EXPERIMENT NO. 1
PREPARATION OF A CALIBRATION CURVE OF SALICYLIC ACID WITH THE APPLICATION OF STATISTICS

Introduction:

Calibration curve is a curve which is prepared from a series of standard solutions to use it as a reference curve to obtain the concentration of an unknown sample of the same drug.

Curve fitting: To fit a straight line among scattered points to represent the linear trend of the points, one can use:

1- *Eye fitting*: often it is possible to fit the data points by eye to a straight line, but this method is not reliable.

2- *Least square fitting*: a commonly preferred method for obtaining estimates of parameters used in curve fitting, is the method of least square.

The least square method is based on the equation which minimizes the sum of the squares of the deviations of the observed values from the line $\left[\sum(y-\bar{y})^2\right]$, where y is the observed value and $\bar{y}$ is the calculated value. In other words, the line of best fit is obtained when the sum of the squares of the vertical distances from the points to the line is a minimum. Such a line is called the linear regression line of y on x, based on the principle of least squares as in Fig. 1.

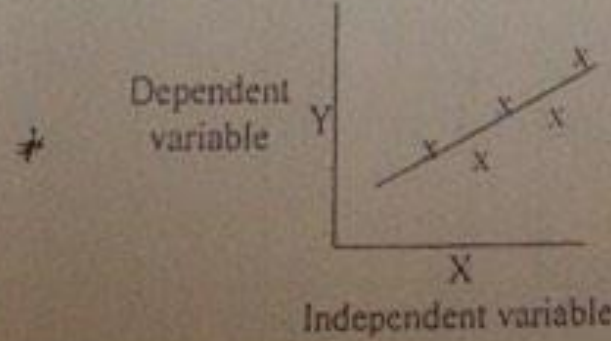


Fig. 1: Schematic plot of curve fitting by least square method.

Spetrophotometric methods:

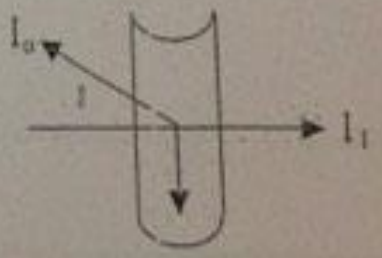
To measure the concentration of a substance in a solution, there are a number of methods one of which is " spetrophotometric methods ".

Spetrophotometric methods: when light falls on a liquid, some is reflected and the rest is partly absorbed and partly transmitted. Spetrophotometric methods of analysis are usually concerned with measurement of the amount of light absorbed or with comparison of the absorption or transmission of two solutions one of which is a standard of known composition.

In pharmaceutical spectrophotometric analysis, visible light, of wavelength 400-760 mμ, or a particular wavelength of ultraviolet light, between 200 and 400 mμ is usually employed.

The measurement of the amount of visible or ultraviolet light of a definite wavelength which is absorbed by a solution involve the use of a spectrophotometer.

The fundamental relationship used in spectrophotometry is Beer and Lambert laws combination:



$$\log \frac{I_o}{I_T} = Kct$$

where : I_o : Incident light.

I_T: Transmitted light.

K: Proportionality constant.

c: Concentration.

t: thickness of substance.

$\log \frac{I_o}{I_T}$: E = Extinction or Absorbance.

$E = -\log_{10} T.$

$T = \text{Transmission} = \frac{I_T}{I_o}$.

solution is placed in the light path, and the light control knob (D) is rotated until the dial read 100 % T.

The blank solution (the medium in which the substance being measured is located) may itself absorb light of certain wavelength. In order to measure the absorbance due to only a particular species in solution, such side effect is compensated by adjusting the dial to read 100 % T or zero absorbance.

4- The sample solution is now placed in the light path. Any change in the reading, is due to the particular light absorbing species in the sample.

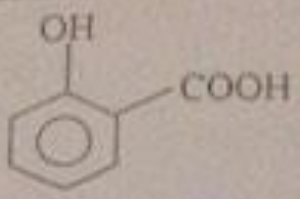
Aim of the experiment:

To prepare a calibration curve of salicylic acid from a series of standard solution to use it as a reference curve to obtain the concentration of unknown sample of the same drug.

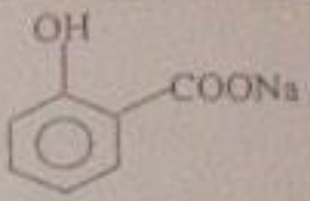
Procedure and Calculations:

1- Prepare 250 ml of a stock solution of sodium salicylate containing the equivalent of 200 mg salicylic acid/ 100 ml.

Note: Sodium salicylate is readily soluble in cold water, so it is used to prepare the stock solution of sodium salicylate after calculating its equivalent content of the acid. While salicylic acid is sparingly soluble in cold water (1 part acid in 550 parts water), but more soluble in hot water (1 part acid in 15 parts of boiling water) from which it can be recrystallized.



Salicylic acid



Sodium salicylate

I- To calculate the number of (mgs) of (S.A) salicylic acid needed to prepare the stock solution:

mg of S.A	mls
200	100
x	250

x = 500 S.A. mg needed = 0.5 g

II- To calculate the equivalent of sodium salicylate to 500 mg salicylic acid.

M. wt of sodium salicylate	M. wt of S.A
160	138
x	0.5

x = 0.5797 g or 579.7 mg (sodium salicylate needed to prepare stock solution containing 200 mg S.A. / 100 ml)

Dissolve 579.7 mg sodium salicylate in about 180 ml water in a beaker then complete the volume to (250 ml by D.W.) using volumetric flask.

2- From the stock solution of sodium salicylate (use volumetric procedure) to accurately prepare solutions containing the equivalent of 50, 40, 30, 20 and 10 mg S.A. / 100 ml, e.g.

To prepare 50 mg/ 100 ml:

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

$$200\,\% \cdot V_1 = 50\,\% \cdot 100$$

$$V_1 = \frac{50 \times 100}{200} = 25 \text{ ml stock solution taken and to be diluted to 100 ml to get 50 mg/100 ml and so on for other concentrations.}$$

For 40 mg/100 ml, we take 20 ml stock solution and dilute it with D.W. to 100 ml.

30 mg/100 ml → 15 ml to 100 ml.

20 mg/100 ml → 10 ml to 100 ml.

10 mg/100 ml → 5 ml to 100 ml.

3- Take 1 ml of each solution add to it 5 ml color developing reagent which is a mixture of:

Mercuric chloride	40 g
Ferric nitrate	40 g
1 N HCl	120 ml (85. 1000ml)
Distilled water	ad to 1000 ml

Mix the solution and determine absorbance in spectrophotometer at 530 µm against a blank.

Notes:

a- A complex will be formed between the carboxyl and the hydroxyl groups in the salicylate with the color-developing reagent resulting the violet color seen when both solutions are mixed.

$$S.A. + Fe^{+3} \rightarrow \text{complex (violet color)}$$

absorb the light at 530 µm

b- Blank in this experiment consists of 1 ml distilled water + 5 ml color developing reagent.

4- Plot the absorbance versus concentration of salicylic acid.

5- Apply the least square method to calculate the slope Ab and intercept.

6- Use the straight-line equation to calculate $\bar{y}$.

7- Draw the line of best fit.