TISHK INTERNATIONAL UNIVERSITY
FACULTY OF APPLIED SCIENCE
DEPARTMENT OF NUTRITION AND DIETETICS

NUTRITIONAL BIOCHEMISTRY II/ LAB
2ND GRADE

SPECTROPHOTOMETRY

ASST. LECT. : AMANI TAHSIN

OUTLINES

- SPECTROPHOTOMETRY DEFINITION
- INTRODUCTION
- TYPES OF SPECTROPHOTOMETER
- DEVICES AND MECHANISM
- BEER-LAMBERT LAW

SPECTROPHOTOMETRY

Is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution.

INTRODUCTION

- Every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation) over a certain range of wavelength.
- Spectrophotometry is a measurement of how much a chemical substance absorbs or transmits.
- Spectrophotometry is widely used for quantitative analysis in various areas (e.g., chemistry, physics, biology, biochemistry, material and chemical engineering, clinical applications, industrial applications, etc).
- Any application that deals with chemical substances or materials can use this technique.

CONT.

- The basic principle is that each compound absorbs or transmits light over a certain range of wavelength.
- This measurement can also be used to measure the amount of a known chemical substance.
- In biochemistry, for example, it is used to determine enzyme-catalyzed reactions.
- In clinical applications, it is used to examine blood or tissues for clinical diagnosis.

SPECTROPHOTOMETER

Is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution.

- With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected.

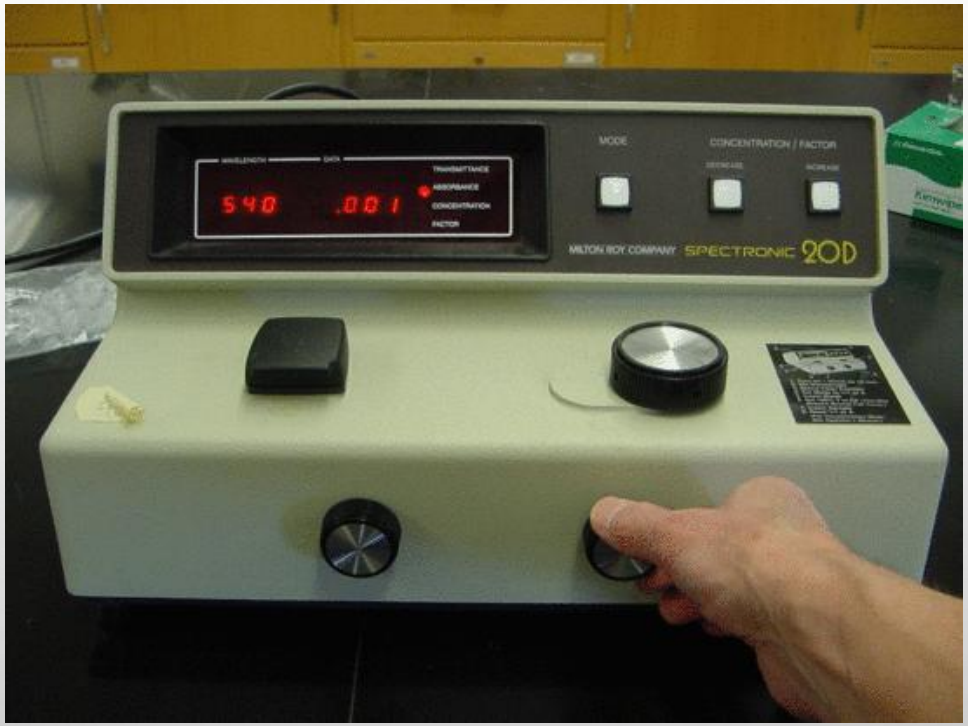
USES OF SPECTROPHOTOMETER

- To determine the absorbance or transmission of characteristic wavelengths of radiant energy (light) by chemical species in a solution.
- Identify organic compounds by determining the absorption maximum.
- Used for color determination within the spectral range.

TYPES OF SPECTROPHOTOMETER

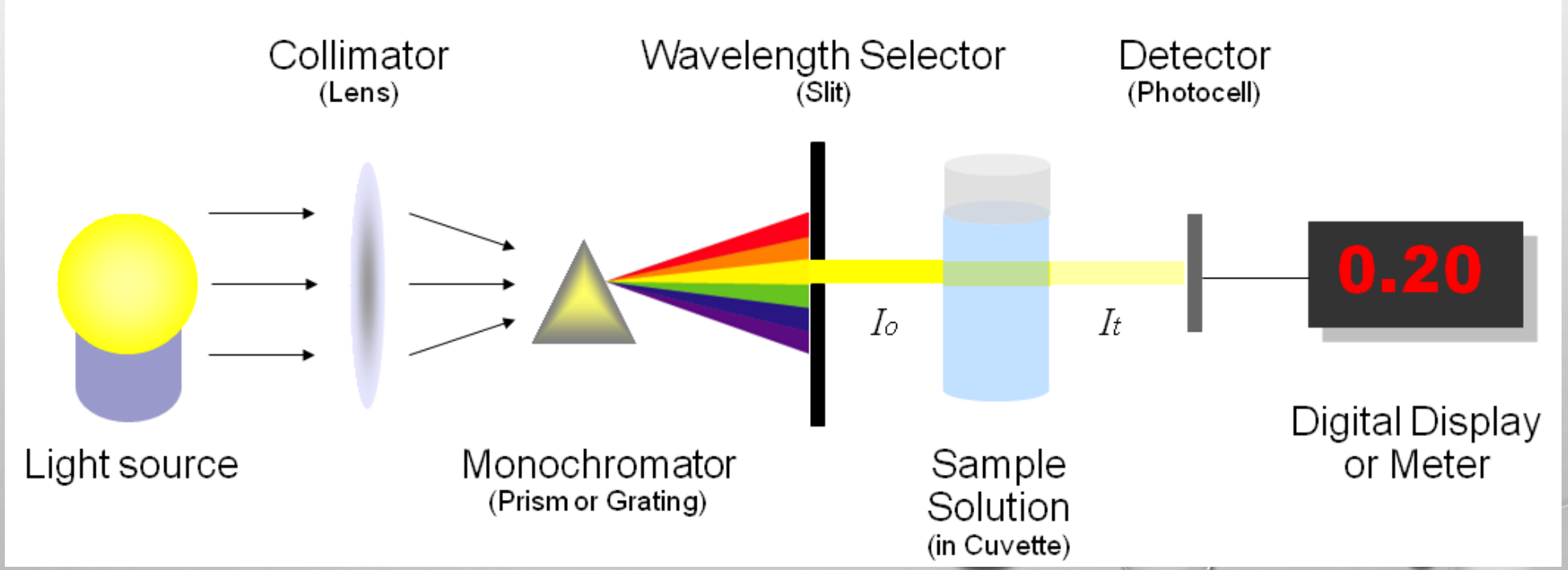
- There are also several variations of the spectrophotometry such as *Atomic Absorption Spectrophotometry* and *Atomic Emission Spectrophotometry.*
- Depending on the range of wavelength of light source, it can be classified into two different types:
- **UV-visible spectrophotometer:** uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.
- **IR spectrophotometer:** uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum.

A SINGLE WAVELENGTH SPECTROPHOTOMETER



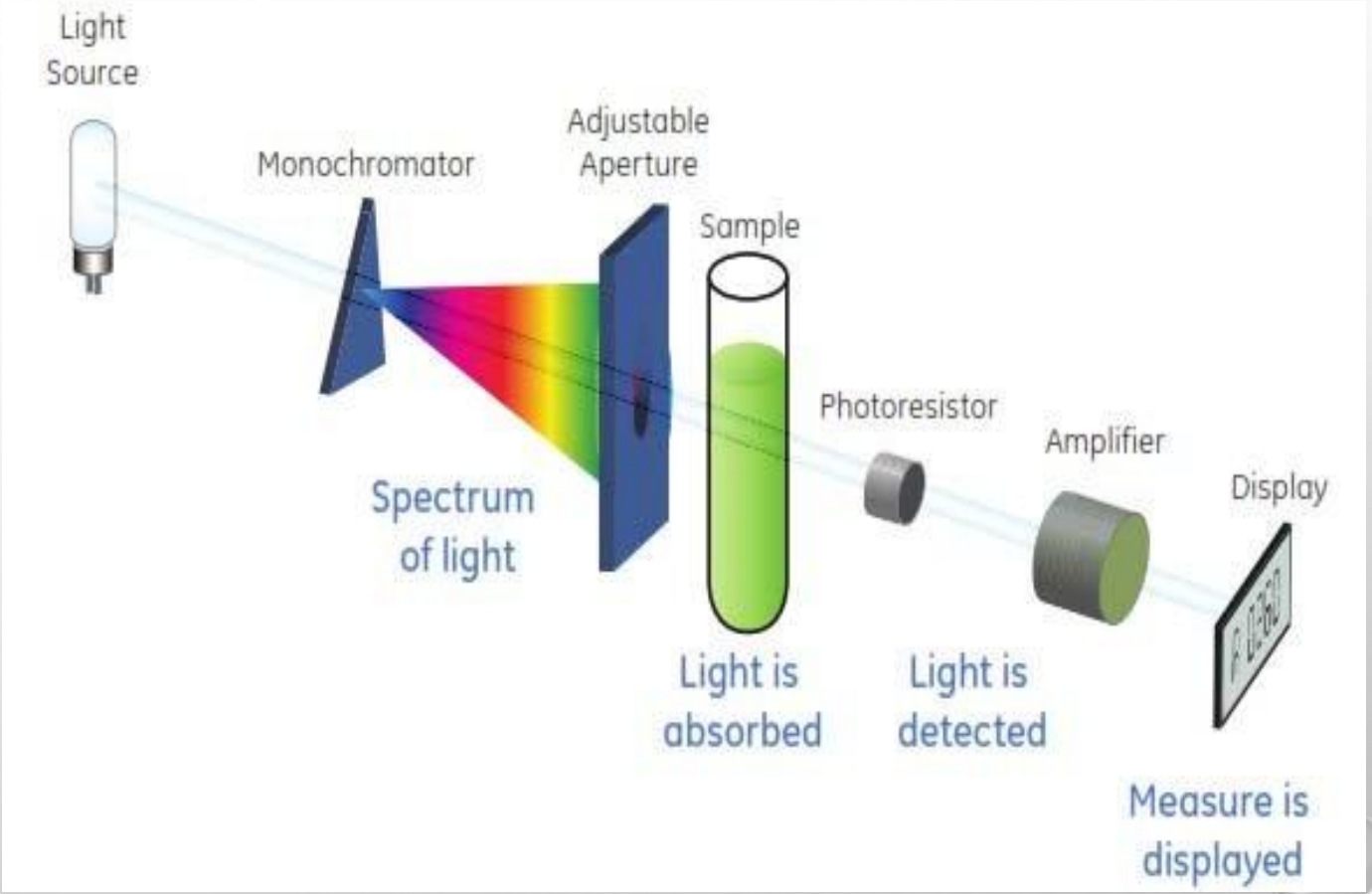
DEVICES AND MECHANISM

The basic structure of spectrophotometers is illustrated below;



It consists of a;

- Light source
- Collimator
- Monochromator
- Wavelength selector
- Cuvette (for sample solution)
- Photoelectric detector
- Digital display (a meter).



CONT.

A spectrophotometer, in general, consists of two devices;

- A spectrometer: Is a device that produces, typically disperses and measures light.
- A photometer: Indicates the photoelectric detector that measures the intensity of light.

MECHANISM

- **Spectrometer**: It produces a desired range of wavelength of light. First a collimator (lens) transmits a straight beam of light (photons) that passes through a monochromator (prism) to split it into several component wavelengths (spectrum). Then a wavelength selector (slit) transmits only the desired wavelengths, as shown in figure 1.
- **Photometer**: After the desired range of wavelength of light passes through the solution of a sample in cuvette, the photometer detects the amount of photons that is absorbed and then sends a signal to a galvanometer or a digital display, as illustrated in figure 1.

The amount of photons that goes through the cuvette and into the detector is dependent on the length of the cuvette and the concentration of the sample. Once you know the intensity of light after it passes through the cuvette, you can relate it to transmittance (T).

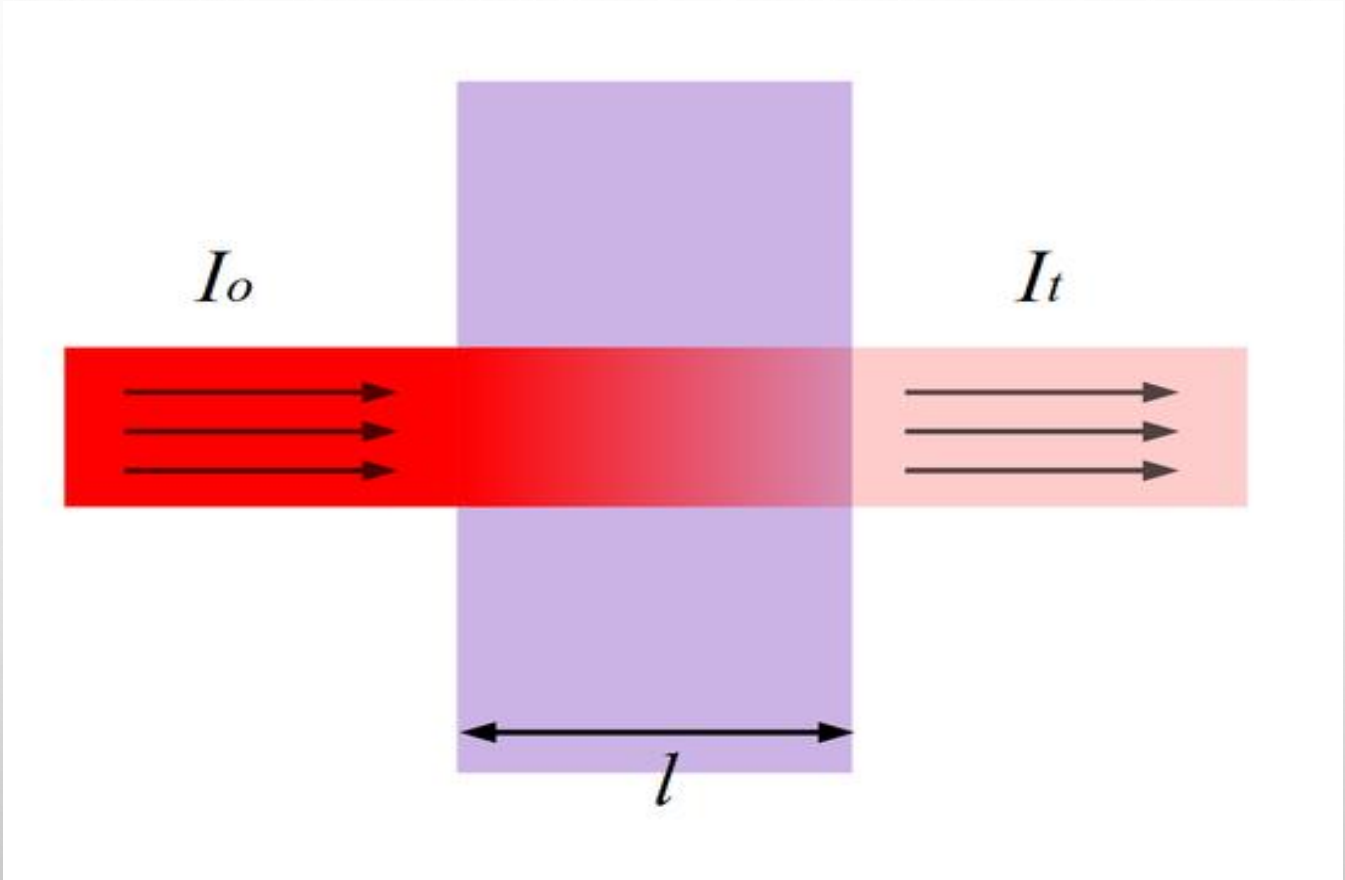
Transmittance is the fraction of light that passes through the sample. This can be calculated using the equation: $\text{Transmittance}(T) = I_t / I_o$

Where It is the light intensity after the beam of light passes through the cuvette and Io is the light intensity before the beam of light passes through the cuvette.

Transmittance is related to absorption by the expression:

$$Absorbance(A) = -log(T) = -log(\frac{I_t}{I_o})$$

Where absorbance stands for the amount of photons that is absorbed. With the amount of absorbance known from the above equation, you can determine the unknown concentration of the sample by using beer-lambert law.


I_o
I_t
l

BEER-LAMBERT LAW

Beer-lambert law (also known as beer's law) states that there is a linear relationship between the absorbance and the concentration of a sample. For this reason, beer's law can *only* be applied when there is a linear relationship. Beer's law is written as:

$$A = \epsilon l c$$

where

- A is the measure of absorbance (no units),
- ϵ is the molar extinction coefficient or molar absorptivity (or absorption coefficient),
- l is the path length, and
- c is the concentration.

HOMEWORK

- RELATIONSHIP BETWEEN %TRANSMITTANCE AND LIGHT PATH LENGTH AND CONCENTRATION