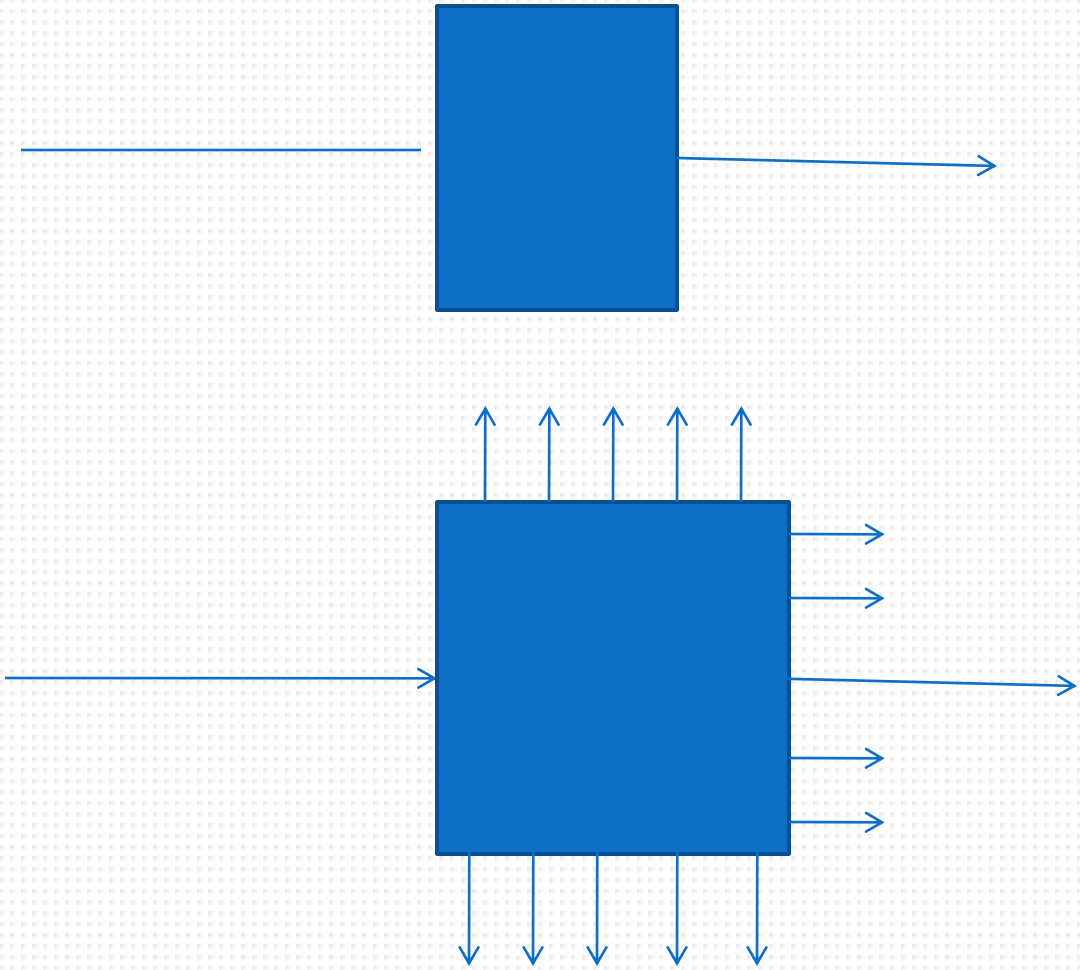
Spectrofluorimetry

Introduction

- Absorption of uv/visible radiation causes transition of electrons from ground state (low energy) to excited state (high energy).
- As excited state is not stable, excess energy is lost by
  - Collisional deactivation
  - Emission of radiation (Photo Luminescence)
- Emission Spectroscopy : emission of radiation is studied.

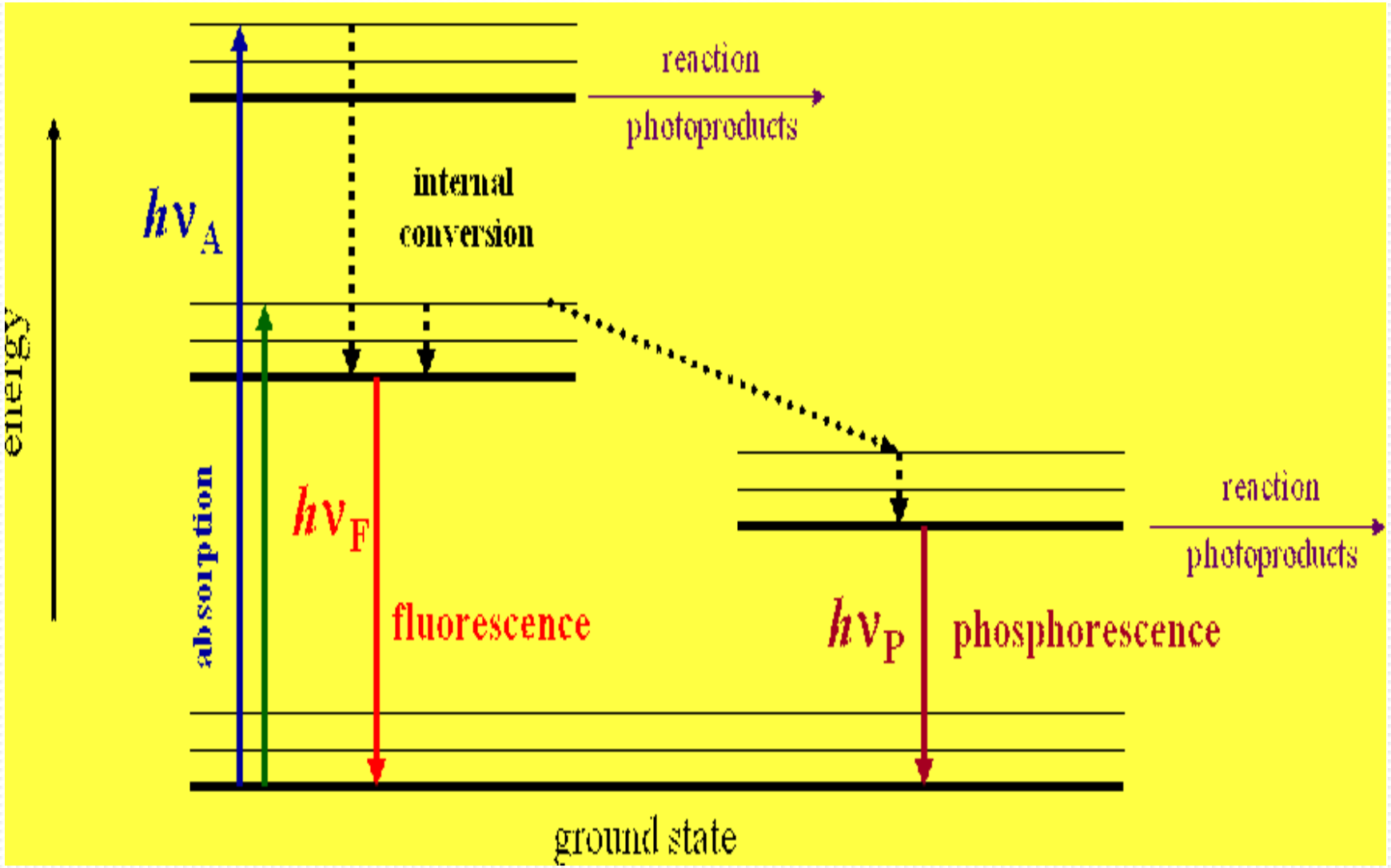


Understanding the terms……..

- Singlet ground state : state in which electrons in a molecule are paired. [↑↓]
- Singlet excited state: state in which electrons are unpaid but of opposite spins. [↑ ↓]
- Triplet state: state in which unpaired electrons of same spin are present. [↑ ↑]
- Excitation process: absorption of energy or light followed by conversion from ground state to excite state.
- Relaxation process: process by which atom or molecule losses energy & returns to ground state.

Photo Luminescence

- Light without heat or cold light
- Basically of 2 types
  - Fluorescence: part of energy is lost due to vibrational transitions and remaining energy is emitted as uv/visible radiation of longer wavelength than incident light.
  - Phosphorescence: under favorable conditions, excited singlet state undergo transition to triplet state. Emission of radiation when e^- undergo transition from triplet state to ground state.


reaction
photoproducts
internal
conversion
$h\nu_A$
energy
absorption
$h\nu_F$
fluorescence
reaction
photoproducts
$h\nu_P$
phosphorescence
ground state

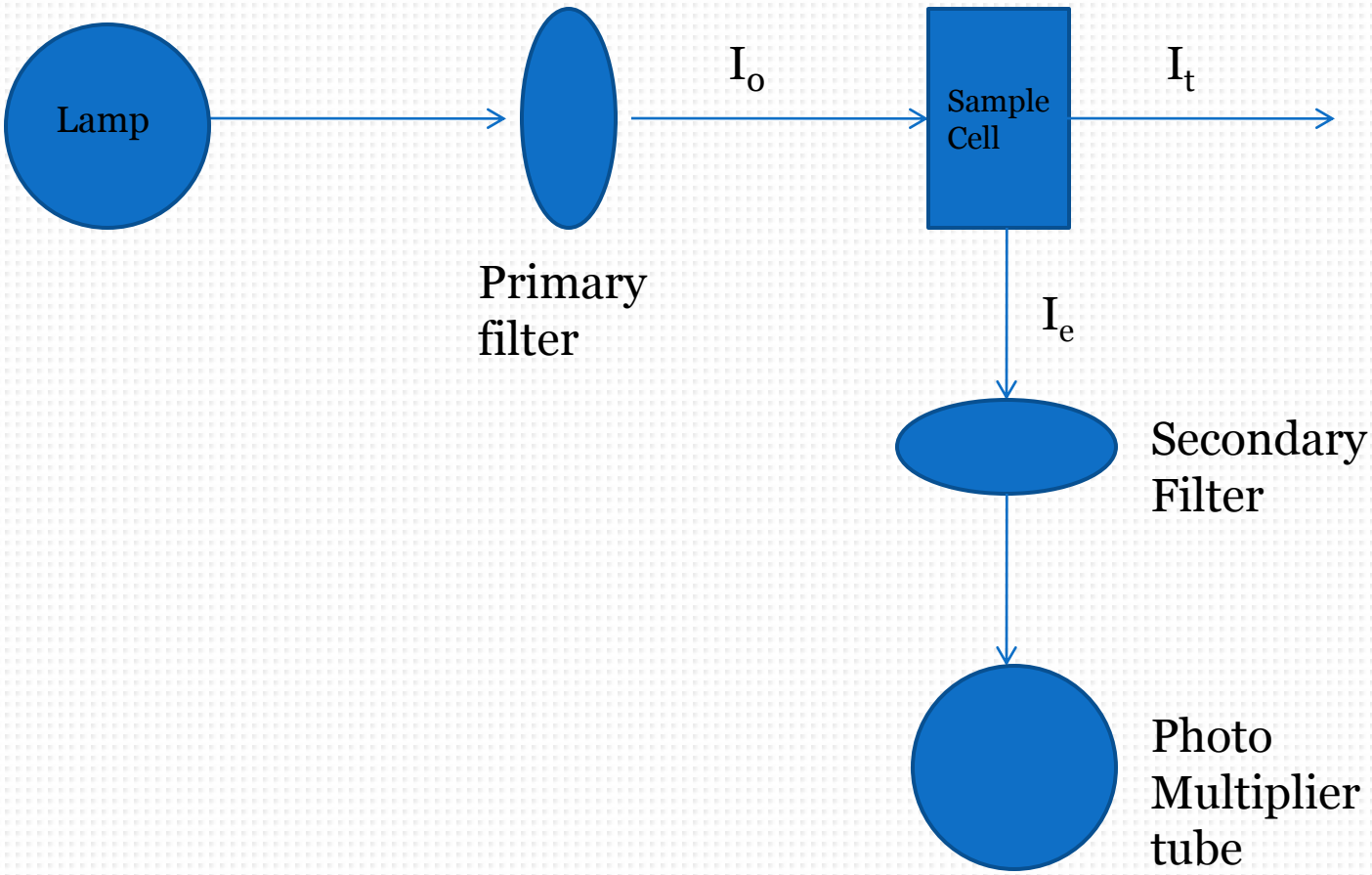
Classification

- Based on the wavelength of emitted radiation when compared to absorbed radiation
  - Stokes fluorescence: wavelength of emitted radiation is longer than absorbed radiation
  - Anti-stokes's fluorescence: wavelength of emitted radiation is shorter than absorbed radiation.
  - Resonance fluorescence: wavelength of emitted radiation is equal to that of absorbed radiation.

Actors affecting fluorescence intensity

- Conjugation: molecule must have conjugation (π electron) so that uv/vis radiation can be absorbed
- Nature of substituent groups:
  - e^- donating groups like NH_2, OH groups enhance fluorescence.
  - e^- withdrawing groups like NO_2, COOH reduce fluorescence.
- Fluorescent intensity is directly proportional to concentration.
- Increase in viscosity leads to decreased collisions of molecules there by increasing fluorescent intensity.
- More rigid the structure of molecule, more the intensity of fluorescence.
- Increase in temp leads to increased collisions b/w molecules decreasing fluorescent intensity.
- Presence of O_2 decreases the fluorescence and so de-aerated solutions must be used and compare result obtained from that of O_2 containing solution.

Instrumentation



- Source of light
  - Mercury vapour lamp : Hg vapour in high pressure (8 atm) gives intense lines on continuous background above 350nm.
  - Xenon arc lamp: gives more intense radiation.
  - Tungsten lamp: used if excitation has to be done in vis region.
- Filters and monochromators
  - In fluorimeter 1^0 filter (absorb vis radiation and transmit uv radiation) and 2^0 filter (absorb uv radiation and transmit vis radiation) are present.
  - In spectrofluorimeters, excitation monochromators and emission monochromator are present.

- Sample cells
  - Sample cells are cylindrical or polyhedral made up of colour corrected fused glass & path length normally 10mm to 1cm.
- Detectors
  - Photo voltaic cell, photo tubes or photo multiplier tubes can be used.

Advantages

- More sensitive when compared to other absorption techniques. Concentrations as low as µg/ml or ng/ml can be determined.
- Precision upto 1% can be achieved easily
- As both excitation & emission wave lengths are characteristic it is more specific than absorption methods.

Applications of Spectrofluorimetry

- Determination of Organic substances
  - Plant pigments, steroids, proteins, naphthols etc can be determined at low concentrations.
  - Generally used to carry out qualitative as well as quantitative analysis for a great aromatic compounds present in cigarette smoking, air pollutant concentrates & automobile exhausts.
- Determination of inorganic substances
- Extensively used in the field of nuclear research for the determination of uranium salts.
- Determination of vitamin B_1 (thiamine) in food samples like meat cereals etc.
- Determination of Vitamin B_2 (riboflavin). This method is generally used to measure the amount of impurities present in the sample.

- Most important applications are found in the analyses of food products, pharmaceuticals, clinical samples and natural products.
- Fluorescent indicators:
  - Intensity and colour of the fluorescence of many substances depend upon the pH of solutions. These are called as fluorescent indicators and are generally used in acid base titrations.
  - Eg: Eosin – pH 3.0-4.0 – colourless to green
  - Fluorescien – pH 4.0-6.0 – colourless to green

Thank You.....