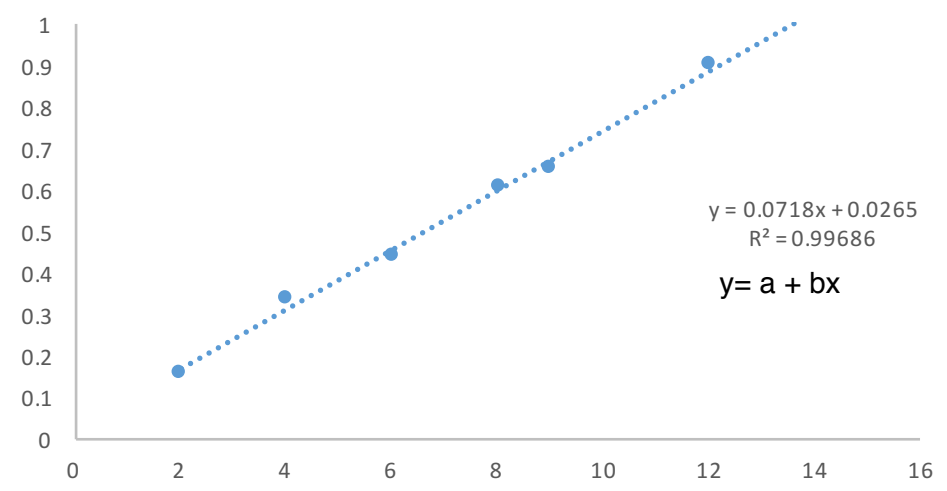


Calibration curve : Is a reference curve prepared from a series of standard solution having different concentration plotted against their absorbance.

Bears -lambert theory

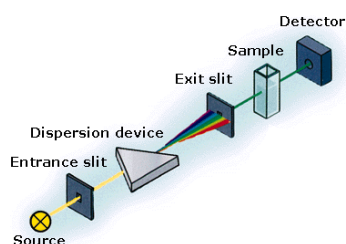
The amount of light absorbed by a sample is directly proportional to its concentration.

Benefit of calibration curve: to obtain the concentration of unknown drug sample from its calibration curve by measuring its absorbance then the concentration could be obtained from the calibration curve equation.



The principle of the measurement:

It was found that when the light falls on a liquid sample, some will be reflected, other part will be transmitted and the rest will be absorbed



λ_{max} : wavelength at which maximum absorbance occur.

Aim of the experiment :

Preparing the calibration curve of Diclofenac Sodium in 6.8 phosphate buffer (pH6.8)

Drug under investigation

Diclofenac Sodium is a nonsteroidal anti inflammatory drugs used for symptomatic treatment of osteoarthritis, ankylosing spondylitis, primary dysmenorrhea, acute gouty arthritis and for relief of pain, including postoperative (e.g., orthopedic, gynecologic, oral) pain, in adults.

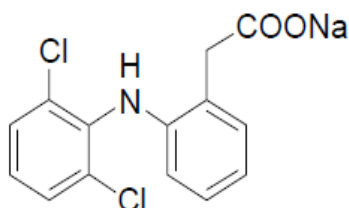


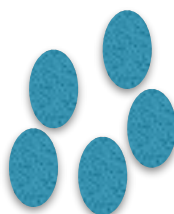
Figure :Chemical Structure of Diclofenac Sodium

Parameters set for plotting of calibration curve

- 1) Beer's & Lambert range: 2-14 mg/ml
- 2) Solvent. Phosphate Buffer 7.4
- 3) λ_{max} for Diclofenac Sodium ... 276nm

Procedure :

- 1-Prepare 100 ml of stock solution of Diclofenac Sodium contains 100 mg in 100 ml of 0.1N Phosphate buffer.
- 2-Make several dilutions in the range from 2 mcg/ml to 14 mcg/ml.
- 3- Set the spectrophotometer to λ_{\max} of ACV (276 nm)
- 4-Measure the absorbance of each dilution and the stock solution
- 5-Use MS Excel to draw the calibration curve.
- 6-Use excel elements to find calibration curve equation and R^2 .



Stock 1. **Stock 2** **standard solution** **Blank (Phosphate buffer 7.4**

1mg /ml **10mcg / ml.** **2-14 mcg/ml**

Definitions:

Stock solution : solution of high and known concentration , from which we can prepare standard solution

Standard solution : solution of known concentration , prepared from stock solution by dilution process

Blank solution : is the solution which contains all the constituent of the sample except for the active ingredient which is required to be measured .

Practical example???

Steps for drawing calibration curve in excel

- 1- Select data range
- 2- From insert select x-y scatter
- 3- From chart design, add chart element , select trendline , linear
- 4- From chart design, add chart element , select trendline , more trendline options , display equation and R^2 .

Notes:

- 1- Type of cells employed in measuring abs in the UV- region is
Because.....
- 2- The same apparatus should be used in the preparation of calibration curve and measuring the abs. of unknown sample because.....
- 3- The values for absorbance should not exceeds 1 because
- 4- The sample should be completely soluble in the solvent employed for the preparation of calibration curve. Complete solubilization could be achieved by adding Such as

H.w.

- 1-What action is required for a sample produces absorbance $\gggg > 1$
- 2- Are spectroscopic methods suitable for determining the unknown conc. of all drugs by measuring the absorbance. Suggest other method
- 3- How to solubilize a drug in case of it is not completely dissolved in the intended solvent for calibration. Explain practical example.

Practical issue :

Find out the solubility value for Drug X in a given solvent , if you know that 0.2963 is the abs obtained after two dilutions for the original sample at ration 1:10 each time. Apply the equation obtained from the curve below .(Answer: 4.489 mg / ml)

