Lab# 1

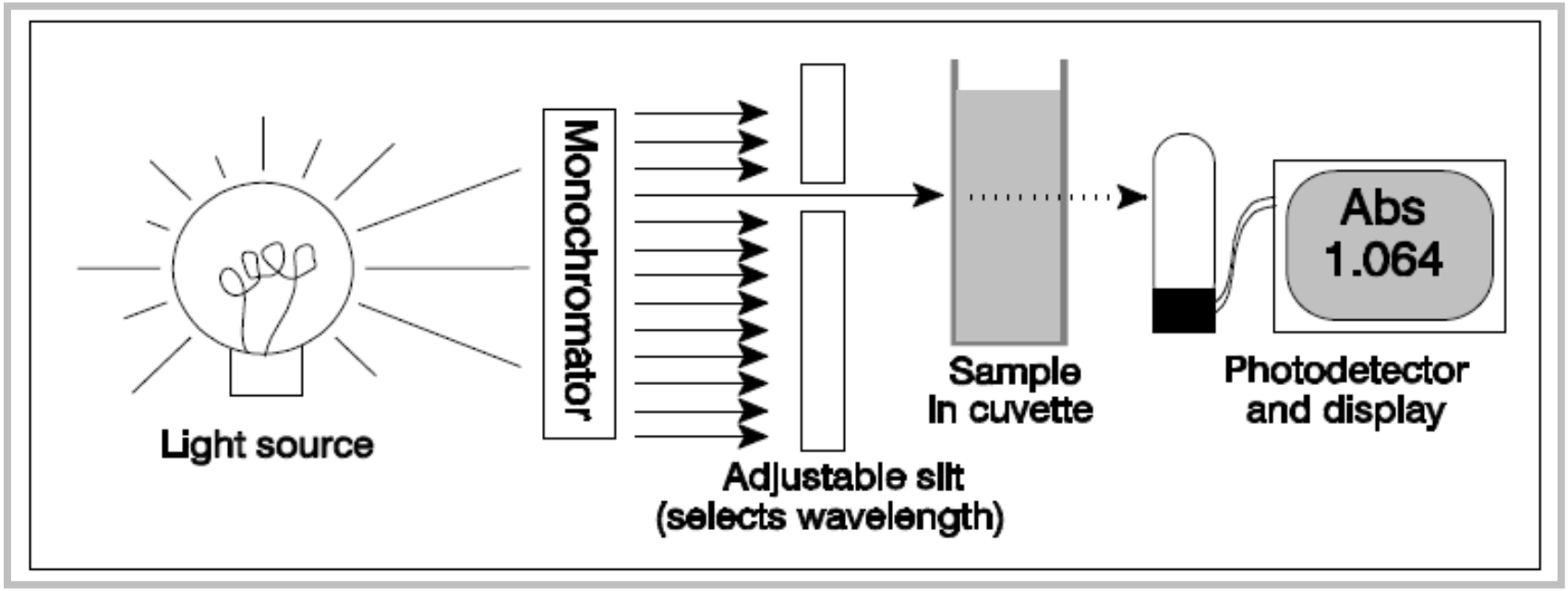
# Scanning spectrophotometry and spectrophotometric determination of concentration

BCH 333 [PRACTICAL]

## Objectives:

1. What is absorption spectrum and determination of λmax.

2. Standard curve of known concentration and determine the concentration for a solution "with unknown concentration".




How a spectrophotometer works.
(spectrophotometer components)

**Spectrophotometer:** it can be used to measure the amount of light absorbed by a solution. How?

-By using the spectrophotometer, we can quantitatively measure the absorbance, and this information can be used to determine the concentration of the absorbing molecule.

-More concentrated solution will absorb more light and transmits less.[why?]

So, the more concentrated solution → high absorbance value.

And Less concentrated solution → less absorbance value.

# Absorption spectrum

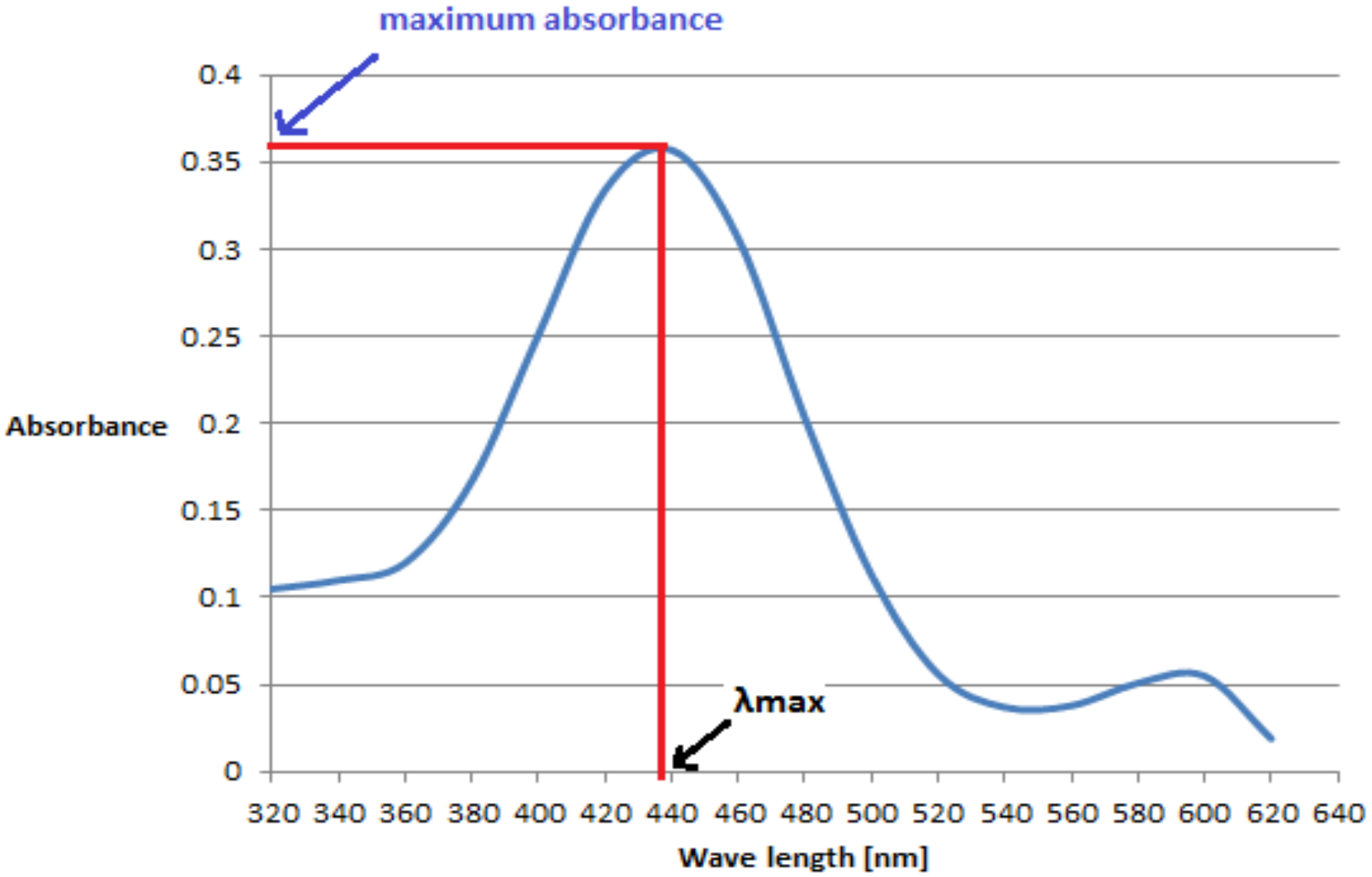
## Absorption spectrum:

The curve that display the action or behaviour of absorption of molecule [solute] at different weave lengths, Known as Absorption spectrum curve.

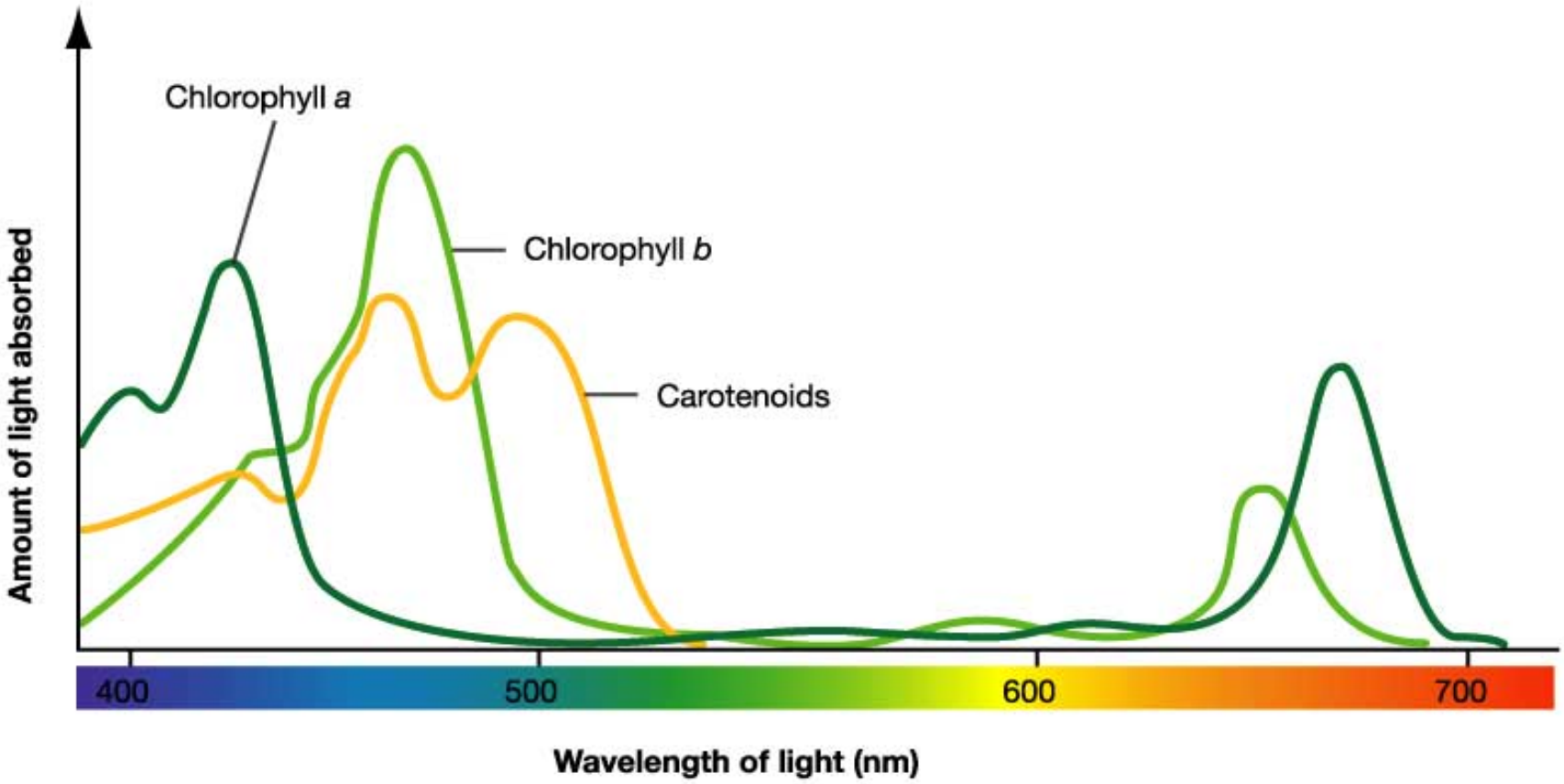
-Every molecule has its own Absorption spectrum, So it's considered as fingerprint for each molecule.

**λmax:** it is the weave length, at which the molecule has the maximum absorbance at this weave length. [best absorbance]. It can be determined from absorption spectrum curve.

[The wavelength of maximum absorption (λmax)]




Absorption spectrum curve and determination of λmax.




Absorption spectrum curve for different molecules.

## What is the benefit of studying the Absorption spectrum :

-Determine λmax.

-Used to identify substances.

-It also can be used to know if there is any contamination with another molecule.

How does a spectrophotometer work?

http://www.youtube.com/watch?v=pxC6F7bK8CU

# Standard curve of Concentrations

# Principle Standard curve of Concentrations:

## Beer-Lambert law:

The absorption of light by a solution is described by the Beer-Lambert law as:

There is linear relationship between absorbance and concentration of an absorbing species.

$A = \varepsilon l c$

A= is the absorbance.
ε = extinction(absorption) coefficient.
l = length of the light path through the solution.
c = concentration of the absorbing substance.

## So, what does standard curve of concentrations mean?

## A standard curve for concentrations:

- It is a graph that shows the relationship between different known concentrations of a substance and the absorbance at a specific wave length.

-Standard curve are most commonly used to determine the concentration of a substance, using serial dilution of solutions of known concentrations.

-So, what is the principle of a standard curve concentrations???

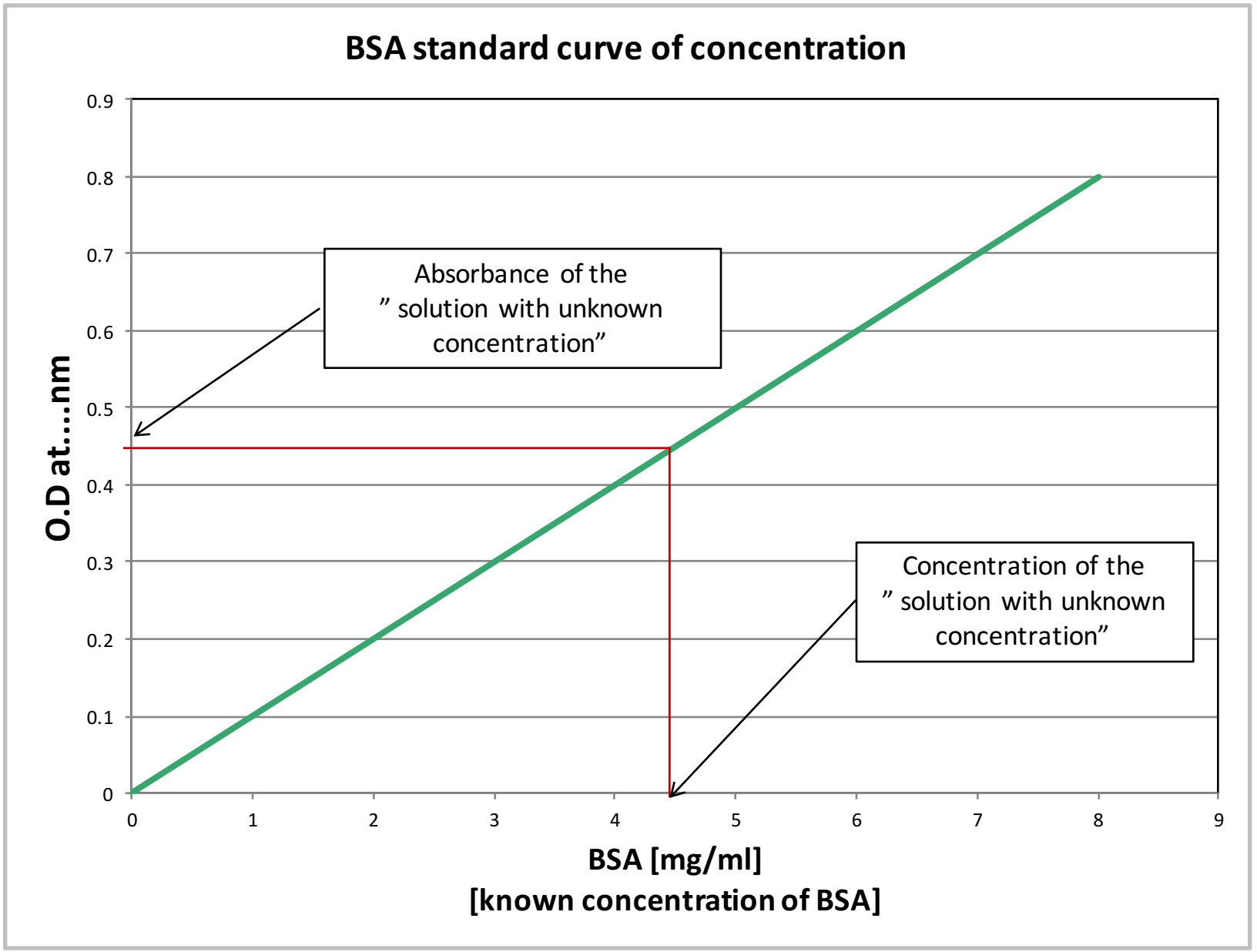
Wait….. what is standard solutions???

## Standard Solution:

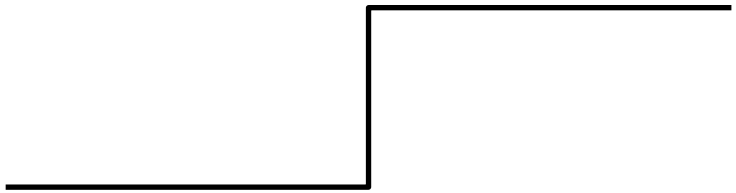
is a solution containing a precisely known concentration of an element or a substance.

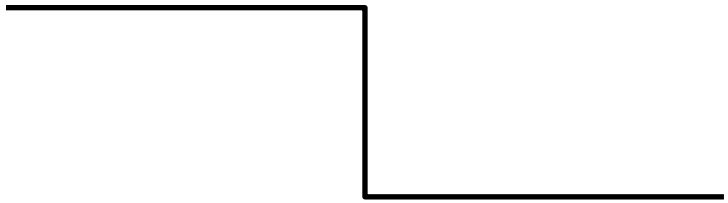
# How to determine unknown concentration of a solution with known absorbance value?

If the unknown concentration of a solution has absorbance value =0.45, the conc. From the curve will be ……??



# To determine the concentration of a solution with "an unknown concentration":





## From standard curve of concentration:

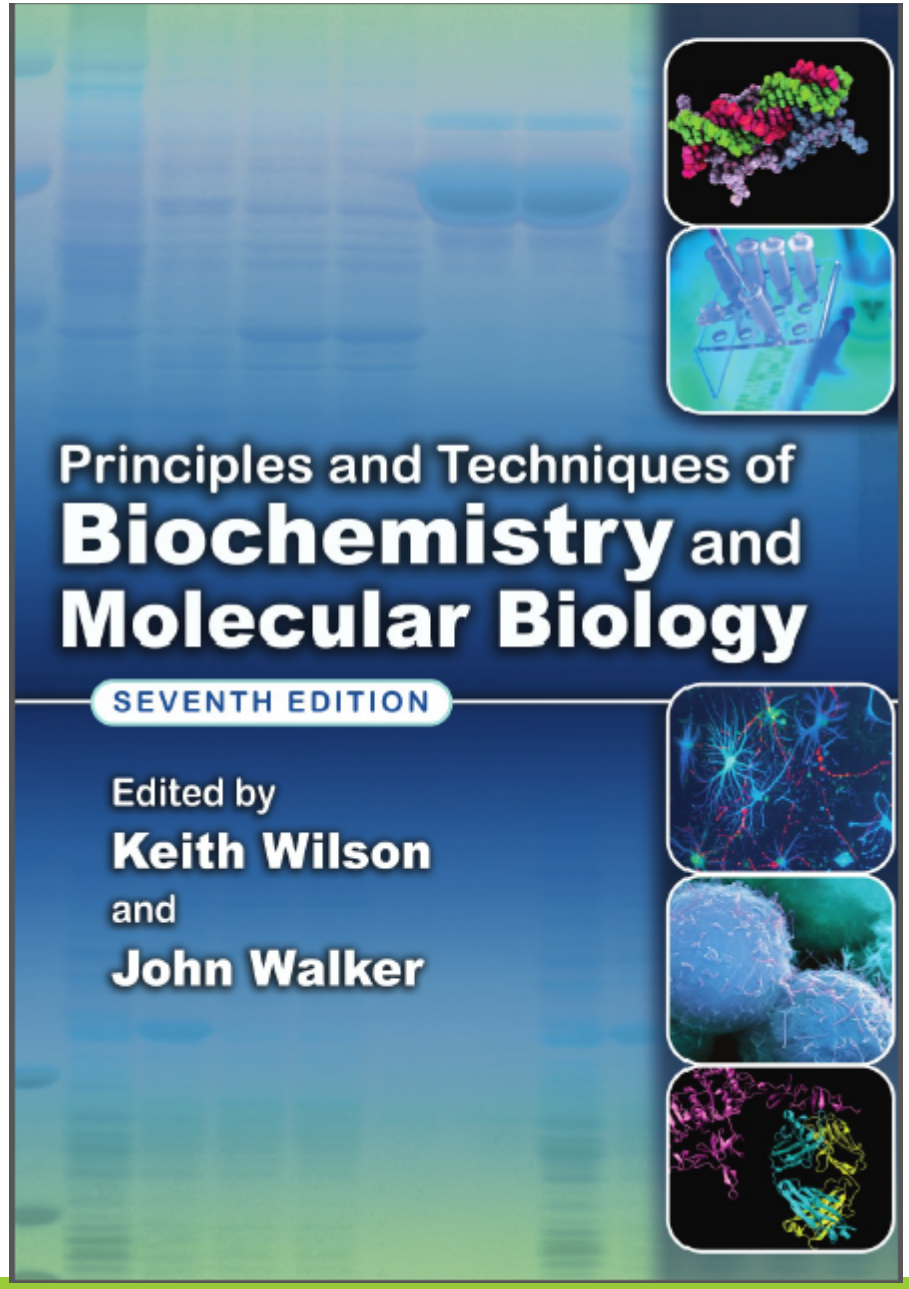
-Measure the absorbance of the "solution with unknown concentration" in order to determine the concentration, from the curve.

## Beer-Lambert law:

Using available information of any standard solution to determine the "ε",

then using these information to get the unknown concentration. Using:A = εlc

Note: "ε" will changed when the weave length changed.


Principles and Techniques of
Biochemistry and
Molecular Biology
SEVENTH EDITION
Edited by
Keith Wilson
and
John Walker

**Spectrophotometry Introduction:**

http://www.youtube.com/watch?v=qbCZbP6_j48

**Spectrophotometry Example**

http://www.youtube.com/watch?v=VqAa_cmZ7OY&feature=relmfu