**STANDARD CALIBRATION CURVE**

*In analytical chemistry, a calibration curve is a general method for determining the concentration of a substance in an unknown sample by comparing the* *unknown to a set of standard samples of known concentration.*

The construction of a curve or straight line by plotting observed or experimental data on a graph is an important method of visualizing relationships between variables.

Fitting a curve to the points on a graph implies that there is some sort of relationship between the variables x and y. Moreover, the relationship is not confined to isolated points but is a continuous function of x and y. In many cases a hypothesis is made concerning the relationship between the variables x and y. Then an empirical equation is formed that best describes the hypothesis. This empirical equation must satisfactorily fit the experimental or observed data.

Physiologic variables are not always linearly related. However, it may be possible to arrange or transform the data to express the relationship between the variables as a straight line.

Straight lines are very useful for accurately predicting values for which there are no experimental observations.

**The general equation of a straight line is:**

**y = a + bx**

Where **a** = y intercept.

**b** = slope.

**y** = dependent variable.

**x** = independent variable.

* Straight line equation could be obtained by the ruler method, the least-squares method, calculator, excel …etc.
* The linear regression calculation using the least-squares method is used for calculation of a straight line through a given set of points.
* Using the least-squares method we can calculate the slope and the intercept by using the following equations, where n = number of data points:

b = 

a = 

**Example :**

A standard calibration curve of aspirin was constructed in acetate buffer fluid and assyed spectrophotometrically at 265 nm . the data obtained are given below:

|  |  |
| --- | --- |
| Concentration X  *(mg/ml)* | Absorbance Y |
| 0 | 0 |
| 0.5 | 0.122 |
| 1 | 0.244 |
| 1.5 | 0.338 |
| 2 | 0.432 |
| 2.5 | 0.548 |

**Using Microsoft Excel:**

|  |  |
| --- | --- |
| **a (intercept)** | 0.0248 |
| **b (slope)** | 0.208 |
| **r** | 0.9988 |



**DISSOLUTION TEST**

* The disintegration test, simply, identifies the time required for the tablets to break up under the conditions of the test and for all particles to pass through the 10 - mesh screen.
* The disintegration test offers no assurance that the resultant particles will release the drug in solution at an appropriate rate. Therefore, dissolution test have been developed for all tablet products.

***The dissolution test:***

is an *in vitro* test for measuring the time required for a given percentage of the drug substance in a tablet to go into solution under a specified set of conditions.

***USP / NF have provided procedures for dissolution testing:***

* **Apparatus I:** in general, a single tablet is placed in a small wire mesh basket fastened to the bottom of the shaft connected to a variable speed motor. The basket is immersed in the dissolution medium contained in 1000 ml flask. The flask is maintained at 37 ± 0.5 oC by a constant temperature water bath. Samples of the fluid are withdrawn at specified time intervals to determine the amount of the drug in solution.
* **Apparatus II:** the same equipment as in apparatus I is used, except that the basket is replaced by a paddle, formed from a blade and a shaft, as the stirring element. The dosage form is allowed to sink to the bottom of the flask before stirring.

**Description of a dissolution test in USP/NF monograph specifies:**

* + The dissolution test medium, and volume,
  + Which apparatus is to be used,
  + The speed at which the test is to be performed (rpm),
  + The time limit for the test, and
  + The assay procedure.

**The test tolerance is expressed as:**

* + - The percentage of the labeled amount of the drug dissolved in the time limit.
    - The results are plotted as concentration vs. time. Values for t 50% , t 90%, and the percentage dissolved in 30 minutes are used as guides. The value for t 50% is the length of time required for 50% of the drug to go into solution.
    - N.B., a common dissolution tolerance in the USP/NF is not less than 75% dissolved in 45 minutes.

1. Drug release of aspirin from one batche each tablet containing (300mg of the drug) was performed using dissolution apparatus І. The dissolution medium was 500 ml of 0.05 M acetate buffer, kept at 37ºC and stirred at 50 rpm. Samples (5 ml) each were withdrawn at specified time intervals and diluted in ratio (1:4), then replaced with fresh aliquots of the dissolution medium, then assayed for its drug contents spectrophotometrically at 265 nm, against a blank of fresh dissolution medium. The following data were obtained:

|  |  |  |  |
| --- | --- | --- | --- |
| Time  (min) | Abs.  at 265 nm | \*Conc. | % released =  (conc./original strength) x 100 |
| 0 | 0 |  |  |
| 5 | 0.299 |  |  |
| 10 | 0.352 |  |  |
| 15 | 0.384 |  |  |
| 20 | 0.386 |  |  |
| 25 | 0.390 |  |  |
| 30 | 0.412 |  |  |

**\*Conc. =**

1. Using the linear regression equation obtained from the standard curve

Conc. =[(abs-a)/b]x dil factor x 500 /100

1. Using the A1%

Conc. =[(abs)/A1%]x dil factor x 500 /100

1. Using the calculator

**STANDARD CALIBRATION CURVE OF ASPIRIN**

1. Grind a small amount of aspirin powder into very fine powder.
2. Prepare a stock solution #1: 100 mg aspirin powder in 100 ml acetate buffer pH 4.5 (1mg/ml).
3. Take 10 ml (10mg) of solution #1 and complete to 100 ml with acetate buffer (0.1mg/ml).
4. Make other dilutions from solution # 1 to get the best fit line and determine the equation:

* 15ml (15 mg) in 100ml acetate buffer.
* 20ml (20 mg) in 100ml acetate buffer.
* 25ml (25 mg) in 100ml acetate buffer.

1. Read the absorbance of all dilutions at 265nm.
2. Plot absorbance VS concentration (mg/ml) which is the standard calibration curve, and then estimate the slope of the line “b” and the y intercept “a” using both the ruler and the calculator method.

**Results:**

|  |  |
| --- | --- |
| Concentration X  *(mg/ml)* | Absorbance Y |
| 0 |  |
| 0.05 |  |
| 0.1 |  |
| 0.15 |  |
| 0.2 |  |
| 0.25 |  |

Slope (b) = .

Y=a+bX

**DISSOLUTION TEST FOR ASPIRIN TABLETS**

**(aspirin® TABLETS)**

***Conditions:***

Apparatus: Apparatus I (Basket)

Media: 500 ml acetate buffer pH 4.5

Temperature: 37oC ± 0.5

Speed: 50 rpm

***Procedure:***

1. Put one tablet into each basket and start the apparatus at the above conditions.
2. Withdraw 5 ml sample with the pipette (using polyethylene tube to filter the sample) at different time intervals (5, 10, 15, 20, 30min.).
3. Dilute one ml of the samples in a test tube with 9 ml acetate buffer i.e dilution factor = 10.
4. Replace the withdrawn amount with 5 ml fresh acetate buffer.
5. Read the absorbance of the diluted samples at 265 nm, against acetate buffer as blank.
6. Tabulate your results as follows:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Time (min)** | **Abs** | **Dilution factor (df)** | **Concentration**  Using the linear regression equation obtained from the standard curve of ASA. | **% released =** |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 30 |  |  |  |  |

1. Plot the % released VS time. From the curve determine the time required for 80% of the dose to be released.

**Limit**:

Not less than 80% of the labeled amount of C9H8O4 is dissolved in 30 minutes.