
# College of pharmacy

## Advanced Pharmaceutical Analysis

## Fifth stage

**Dr. Maytham Ahmed**

**Lab Lecture 1**

## Introduction &demonstration to visible spectrophotometry

## Principle of Spectrophotometer

The spectrophotometer technique is to measure light intensity as a function of wavelength. It does this by diffracting the light beam into a spectrum of wavelengths, detecting the intensities with a charge-coupled device, and displaying the results as a graph on the detector and then on the display device.

1. In the spectrophotometer, a prism (or) grating is used to split the incident beam into different wavelengths.

2. By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution.

3. The spectrophotometer is useful for measuring the absorption spectrum of a compound, that is, the absorption of light by a solution at each wavelength.

# Instrumentation of Spectrophotometer

The **essential components** of spectrophotometer instrumentation include:

**1.** A table and cheap radiant **energy source**

•Materials that can be excited to high energy states by a high voltage electric discharge (or) by electrical heating serve as excellent radiant energy sources.

**2.** A **monochromator**, to break the polychromatic radiation into component wavelength (or) bands of wavelengths.

•A monochromator resolves polychromatic radiation into its individual wavelengths and isolates these wavelengths into very narrow bands.

**Prisms:**

• A prism disperses polychromatic light from the source into its constituent wavelengths by virtue of its ability to reflect different wavelengths to a different extent.

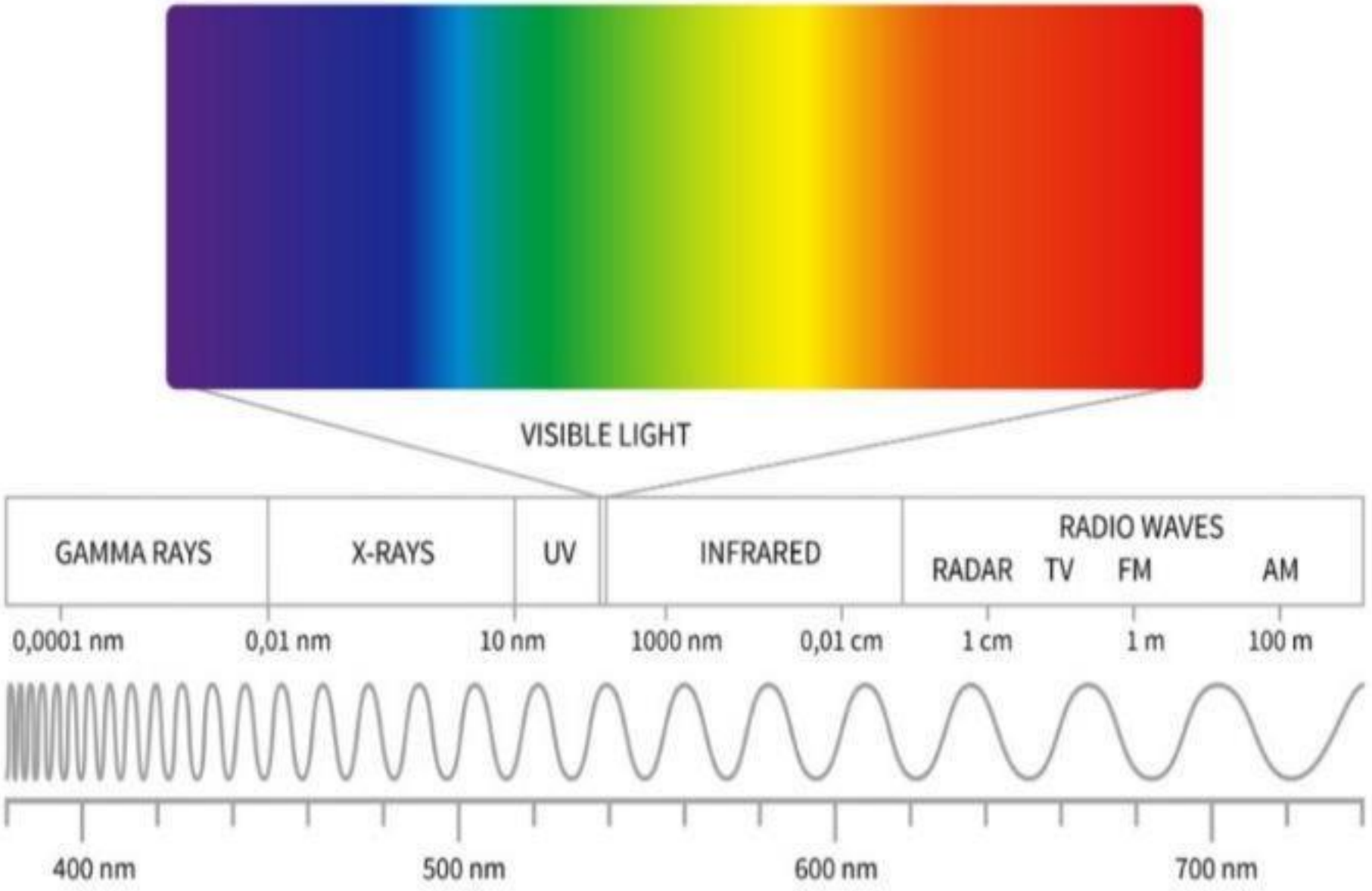
**Grating:**

• Gratings are often used in the monochromators of spectrophotometers operating ultraviolet, visible and infrared regions.

**3. Transport vessels** (cuvettes), to hold the sample

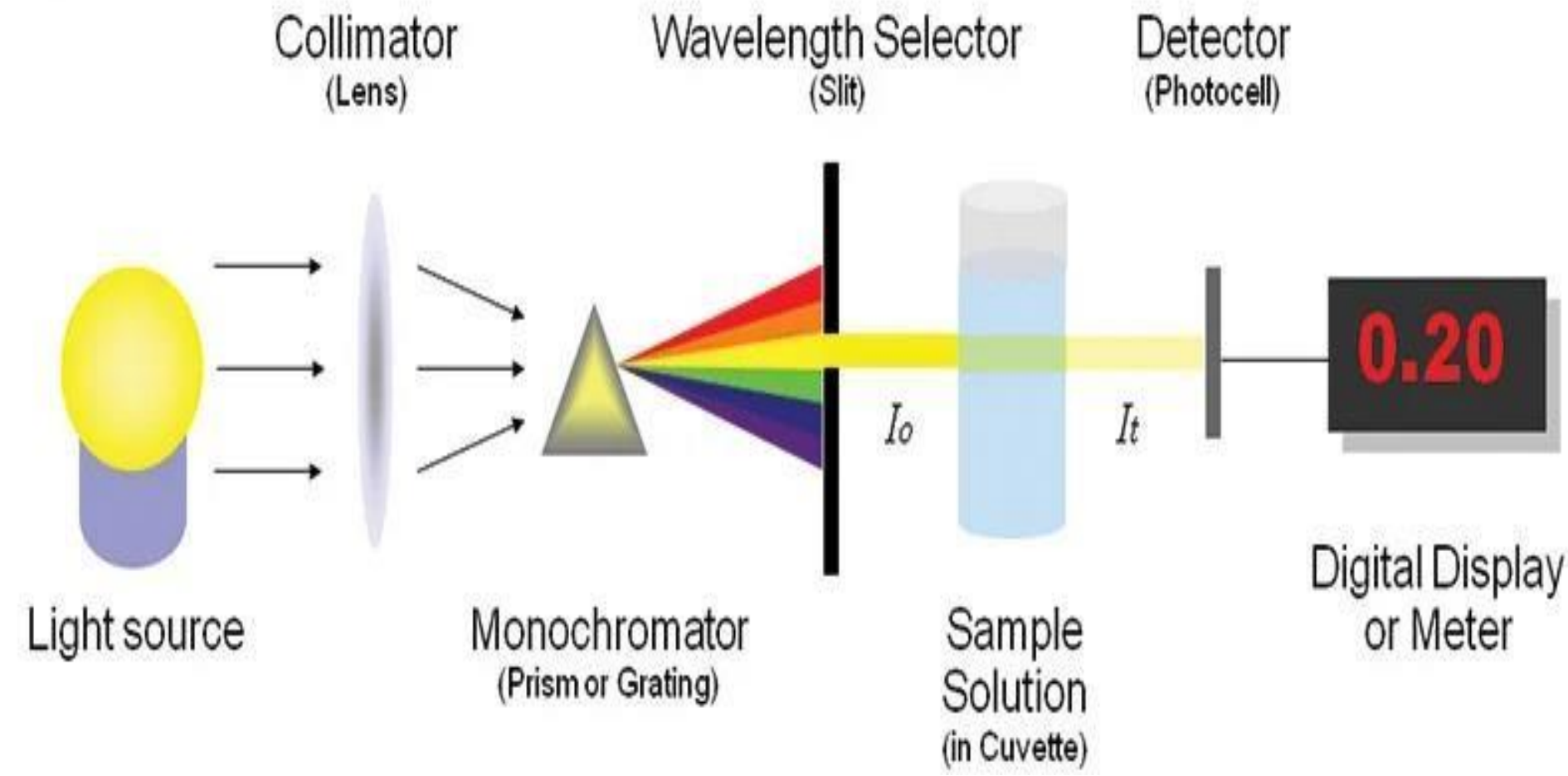
•Samples to be studied in the ultraviolet (or) visible region are usually solutions and are put in cells known as “CUVETTES”.

# VISIBLE SPECTRUM



# Spectrophotometer

## Principle, Instrumentation, Applications

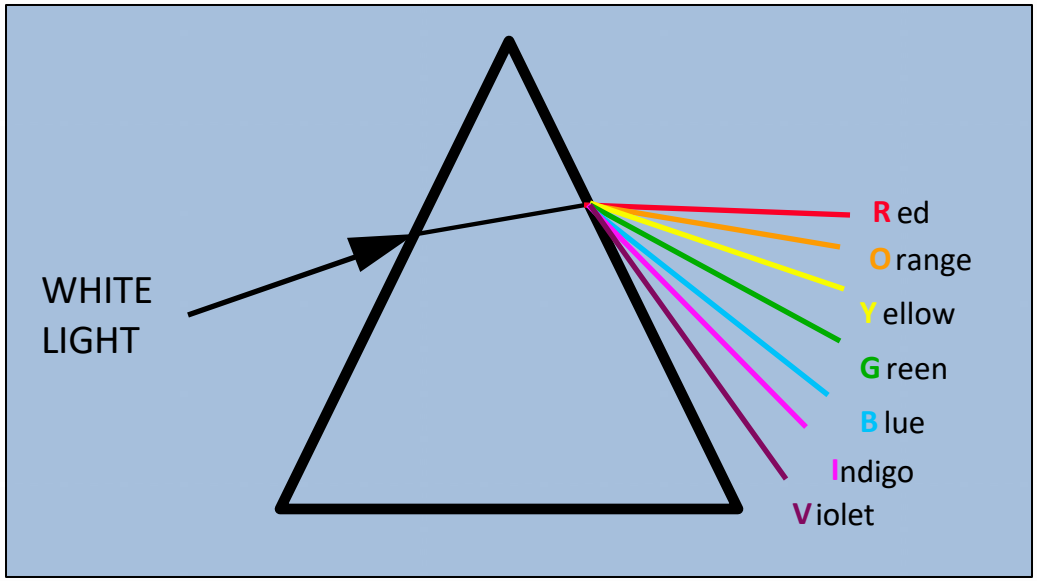


# VISIBLE SPECTROSCOPY

**COMPOSITION OF WHITE LIGHT**

- **Sunlight is white light and covers a wavelength range of 380-750nm. A simple physics experiment shows that white light is actually a composition of a range of colours i.e., light of different energies and hence wavelengths.**

**When white light falls on an object the colour detected by the eye will depend upon the ABSORPTION/REFLECTION properties of the material in the object;**




- **If the material completely REFLECTS all light it appears WHITE**
- **If the material absorbs a constant fraction of the light across the spectrum it appears GREY.**
- **If the material completely ABSORBS all the light it appears BLACK**

# Colors & Wavelengths

| COLOR | WAVELENGTH (λ in nm) |
| --- | --- |
| Violet | 380 – 435 |
| Blue | 436 – 480 |
| Greenish-blue | 481 – 490 |
| Bluish-green | 491 – 500 |
| Green | 501 – 560 |
| Yellowish-green | 561 – 580 |
| Yellow | 581 – 595 |
| Orange | 596 – 650 |
| Red | 651 – 780 |

# THE LAWS OF SPECTROPHOTOMETRY

**There are two very important basic laws and a third one which is a combination of the two.**

**LAMBERTS LAW – ABSORBANCE (A) proportional to the PATHLENGTH (l) of the absorbing medium.**

**BEERS LAW - ABSORBANCE (A) proportional to the CONCENTRATION (c) of the sample.**

**BEER- LAMBERT LAW - ABSORBANCE (A) proportional to c x l**

$$A \propto cl$$

$$A = Ecl$$ **(A is a ratio and therefore has no units)**

**The constant E is called the MOLAR EXTINCTION COEFFICIENT**

# UV / VISIBLE SPECTROSCOPY - THEORY

## IMPORTANCE OF THE BEER LAMBERT LAW

**A = Ecl** **but if E and l are constant**

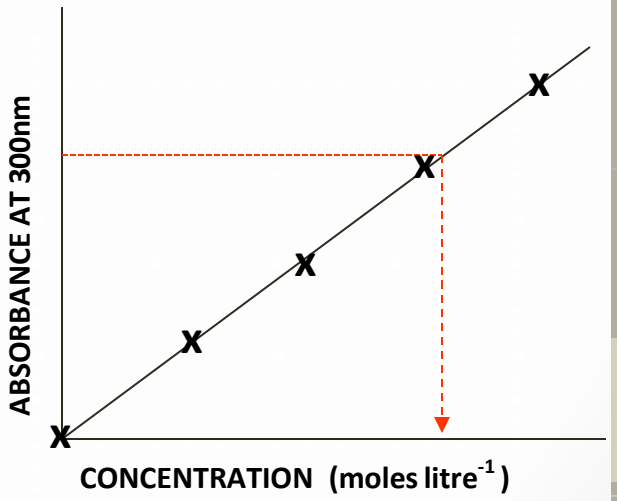
**ABSORBANCE ∝ CONCENTRATION and should be linear relationship**

**Prepare standards of the analyte to be quantified at known concentrations and measure absorbance at a specified wavelength.**

**Prepare calibration curve.**

**From measuring absorbance of sample**

**Concentration of analyte in sample can be obtained from the calibration curve**



# UV / VISIBLE SPECTROSCOPY - THEORY

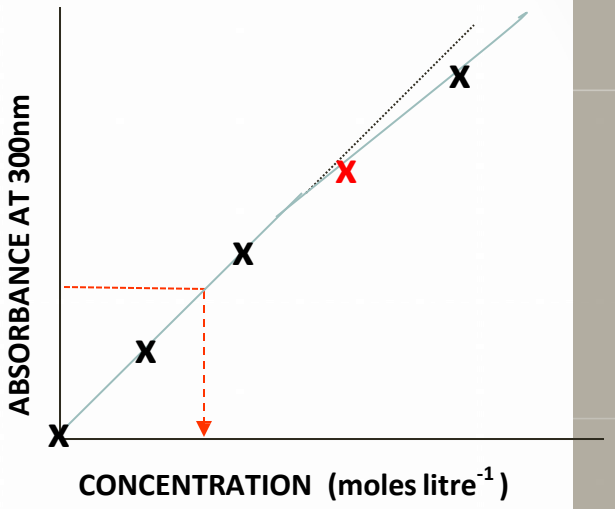
## RULES FOR QUANTITATIVE ANALYSES

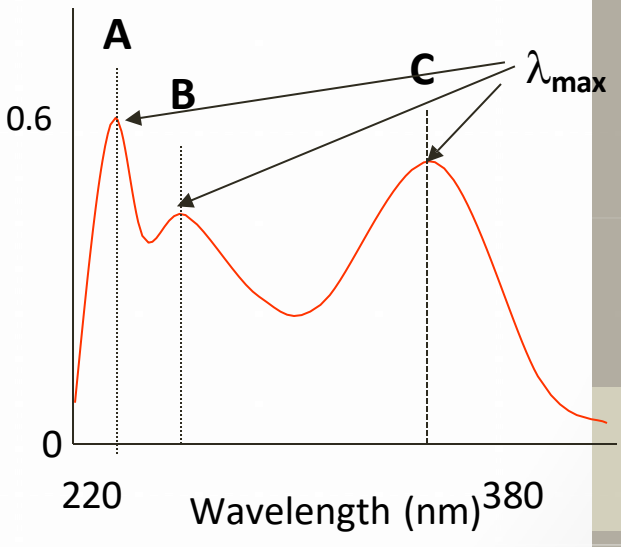
**1- At high concentrations the calibration curve may deviate from linearity – Always ensure your concentration of the sample falls within the linear range – if necessary dilute sample.**

**2- Absorbance not to exceed 1 to reduce**

**error* CHOOSE CORRECT WAVELENGTH.**

**3-Need to choose wavelength more specific to compound (SELECTIVITY) and if more than one select one with highest absorbance as this gives less error – hence use C .**

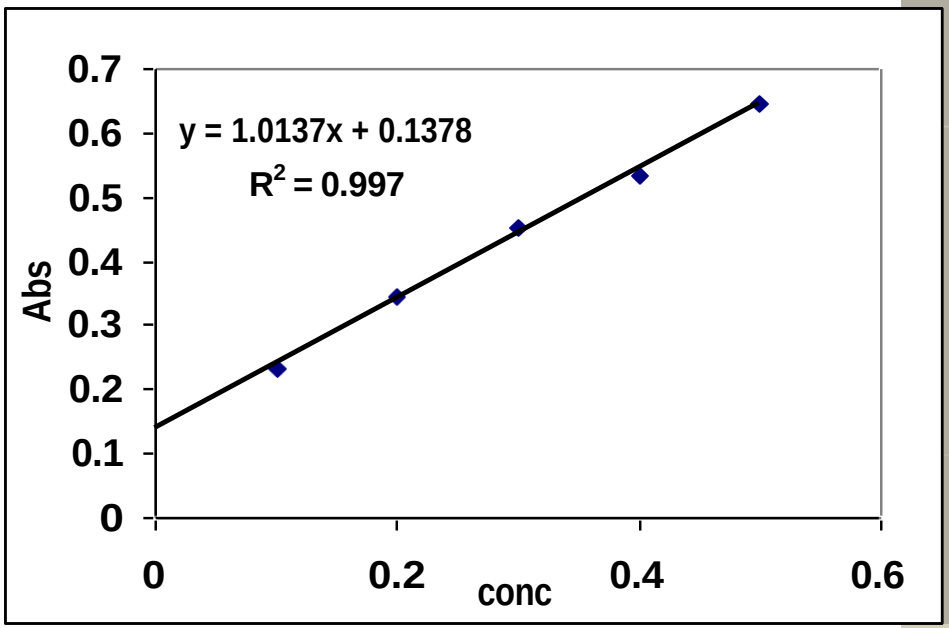


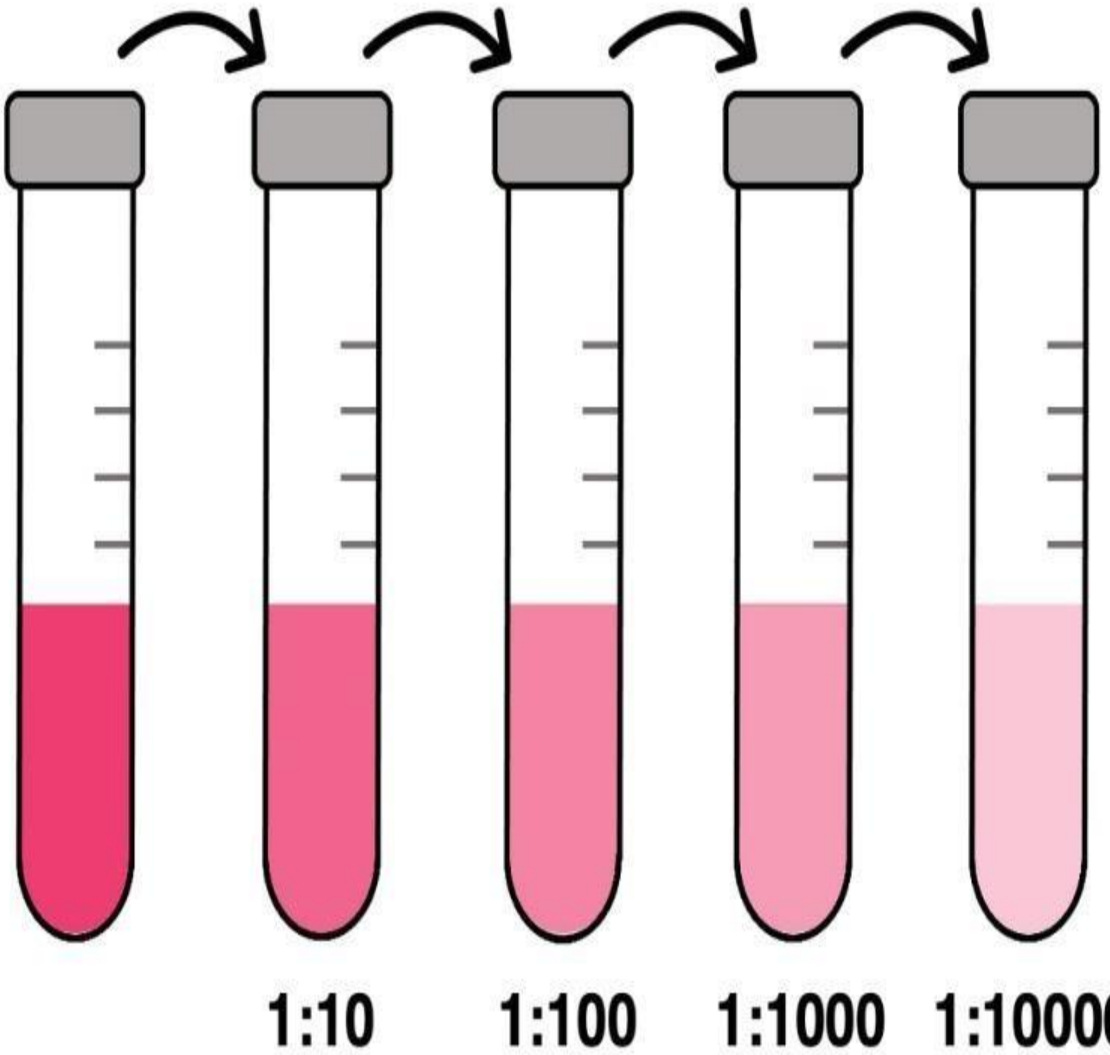
# Example of calculations for photometry

**Given the following set of data for a compound C:**
**Can you give the least square equation better fitting the curve?**
**(Conc=X, Abs=Y)**

| Conc (M) | Abs |
|---|---|
| 0.1 | 0.2322 |
| 0.2 | 0.3456 |
| 0.3 | 0.4532 |
| 0.4 | 0.5331 |
| 0.5 | 0.6453 |



# Serial Dilution



# Thanks for listening