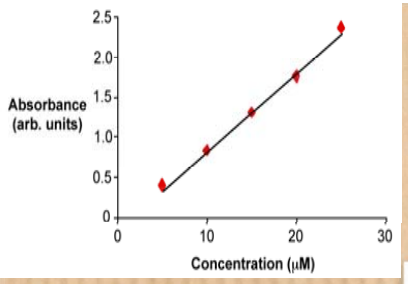


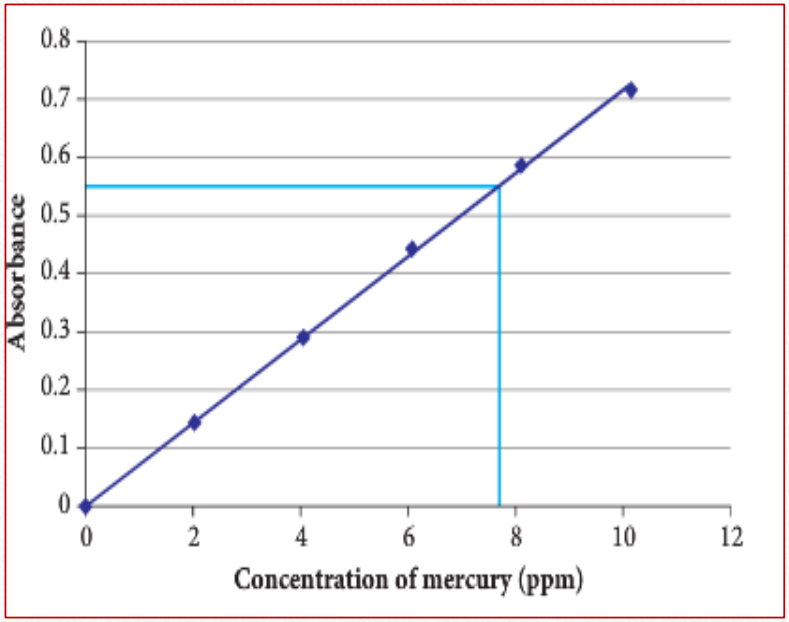
Lab4

PREPARATION OF CALIBRATION CURVE

Calibration curve definition

✓ Calibration curve:

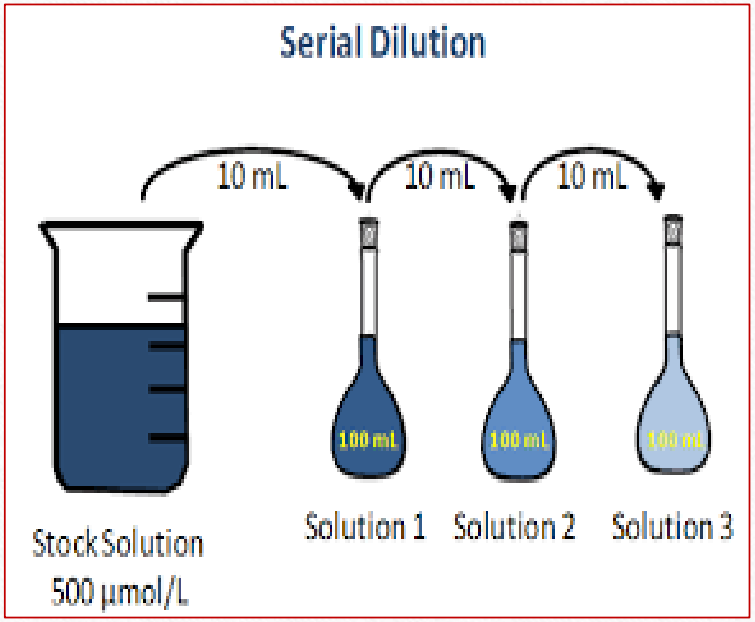
- It is the curve prepared from a series of standard solution
- It used as a reference curve to obtain the concentration of unknown sample of the same drug
- X axis s the conc· of sample
- Y axis is the absorbance



Stock solution

✓ **Stock solution:**

Solution of known and high conc. from which we prepare standard solution

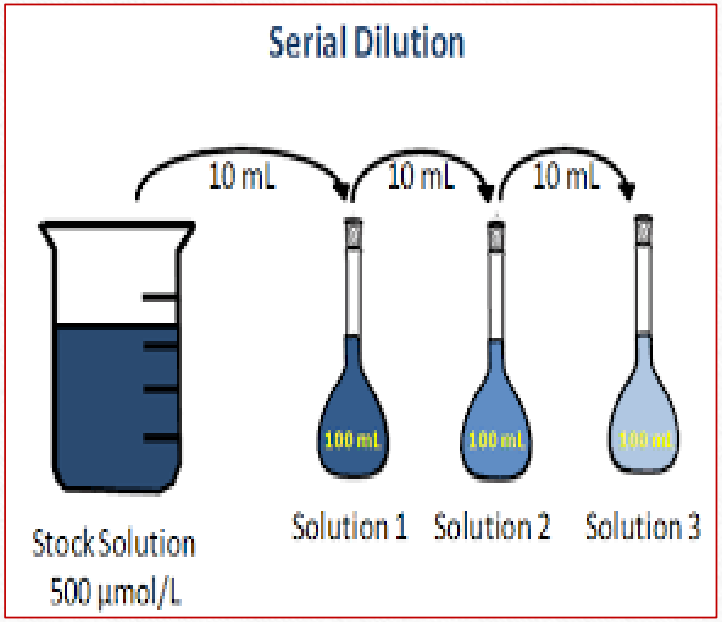


Standard solution

✓ **Standard solution:**

Solution of known conc. Prepared from stock solution using dilution equation:

$$C_1 \cdot v_1 = C_2 \cdot v_2$$



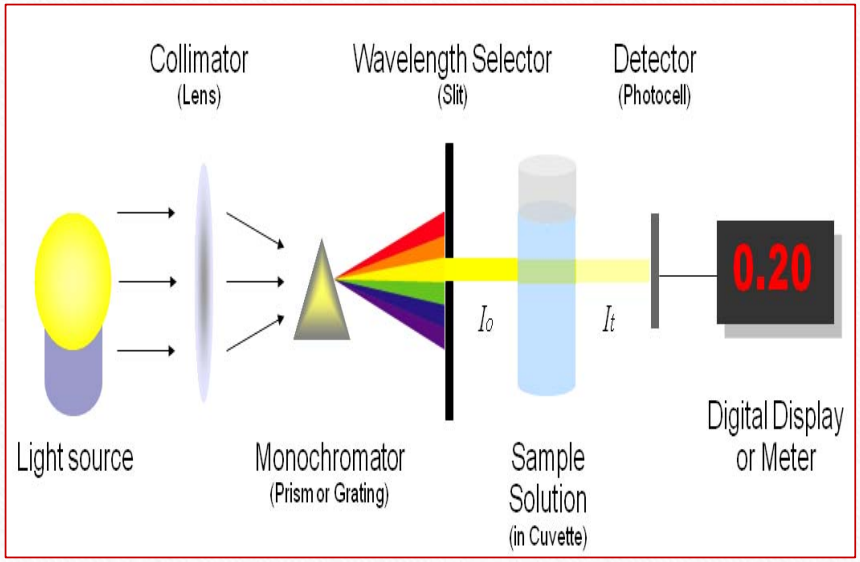
Blank solution

✓ **Blank solution:**

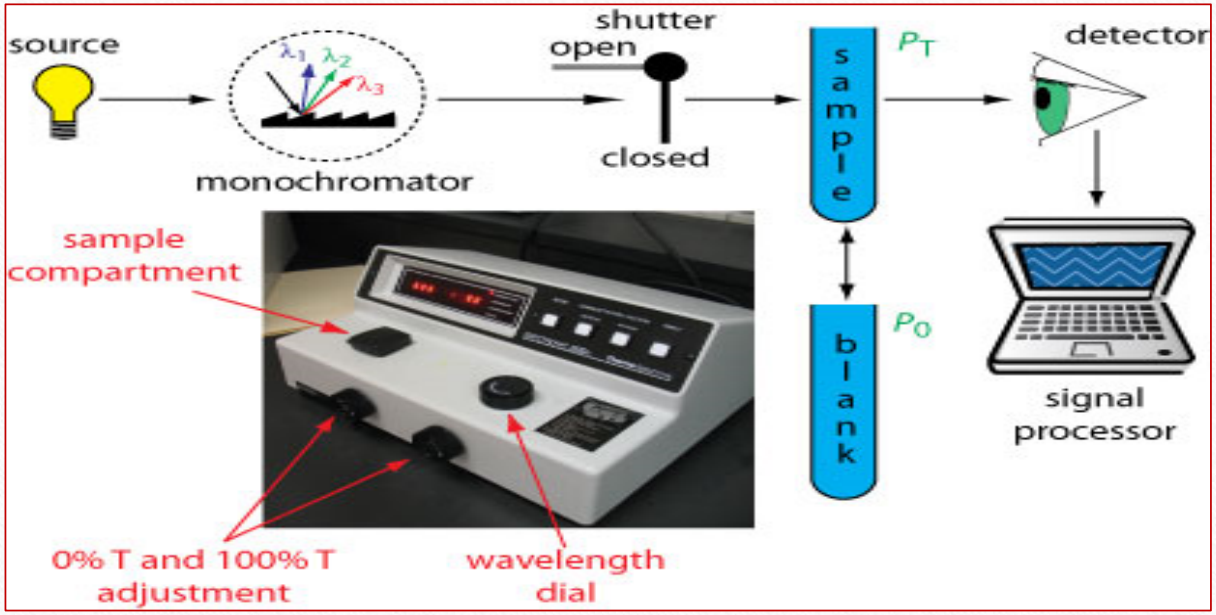
Its the solution which contain all the constituents of the sample except the active ingredient which is required to be measured

Spectrophotometry

- It is a method to measure how much a chemical substance absorbs light
- The basic principle is that each compound absorbs or transmits light over a certain range of wavelength.
- In our work usually the analysis concerned with the absorbed amount of light



Spectrophotometry



Spectrophotometry

- Depending on the range of wavelength of light source, it can be classified into two different types:

1. UV-visible spectrophotometer:

- uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.

2. IR spectrophotometer:

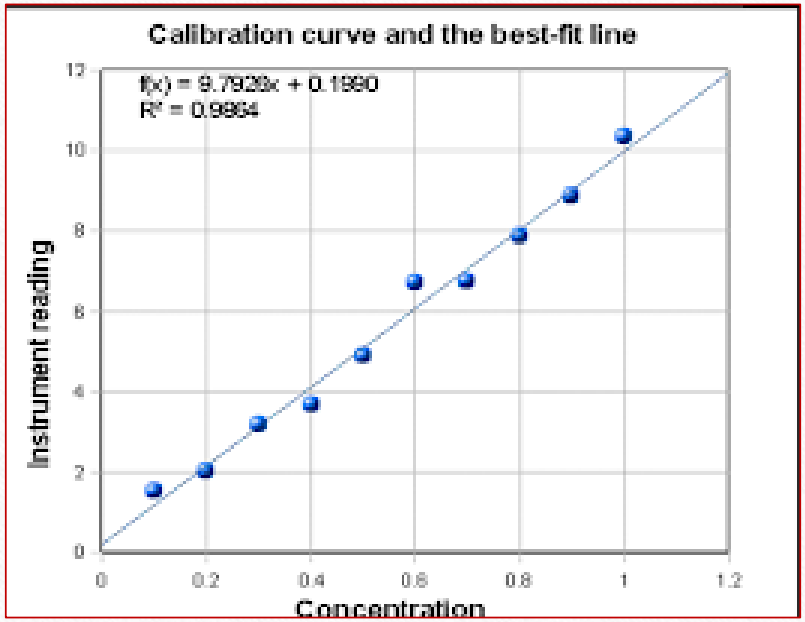
uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum.

Methods to obtain the unknown conc.

1. Curve fitting method:

1. Curve fitting method:

- To fit a straight line among scattered points
- Not reliable



Methods to obtain the unknown conc.
2. Least square fitting method:

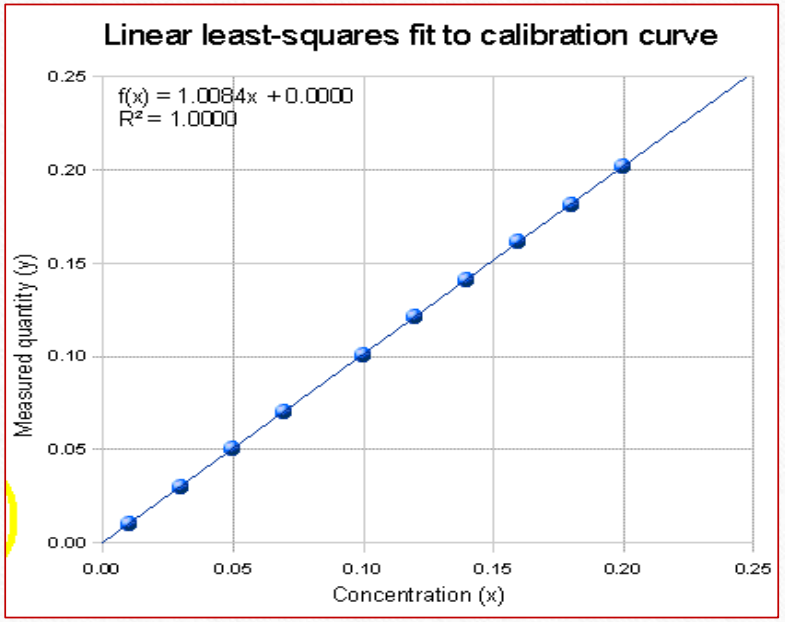
2. Least square fitting method:

This method is based on the equation which minimizes the sum of the squares of deviation of the observed values from line.

$$\sum (y - \bar{y})^2$$

Where: y =observed value

ȳ = calculated value



Methods to obtain the unknown conc.
2. Least square fitting method:

X conc(mg/ml)	Y (abs.)	X^2	X*Y
X1	Y1	$X1^2$	X1*Y1
X2	Y2	$X2^2$	X2*Y2
X3	Y3	$X3^2$	X3*Y3
X4	Y4	$X4^2$	X4*Y4
X5	Y5	$X5^2$	X5*Y5
Σx	Σy	Σx^2	$\Sigma x*y$
$(\Sigma x)^2$			

Methods to obtain the unknown conc.
2. Least square fitting method:

$$\bar{y} = c + bx$$

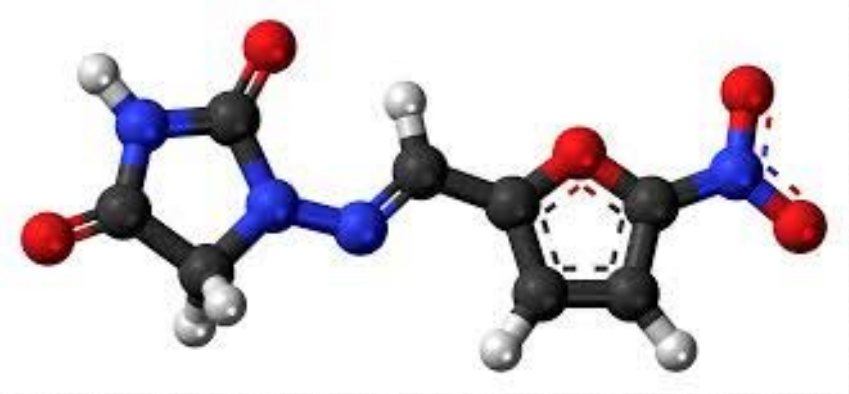
$$b = \frac{\sum(x \cdot y) - \sum(x) \cdot \frac{\sum(y)}{n}}{(\Sigma x^2) - \frac{(\sum x)^2}{n}}$$

$$c = \frac{\sum y - b(\Sigma x)}{n}$$

X conc(mg/ml)	Y (abs.)	X^2	X*Y	$\bar{y}$
X1	Y1	$X1^2$	X1*Y1	$\bar{y}$1=c+bx1
X2	Y2	$X2^2$	X2*Y2	$\bar{y}$2=c+bx2
X3	Y3	$X3^2$	X3*Y3	$\bar{y}$3=c+bx3
X4	Y4	$X4^2$	X4*Y4	$\bar{y}$4=c+bx4
X5	Y5	$X5^2$	X5*Y5	$\bar{y}$5=c+bx5
Σx	Σy	Σx^2	$\Sigma x*y$	
$(\Sigma x)^2$				

From these variables using the above equations we can obtain c & b, then by substitute each X value we can get $\bar{y}$ (calculated value)

Calibration curve of Nitrofurantoin

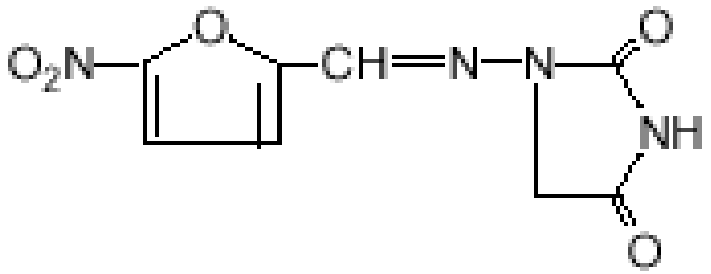


Calibration curve of Nitrofurantoin

✓ Chemical name of drug:

1-[[[5-nitro-2-furanyl]methylene] amino]-2,4-imidazolidinedione

✓ Chemical structure:



Calibration curve of Nitrofurantoin

✓ **Solubilty of drug:**

It is slightly soluble in water and ethanol, and souble in DMF (dimethyl formamide)

✓ **Action of drug:**

Nitrofurantoin is an oral antibiotic widely used either short term to treat acute urinary tract infections or long term as chronic prophylaxis against recurrent infections.

Calibration curve of Nitrofurantoin experimental procedure

1. Stock solution preparation

Preparation of 100mg/ml stock sol. Of nitrofurantoin can be done via take 10g (10000mg) to dissolve up to 100 ml of DMF (suitable solvent)

10000mg/100ml=100mg/ml

Calibration curve of Nitrofurantoin experimental procedure

2· Standard solution preparation

From stock sol· above prepare standard sol of deferent conc·(1·5, 2,3,4,&5mg/ml),Take certain volume from the stock then complete the volume to 100 ml by addition of DMF

To get standard sol with 1·5 mg/ml conc·:-

C1V1=C2V2 ---------- 100*V1=1·5*100 -------So V1 = 1·5 ml of stock sol· Completed to 100ml with DMF

And so on for other standard conc· (2, 3, 4, & 5mg/ml)

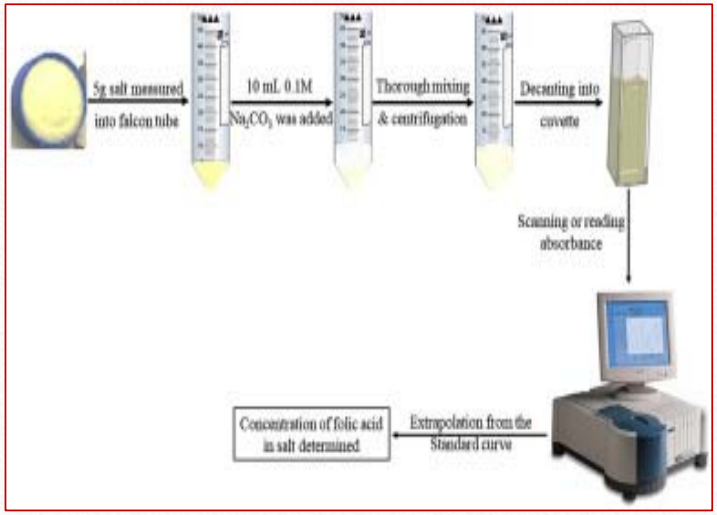
X conc(mg/ml)
1.5
2
3
4
5

Calibration curve of Nitrofurantoin experimental procedure

3· Absorbance measurement

Determine the absorbance of suitable blank, 1·5,2,3,4,&5 mg /ml standard solutions by spectrophotometry at 370 nm

X conc(mg/ml)	Y (abs.)
1.5	0.02
2	0.04
3	0.08
4	0.12
5	0.16



Calibration curve of Nitrofurantoin experimental procedure

4· Apply the least squares method: Calculate b, c, then ȳ depending on the least squares method ·

$$b = \frac{\sum(x \cdot y) - \sum(x) \cdot \frac{\sum(y)}{n}}{(\Sigma x^2) - \frac{(\sum x)^2}{n}} = 0.007$$

$$c = \frac{\sum y - b(\Sigma x)}{n} = 0.06$$

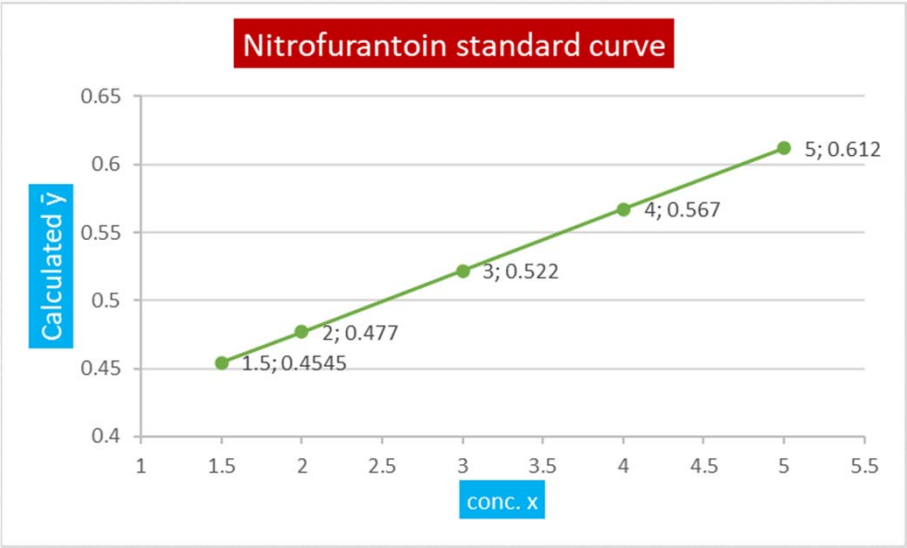
$$\bar{y} = c + bx = ?$$

X conc(mg/ml)	Y (abs.)	X^2	X*Y	ȳ
X1	Y1	$X1^2$	X1*Y1	ȳ1=c+bx1
X2	Y2	$X2^2$	X2*Y2	ȳ2=c+bx2
X3	Y3	$X3^2$	X3*Y3	ȳ3=c+bx3
X4	Y4	$X4^2$	X4*Y4	ȳ4=c+bx4
X5	Y5	$X5^2$	X5*Y5	ȳ5=c+bx5
Σx	Σy	Σx^2	$\Sigma x*y$	
$(\Sigma x)^2$				

Calibration curve of Nitrofurantoin experimental procedure

5· Plot ȳ vs conc standard curve

X conc(mg/ml)	Y (abs.)	X^2	X*Y	ȳ
1.5	0.02	2.25	0.03	0.4545
2	0.04	4	0.08	0.477
3	0.08	9	0.24	0.522
4	0.12	16	0.48	0.567
5	0.16	25	0.8	0.612
15.5	0.42	56.25	1.63	
240.25				



THANK YOU FOR YOUR ATTENTION