

The previous equation is the **heart of spectrophotometry** as applied to analytical chemistry, it is called Beer-Lambert law or simply **Beer's law**

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1- Concentration of the analyte is given in unit mol/L (M)

2-The path length, b, in cm

3- $\epsilon$ , is called the molar absorptivity or molar absorption coefficient

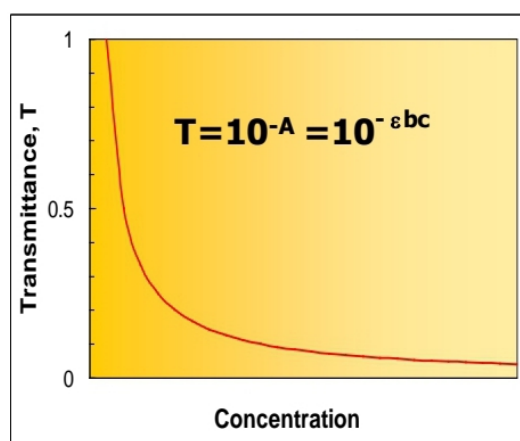
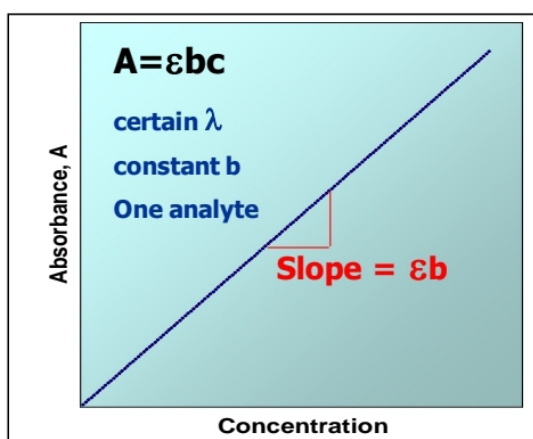
**"Absorbance of 1 M solution measured in a cell of 1 cm path length"**

$$\epsilon = \frac{A}{bc} = \frac{1}{\frac{\text{mol}}{\text{L}} \text{ cm}} = \text{L mol}^{-1} \text{ cm}^{-1} = \text{M}^{-1} \text{ cm}^{-1}$$

$\epsilon$ , is characteristic for each substance at a particular wavelength,  $\lambda$ .

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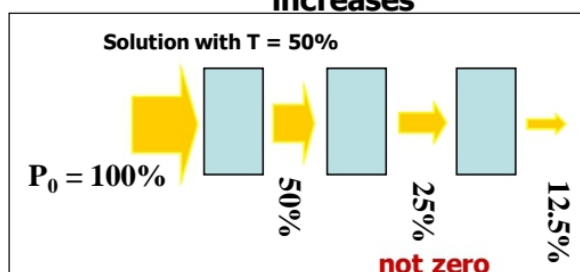
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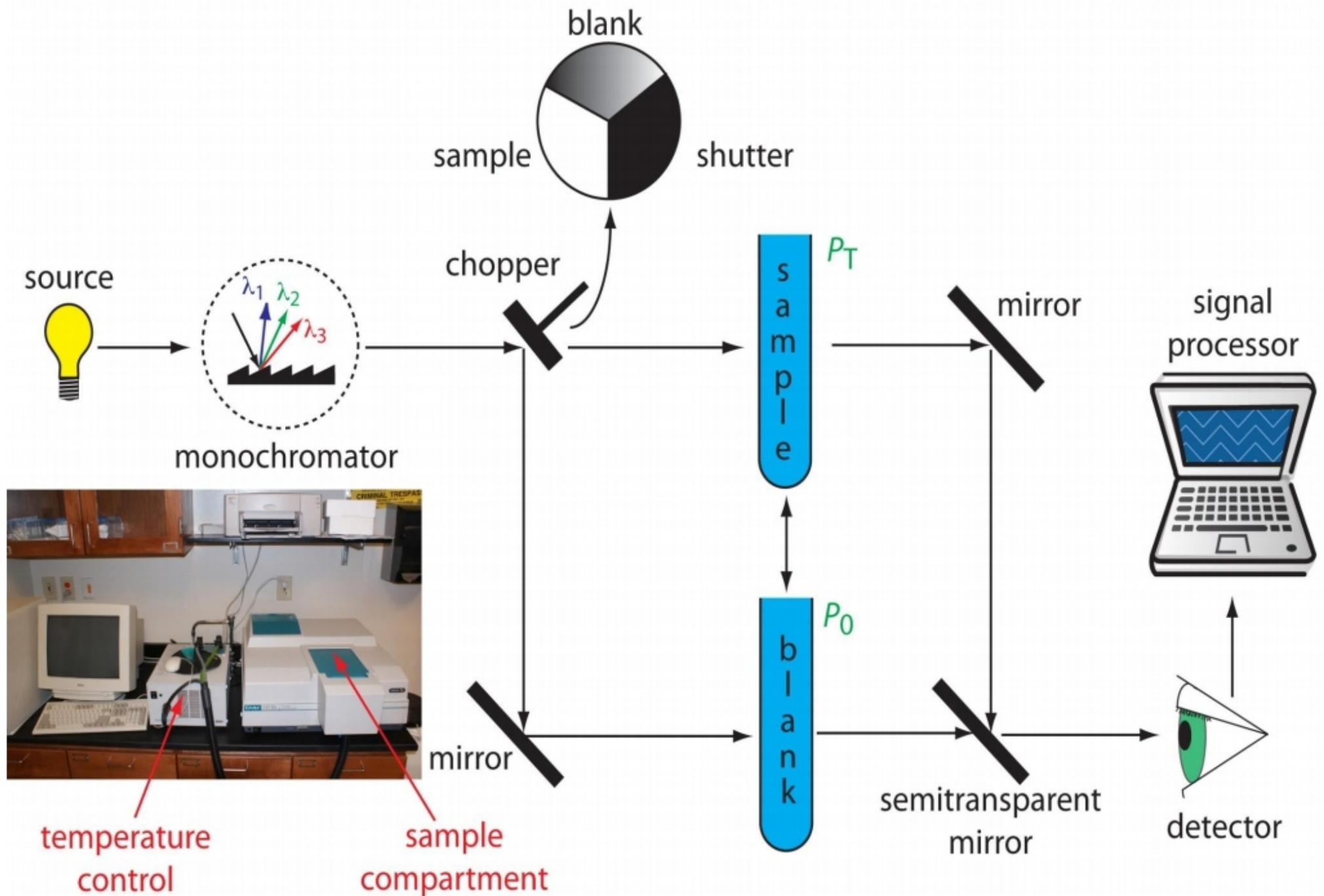


Beer's law is a relation between absorbance and concentration which is a straight line passes by origin at constant pathlength, b, and at certain wavelength,  $\lambda$ .

**Beer's law is obeyed for monochromatic light**

**Transmittance decreases exponentially as concentration increases**





قانون بير

$$A = \epsilon bc$$

A:-absorbance(unit less) ما له وحدة

molar absorbitivity L/mol.cm :- $\epsilon$

C:- التركيز mole/L

B:- سمك العينة وتقاس بوحدات المسافة cm

→ قانون بير هو العلاقة بين الامتصاص والتركيز وهو خط مستقيم يمر بنقطة الأصل

## 2

طول مسار ثابت  $b$ ، وعند طول موجي معين  $X$ .

• تتناقص النفاذية (transmittance) بشكل كبير مع زيادة التركيز (C)

Slope الميل يساوي  $\epsilon b$  ومن احد أفكار الامتحان يعطيك الميل

ويحكيك بده مثلا  $A$  ويكون معطيك التركيز

الميل يساوي  $\epsilon b$  ضرب التركيز ويطلع معك ال absorbance

Monochrometer :- هو جهاز يختار الفوتون المناسب اللي إله

طول موجي مناسب عشان يدخل العينة ويحفزها

Beer-Lambert's law proves a direct correlation between the absorbance (A) of a molecule to the concentration (c) and the path length (b).

### Derivation of Beer Lambert Law.

This relationship is a linear for the most part. However, under certain circumstances the Beer relationship gives a **non-linear relationship**.

These deviations from the Beer Lambert law can be classified into three categories:

**Real Deviations** - These are fundamental deviations due to the limitations of the law itself.

**Chemical Deviations**- These are deviations observed due to specific chemical species of the sample which is being analyzed.

**Instrument Deviations** - These are deviations which occur due to how the absorbance measurements are made.

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## **1- Real Deviation**

Beer law and Lambert law is capable of describing absorption behavior of solutions containing relatively low amounts of solutes dissolved in it (<0.01 M or 10 mM).

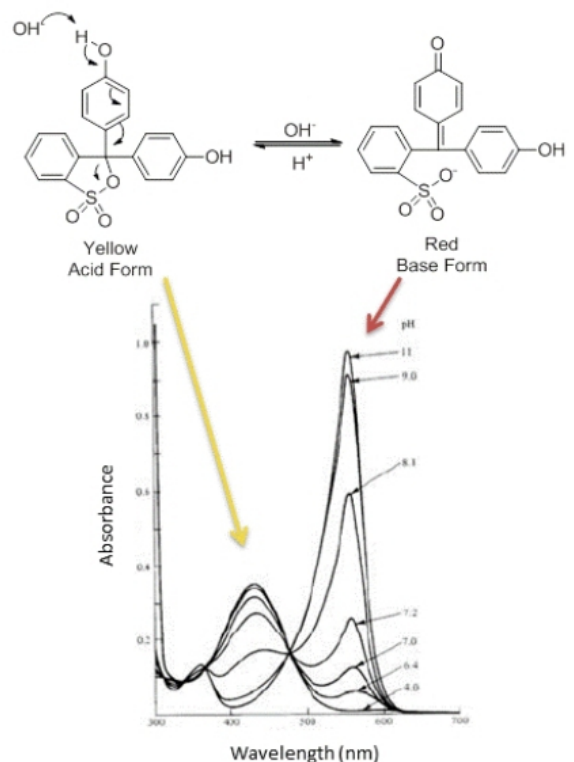
When the concentration of the analyte in the solution is high (> 0.01 M or 10 mM), the analyte begins to behave differently due to **interactions** with the solvent and other solute molecules and at times even due to **hydrogen bonding interactions**.

It is also possible that the concentration is so high, that the molecules **create a screen** for other molecules thereby shadowing them from the incident light.

## 2- Chemical Deviations

Chemical deviations occur due to **chemical phenomenon** involving the analyte molecules due to association, dissociation and interaction with the solvent to produce a product with different absorption characteristics.

For example, **phenol red** undergoes a resonance transformation when moving from the acidic form (yellow) to the basic form (red). Due to this resonance, **the electron distribution** of the bonds of molecule changes with the pH of the solvent in which it is dissolved.



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## 3- Instrumental Deviations

### **A] Due to Polychromatic Radiation**

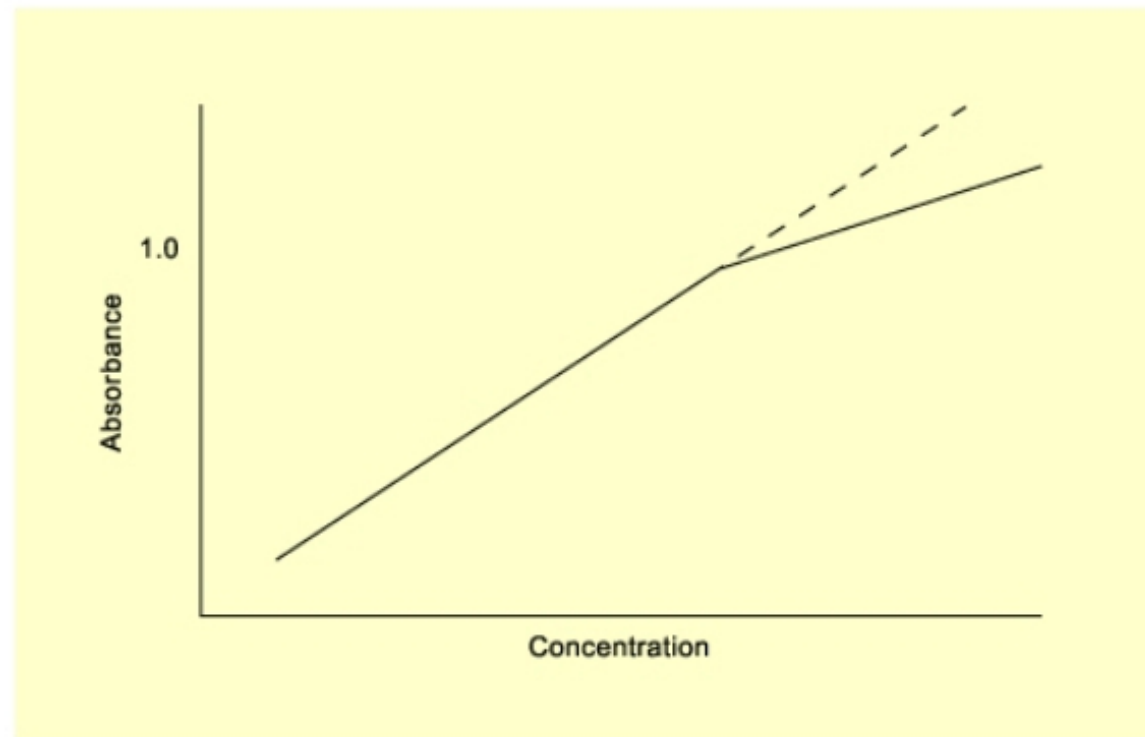
Beer-Lambert law is strictly followed when a monochromatic source of radiation exists. In practice, however, it is common to use a **polychromatic source** of radiation with continuous distribution of wavelengths along with **a monochromators** to create a monochromatic beam from this source.

### **B] Due to Presence of Stray Radiation**

Stray radiation or scattered radiation is defined as radiation from the instrument that is **outside the selected wavelength** band selected. Usually, this radiation is due to reflection and scattering by the surfaces of lenses, mirrors, gratings, filters and windows. If the analyte absorbs at the wavelength of the stray radiation, a deviation from Beer-Lambert law is observed similar to the deviation due to polychromatic radiation.

## C] Due to Mismatched Cells or Cuvettes

If the cells holding the analyte and the blank solutions are having different path-lengths, or unequal optical characteristics, it is obvious that there would be a deviation observed in Beer-Lambert law.



**Deviations from Beer's Law**

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عيوب قانون بير لامبرت تُقسم إلى ثلاث أصناف :- ١-real  
deviation ٢- chemical deviation ٣-instrumental  
diviation

1-real diviation:- لحد معين يكون دقيق لكن بعد هيك بفقد

العلاقة الخطية بين  $b$  و  $A$  و  $c$

> 0.01 - 10mM. بعد القيم هاي بصير تفاعلات بين المواد وتعطي  
قراءات خاطئة وأقل من الحقيقية وممكن هاي التفاعلات تؤدي إلى  
تكوين روابط هيدروجينية وإذا كان التركيز عالي ممكن يعمل  
screen عن particales الثانية وما تتم قراءتها

2-chemical deviation:- هي ناتج خطأ من تفاعلات كيميائية

اثناء التفكك أو التحلل

مثلا Red phenol بحالة الوسط القاعدي يكون لونه احمر لكن لما  
ينتقل للوسط الحمضي مثلا نتيجة تغير ال (PH) بصير لونه اصفر  
وهيك ممكن يعمل مشكلة ويغير ال structure

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3-instrumental deviation:- خطأ بالجهاز نفسه

polychromatic radiation- هو يكون مسؤول عن إدخال الفوتون

لداخل العينة ف ممكن يصرف فيه مشكلة واضطر

استخدم monochrometer

-stray radiation:- ضوء خارجي بأثر على قراءة العينة

-cuvetts:- هو الوعاء الي بحط في العينة الي بدي أقرأها ف اذا

اخترت الوعاء الغلط او الغير مناسب لهذه العينة رح يعمل قراءة غلط

لان كل cuvvet له طول موجي وخصائص بصرية تختلف من وعاء