Spectrophotometer:-

PAGE NO.:
DATE: / /

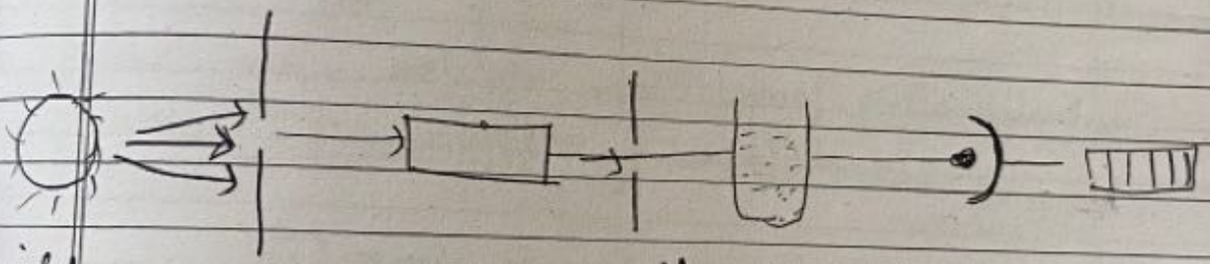
Instrument... uses isolated narrow wavelengths range of spectrum

By filter ... By prisms / grating

Filter photometer → spectrophotometer.

spectrophotometer → single beam, Double Beam.

(1) Single beam spectrophotometer:-



light source — slit — mono-chromator — exit slit — Cuvette — Detector — meter

Beam of light is passed
↓
passes through slit
↓
monochromator isolates desired region of spectrum
↓
passes through absorp° cell (Cuvette)
↓
Radiant energy is absorbed depending nature of & conc. of substance in soln.

↓

light is not absorbed is transmitted to detector

↓

Converted light energy to electrical energy.

↓

Recorded and displayed on meter.

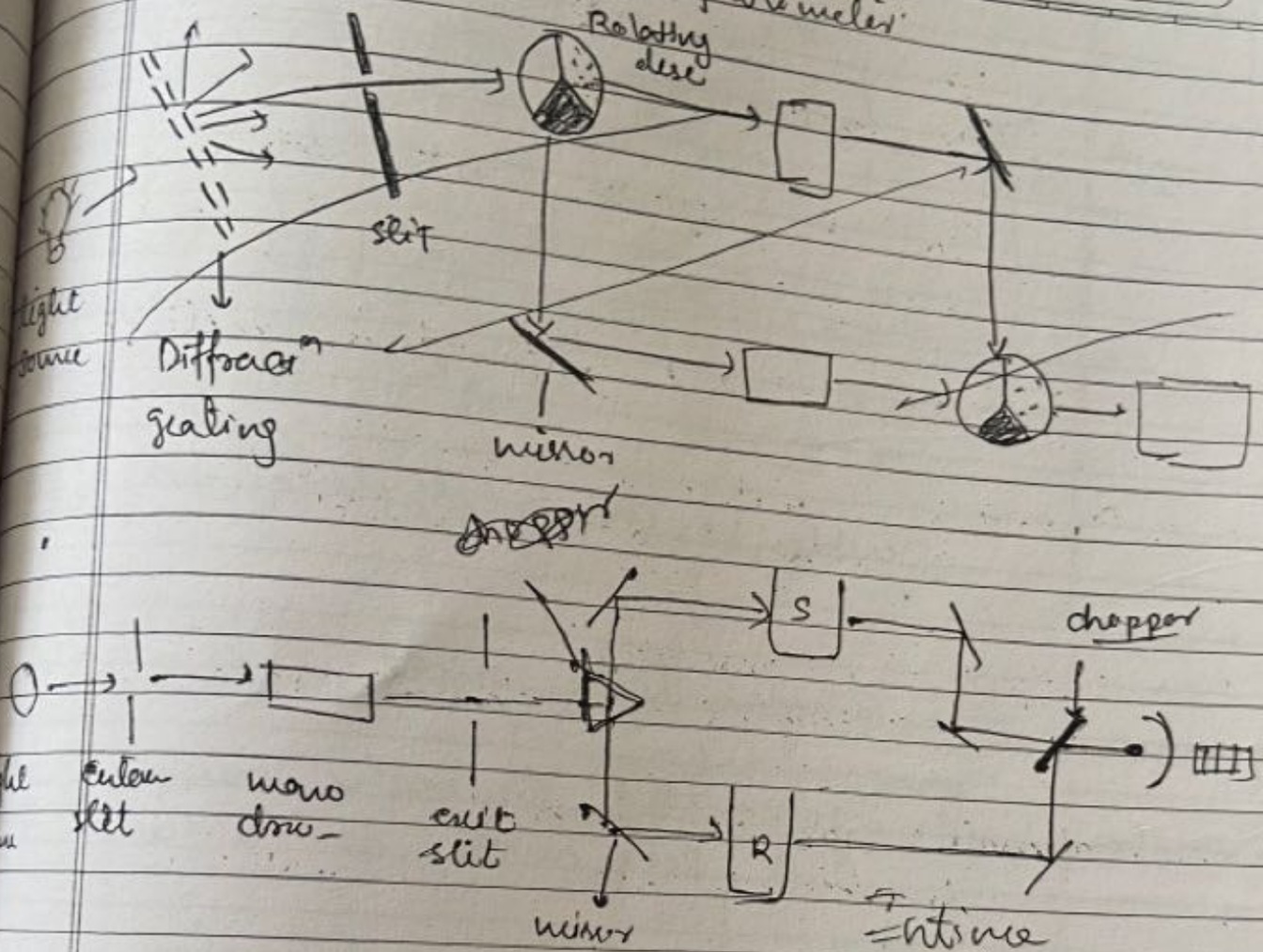
(1) Opaque block in place of cuvette
↳ No light → 0% T noted

(2) Reagent blank inserted
↳ containing no substance → 100% T noted

(3) Calibrating solutions inserted
↳ different reference T noted.

(4) Lastly unknown sample inserted
↓
Transmittance compared to all previous ref. T.

(2) Double beam spectrophotometer



chopper → a rotating wheel with alternate silver section and cut out section

↓

Pass the light transmitted from sample cuvet and ref cuvet alternately to common detector.

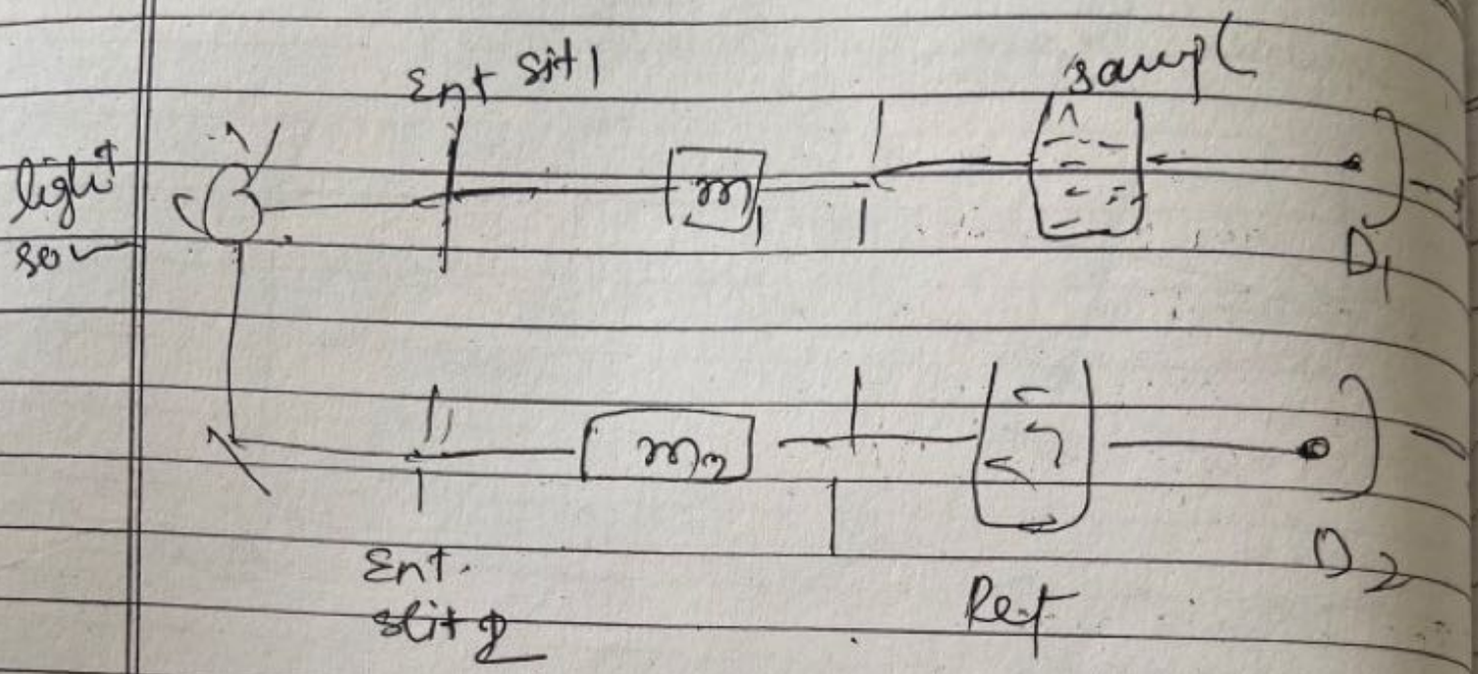
↓

In time spectrophotometer

↓

↓ light source variation and

↓ sensitivity changes in detector



Double beam spectrophot in spa

↓

Single light source

passed ↓

~~transmitted~~ to both sample and Ref. cuvet at a same ti

↓

ē use of 2 mono chromator, 2 detector

↓

fluctuation in light source b/w sample and Ref. cuvet is eleminated.

* Components of spectrophotometer

PAGE NO.:
DATE: / /

① light source
- incandescent lamp
- xenon arc lamp
- Cathod lamp.
- laser source.
- light emitting diodes.

② Spectral isolat"
- Filter
- prism
- grating

③ Cuvets

④ Photo detectors → photo multiplier tubes
- photo diodes array
- CCD (charged couple detector)

(I) Light sources :-

→ source of visible light ↓

Incandescent bulb
(Tungsten light bulb)
↓
+ low pressure halogen vapor (iodide)
ex:- quartz - halogen lamp
↓
halogen → ↑ life time of tungsten

→ Source of UV spectrum : gas discharge lamps
↙ vapor ↘ ↑ electricity to ionized
mercury lamp : Xenon/Mercury arc lamp
↙ ↘ ↓
high pressure low pressure Intense 254 nm resonance line
↓ ↓
limited used in HPLC → alternate is zinc lamp
↓
214 nm
↓
close to peptide bond absorption

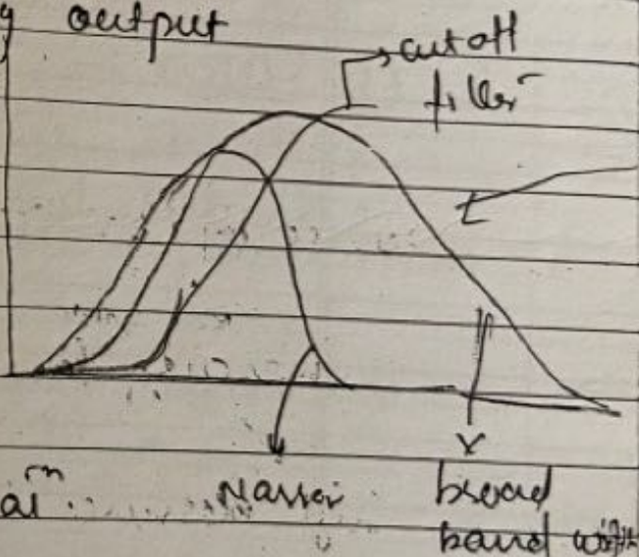
→ Laser source is light amplification by stimulated emission of radiation
↙ device that controls the way that energized atom release photon
provide intense light + narrow wavelength
different laser → diff. material used → diff ↑ light are

→ 6 properties of laser source that distinguish it from conventional source.

① spatial coherance → allows beam diameter in range of several inches.
② monochromatic light produce.
③ have pulse width that vary from micro sec to nano & pico seconds. in duration.

ex:- ① Argon ion laser
↓
produce 25 mW energy output at 480 nm

② Helium-neon
Helium-Cadmium → low cost
ease of operation



(II) Spectral Isolation:- Monochromatic devices

filter
prism
grating
} → lenses
tow lens and slits are inserted before and after monochromatic device to

↙ to render parallel rays of light

↘ to isolate narrow portion of light

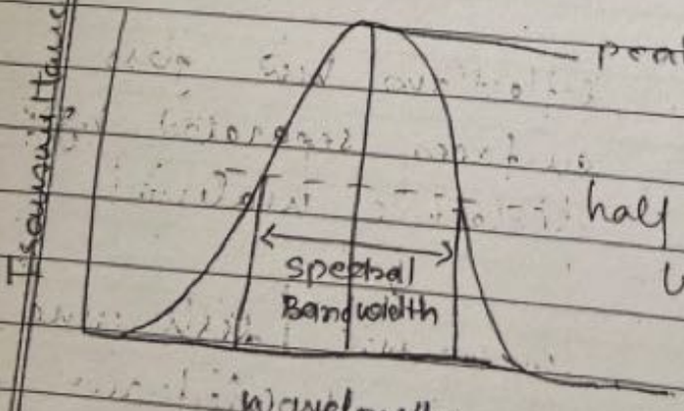
(1) Filters :-

- Simplest type of filter → thin layer of coloured glass

↓

Certain metal complexes / salts are dissolved in glass, produces colours of predominant wavelength transmitted.

- Spectral Bandwidth :



Transmittance Curve.

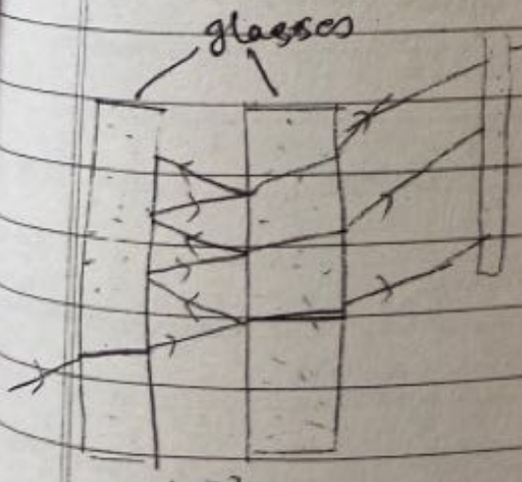
→ Spectral bandwidth is defined as width (nm) of transmittance curve at a point equal to half the peak transmittance.

- Spectral bandwidth ~~spe~~ indicate spectral purity.
  ↓
  higher the bandwidth, wider the range of wavelength transmitted.
  ↓

- wide bandpass filter - 30 nm (glass filter) ordinary
- ~~Narrow bandpass~~ - ~~5-15 nm~~.
- Interference / dichroic filter - ~~nnnn~~. 5-15 nm

⇒ other glass filter includes
- Narrow ~~band~~ pass
  ↓
  By combining ≥ 2 sharp cut-off filter
- cut off filter → sharp rise in transmittance over narrow range
  → eliminate light below given frequency.
- Interferenced Dichroic filter

- Interference / Dichroic filter:



→ to eliminate undesired wavelength
Reflections b/w glass surfaces separated by dielectric material
↓
Constructive / destructive wavelength interference
↓
Narrow spectral ~~bandwidth~~ range (5-15 nm) of wavelength is ~~passed~~ transmitted

↓
Dielectric material spacing

Reflectⁿ Refractⁿ Refractⁿ →

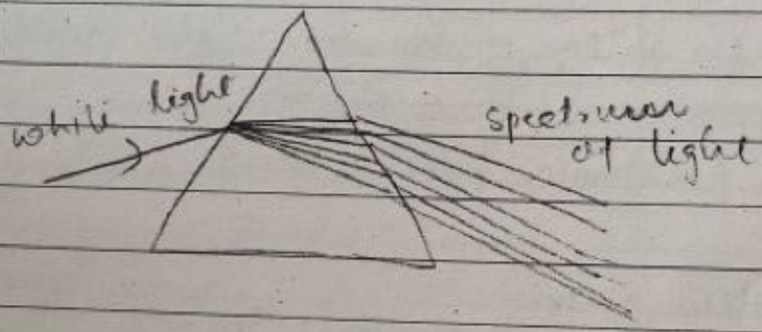
② Prism :-

↓

Separate white light into continuous spect

↓

shorter wavelength are bent / refracted more than longer wavelength



③ grating :-

→ Preparation of grating

① thin layer of ~~alu~~ aluminium - copper on glass plate (metal coating)

↓

Ruling parallel grooves on metal coating

↓

less accurate (1000 - 2000 lines/mm)

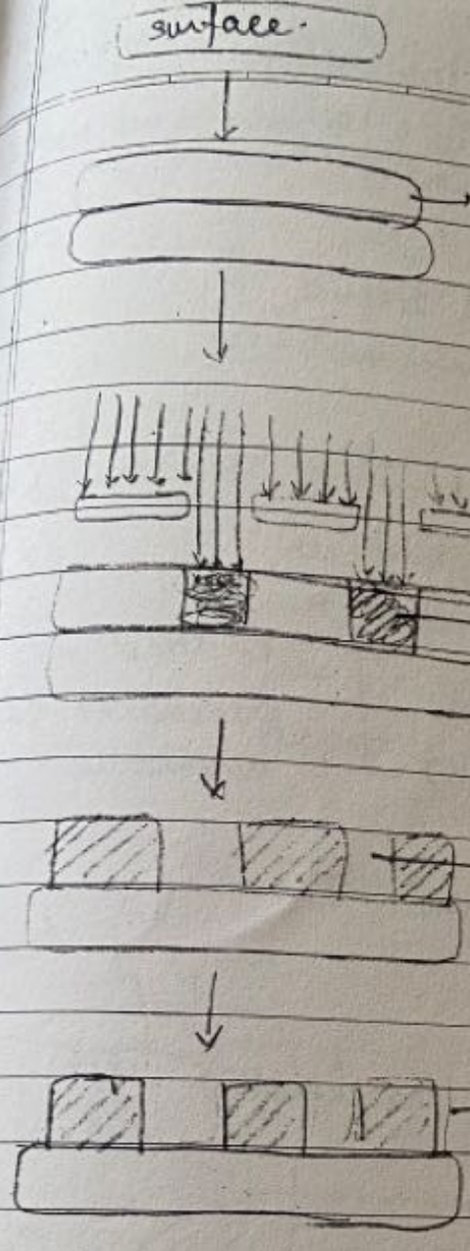
② Holographic grating using laser.

photosensitive material → called photo resist

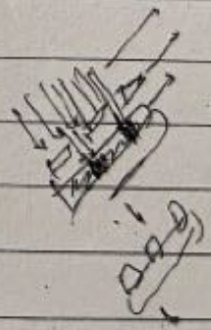
↓

applied over surface

- extremely accurate
- low light scattering



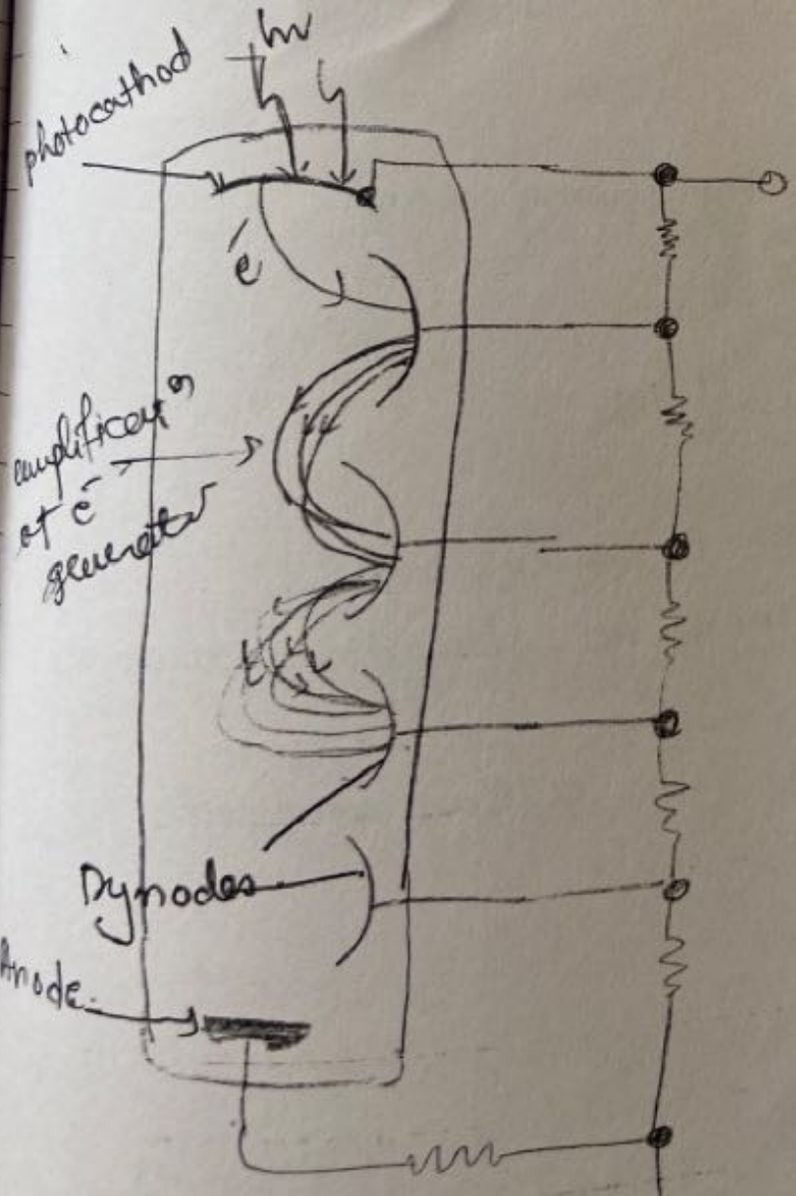
grating
↓
Illuminated
↓
reflects light
↓
gives rise to ting spectrum
↓
arrays of parelled waves formed that reinforce (or cancel ∂.
↓
Net result: uniform linear spectrum



Disadvantage → sensitive to stray light
↓
should be fully shielded.

(e) Burnt out at room temp.
(3) ~~sensitive e~~

Dark current :- when voltage is applied in absence of incident light, it produces some current → called dark current
↓
appears as background noise.
↓
should be at lowest level.



Photodiodes :-

solid photodetectors fabricated from photosensitive semiconductive materials

e.g. ① silicon
② gallium arsenide
③ lead sulfide.

↓

Absorb light over characteristic wavelength

↓

All diodes are charged ~ 5 V. and they discharge when they struck by light

↓

Sequentially scanned and recharge ~ 5 V

↓

Amt of energy required for recharging is proportional to quantity of light striking that diode.

↓

Resultant data is processed by many ways

① signal averaging
② background substraction.
③ Correction of scattered light

When photodetectors consist of 2-dimensional array of diodes they are know as photodiode array.

↓

each photodetector in array responds to specific λ.

e.g.

* Photodetector

Device that ~~converts~~ converts light into electrical signal that is proportional to number of photon striking its photosensitive surface.

(1) Photomultiplier tube
(2) Photodiodes.
(3) ~~Readout devices~~

(1) Photo multiplier Tube:
contain (1) cathod (photosensitive)
(2) ~~light sensitive~~ metal
(3) series of dynodes. (15)
] - enclosed in an evacuated glass enclosure

Photon strikes the photosensitive cathod
↓
emit electrons that are accelerated towards dynodes
↓
as electrons strikes the dynode, additional electrons are generated at each dynodes.
↓
cascading effect creats $10^5 - 10^7$ electrons for each photon hitting first cathod.
↓
this amplified signal is finaly collected (at anode → measured.

Advantage - (1) extremely rapid response time
(2) very sensitive.
(3) slow to faligue

Disadv

Dark

photoc

ampli of

An