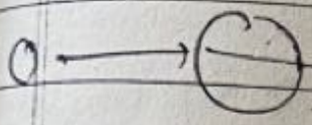
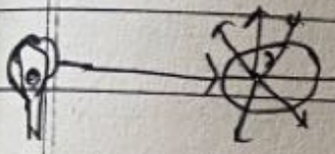
Nephelometry / Turbidimetry:

- Absorbance.



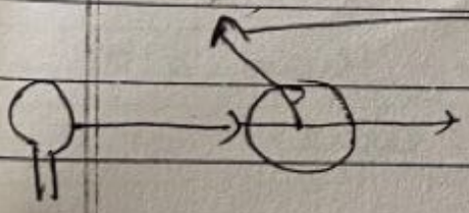
→ Measure transmittance dlt absorption of incident light

- turbidimetry



→ Measure ↓ intensity after dlt scattering

- Nephelometry



→ measure intensity of scattered light.

Turbidimetry and Nephelometry.

November 21/06

principle

Tue Radiant light when passes through a solution encounters a molecule in an elastic collision.

↓

Causes light scattering in all direction.

→ fluorescent ✱

Incident light → low λ

emitted light → high λ

scattering ↓

Incident light } same λ

emitted light }

→ Factors influencing light scattering.

① Partical size :-

Wed

Small size particle

↓

smaller than λ of incident light (1/10th of λ)

↓

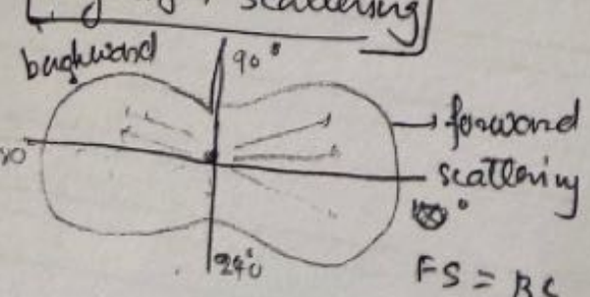
each particle is subjected to same electric field

↓

Reradiated and scattered light waves are in phase and reinforce eachother.

↓

Rayleigh scattering



FS = BS

longer size particle

↓

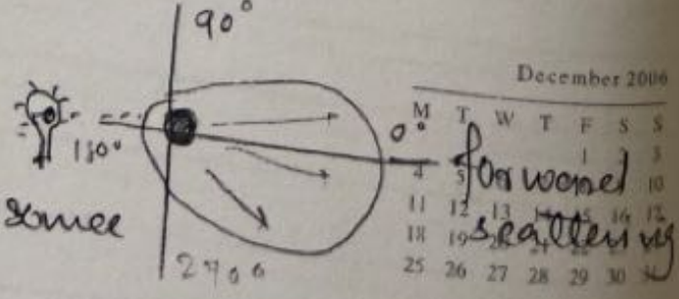
large size than λ of incident light

↓

radiated wave no longer in phase ✱

+

reinforcement in one direct destructive interference in other direct.



FS > BS.

⇒ Theory of electrophoresis

Depending on charge they carry, ionised solu move towards cathode / anode in electrophoresis system

ex → Positive ion → migrate to cathode.
Neg. ion → Anode.

- Amphalyte – having either positive or Negative (protein, NA) charge.
  ↓
  depends upon pH of solution

Acidic solutⁿ
↓
pH less than its pI
↓
becomes +ve charged
migrate towards cathod

Alkaline solⁿ
↓
pH more than its pI
↓
becomes -ve charge
↓
Anode.

→ Rate of migration:-
driving force $F = XQ = \frac{(EMF)Q}{d}$

where F = force exerted on ion.
X = current
Q = net charge
EMF = Electro motive force (voltage)
d = length of electrophoretic medium

Basic Concept:-

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- Electrophoresis
  migration of charged molecules/particles of any size in a liquid medium ↳ influence of an electric field.

- Ionophoresis / Isotachophoresis → similar term but refers to migration of small ions.

- Zone electrophoresis
  migration of charge protein molecules in porous supporting media like agarose gel film, such that each protein zone is sharply separated by zone of protein free area.

  ↓

  zones are visualized by staining ⊂ protein specific stain

  ↓

  electrophoregram

  ↓

  scanned and quantified by densitometer

Use → to seperate protein in serum, urine, CSF, other physiological fluids, RBC, tissue and nucleic acid.

→ steady acceleration of migrating ion is counter acted by resisting force;

$$F' = 6\pi r \eta v$$

where F' = resisting force
r = ionic radius of solute
η = viscosity of medium.
v = rate of migration = length travelled / sec.

$$F = F'$$

∴ resultant of 2 forces is constant velocity.

$$\therefore 6\pi r \eta v = x q.$$

$$\therefore \frac{v}{x} = \frac{q}{6\pi r \eta} = \boxed{\mu}$$

→ μ is defined as electrophoretic mobility.
↳ rate of migration / unit field strength

directly
μ α net charge.
inversely α to size of molecule and viscosity of medium.

⇒ Factors affecting rate of motility
① Net charge of molecule
② Size and shape of molecule
③ Strength of electric fei field
④ Support media properties
⑤ Ionic strength of buffer
⑥ Temp.
⑦ end osmosis

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* Electroendosmosis :-

electrophoretic support medium such as gel

↓

In contact with water, takes negatively charge caused by adsorptⁿ of hydroxyl ion.

↓

Fixed to surface and immobile

↓

Positively ions in solution

* Wick flow - movement of ~~support~~ buffer in to support medium

Passage of current throug resistive medium

↓

Heat generatⁿ

↓

evaporatⁿ of solvent from medium. (drying

↓

Draws buffer into medium

↓

Affect protein migration