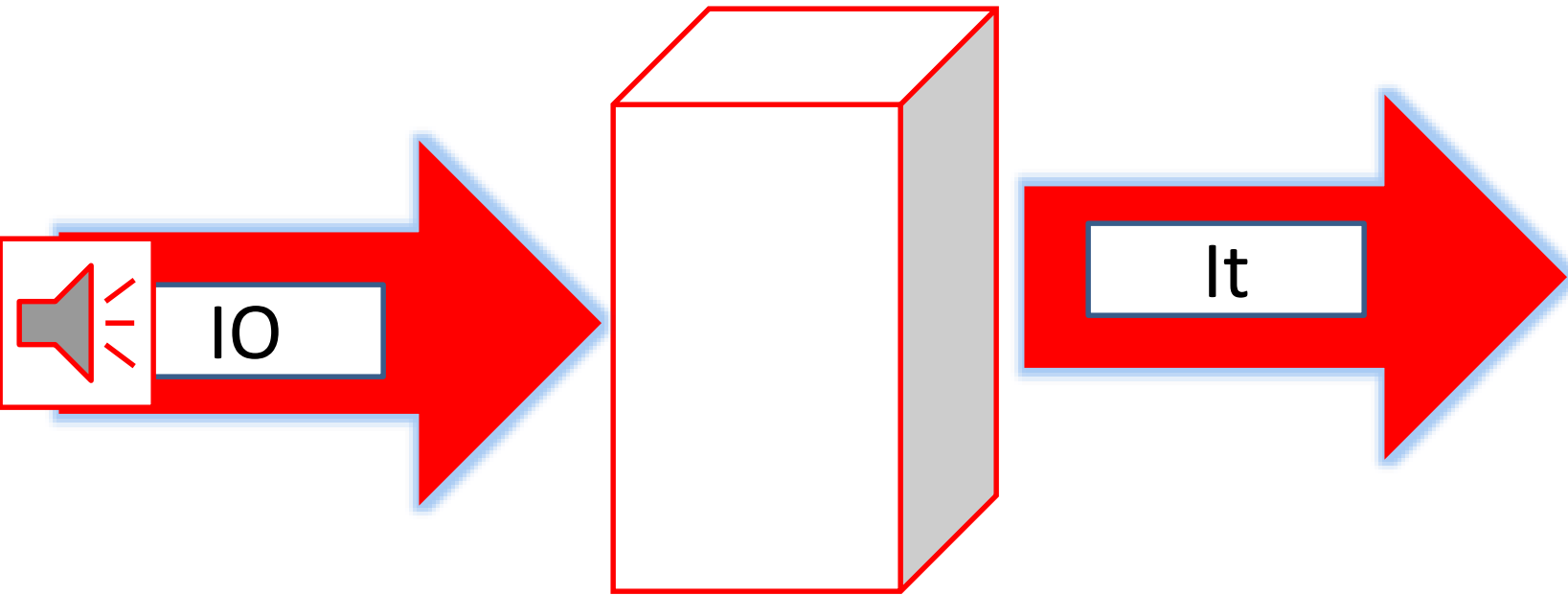
The Basic components of Instruments are



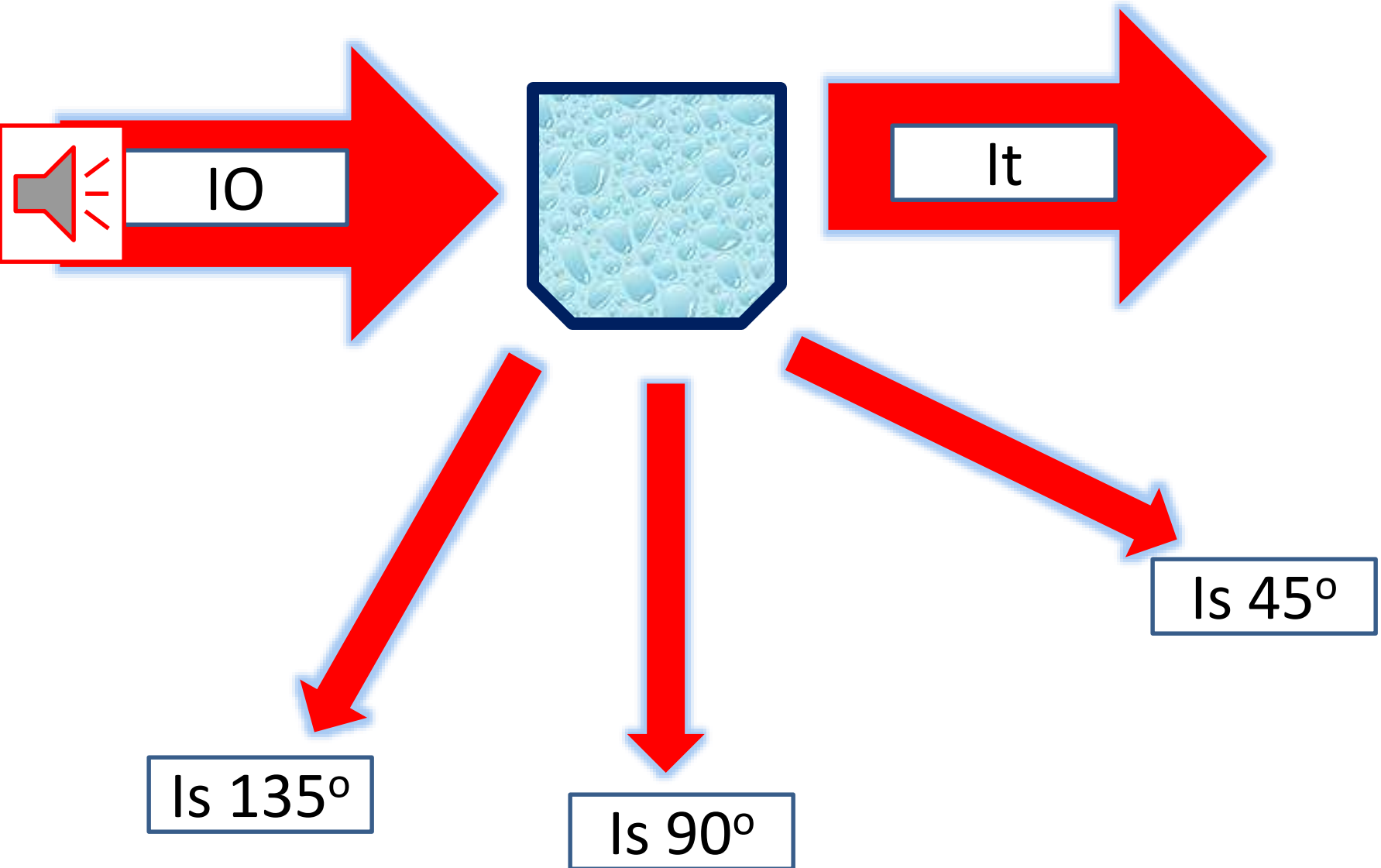
Instrumentation

- **Radiation source** : Ordinary tungsten filament lamp or mercury arc lamp can be used as source of radiation.
- **Sample cell**: The cells made from glass or plastic are used for study. Rectangular cell are used in Turbidimeter and Semi octagonal sample cell are used in Nephelometer .

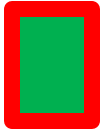
Sample Cell in Turbidimeter



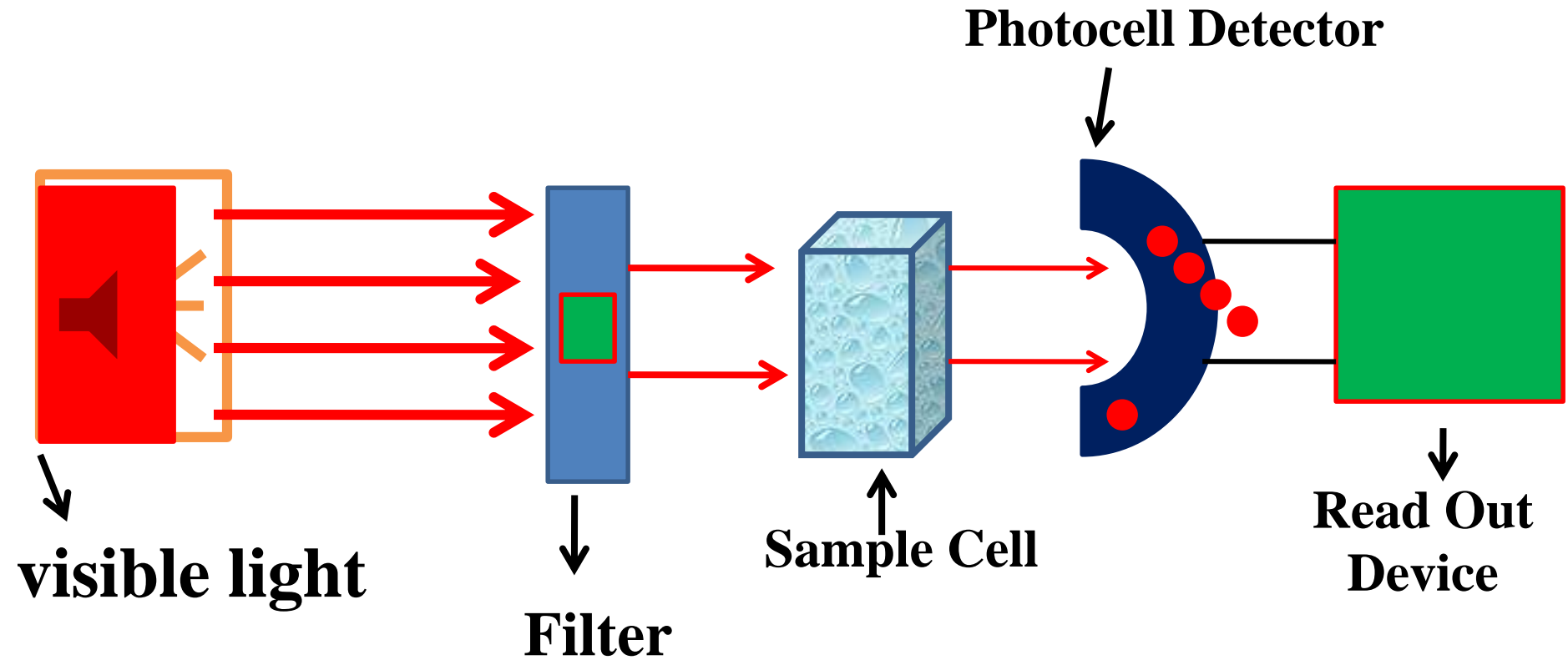
Sample Cell in Nephelometer



- **Detector: Photocell can be used as detector**



Turbidimeter



CHOICE OF THE METHOD

- Choice Of The Method depends upon the amount of light scattered by suspended particles present in solution.
- **TURBIDIMETRY** - high concentrated suspensions.
- **NEPHELOMETRY** - low concentrated suspensions - more accurate results

APPLICATIONS

Clinical Applications

- Determination of the concentration of total protein in biological fluids such as urine and CSF which contain small quantities of protein (mg/L quantities) using trichloroacetic acid
- Determination of amylase activity using starch as substrate. The decrease in turbidity is directly proportional to amylase activity.
- Determination of lipase activity using triglycerides as substrate. The decrease in turbidity is directly proportional to lipase activity.

Clinical applications of nephelometry.

- Widely used to determine concentrations of unknowns where there is antigen-antibody reactions such as
 - Determination of immunoglobulins (total, IgG, IgE, IgM, IgA) in serum and other biological fluids
 - Determination of the concentrations of individual serum proteins; hemoglobin, haptoglobin, transferrin, c-reactive protein, α 1-antitrypsin, albumin (using antibodies specific for each protein)
 - Determination of the size and number of particles (laser-nephelometry)

Limitations

- Antigen excess
- Matrix effect