

Spectrophotometer and Micropipettes Lab 1

By

Lec. Aseel Ghassan Daoud

What is a spectrophotometer?

- **Spectrophotometry is a tool that depends on the quantitative analysis of molecules depending on how much light is absorbed by colored compounds.**
- **It can measure the intensity of a light beam at different wavelengths.**
- **It is most commonly applied to ultraviolet, visible, and infrared radiation.**

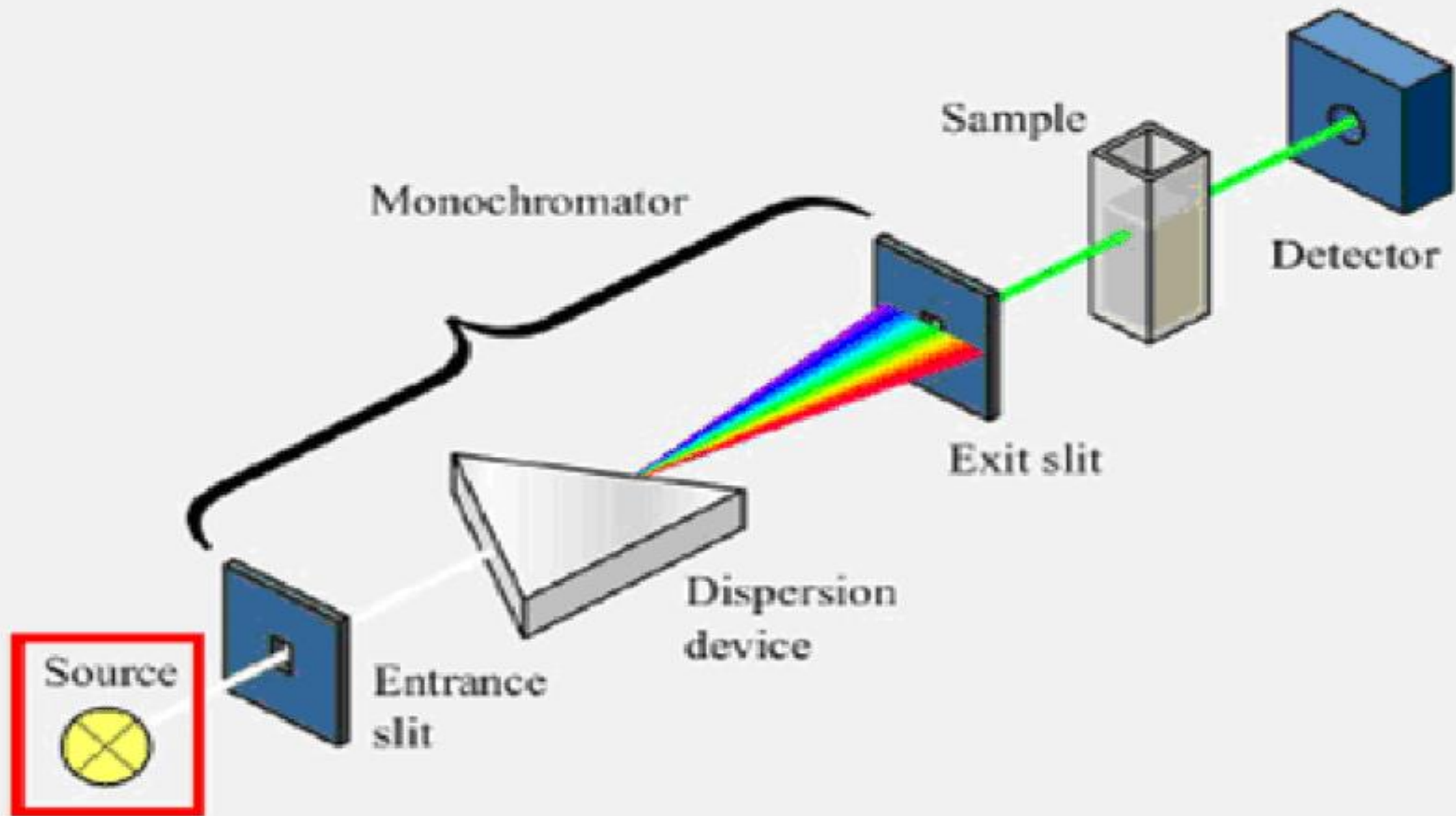
- **It can be used for the measurement of transmittance or absorbance of solutions, transparent or opaque solids, and gases.**
- **Most of biochemical materials are colored, they can absorb visible light therefore, it can be measured by colorimetric procedures**
- **Visible region 400–700 nm spectrophotometry is used extensively in colorimetry science**

- **Spectrophotometry is an important technique used in many biochemical experiments that involve DNA, RNA, and protein isolation, enzyme kinetics and biochemical analyses.**
- **A brief quantity of these materials can be sufficient to be measured by this technique.**
- **It is done by comparing the absorbency of a blank sample that does not contain a colored compound to a sample that contains a colored compound.**

Advantages:

- **It requires only micro-volumes of a substance to be measured**
- **Highly accurate and precise**
- **Very sensitive**
- **Inexpensive**
- **Simple**





Micropipettes:







Working Procedure (example):

- 1. Bring reagents and samples (serum) to room temperature.**
- 2. Pipette into labeled test tubes**

Tubes	Blank	Sample	Standard
Working reagent R	1 ml	1 ml	1 ml
Sample	-	50 μ	-
Standard	-	-	50 μ

3. Mix, and let stand the tubes for 10 min. at room temperature or 5 min. at 37 °C

4. Read the absorbance (A) of the sample at a given wavelength ex: 550 nm.

Calculations:

A (sample) * concentration of standard (known) mg/dL = conc. of sample mg/dL

A (standard)

THANK YOU

