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- Diffusion parameters
- Dissolution parameters
- Pharmacokinetic parameters
- Similarity factors- f2 and f1
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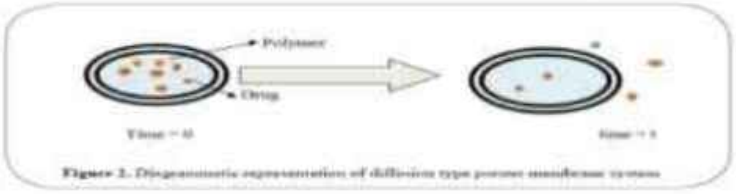
DIFFUSION PARAMETER

Defination:

- Diffusion is a process of the mass transfer of the individual molecule of a substance brought about by random molecular motion associated with a driving force like concentration gradient i.e. generally from higher concentration to lower concentration.

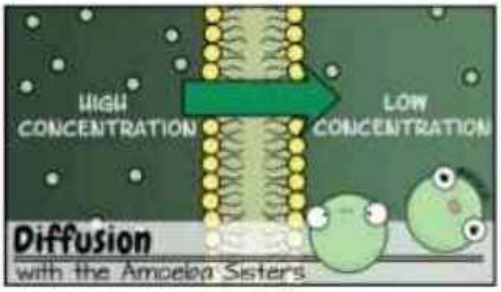
- Free diffusion of the substance through liquids, solids and the membranes are for special interest in designing of a dosage form.

- Studying the diffusion parameters will help us to understand the PERMEATION AND DISTRIBUTION of drug molecules in living systems.



The need to study diffusion parameters:

- In controlled release systems.
- Whether or not the tablet matrix disintegrates, the rate at which solvent penetrates the matrix influences in terms of the drug release rate as well as the total drug released.
- The solvent/water penetration rate into the tablet often correlates well with the disintegration rate.
- For ex- A swelling gel layer, formed during the penetration and acting as a diffusion barrier for active ingredients, may also affect their dissolution rate.



Fick's Law of Diffusion-

- The amount of material, M of material flowing through a unit cross section, S, of a barrier in a unit time, t, is known as flux, j;

$$J = \frac{dM}{S.dt}$$

- The flux, in unit turn, is proportional to the concentration gradient, dC/dx;

$$J = -D.\left(\frac{dC}{dx}\right)$$

- Where D is the diffusion coefficient in cm2/sec.
- C is the concentration in g/cm3.

- D is affected by the concentration ,temp., pressure, solvent property, and chemical nature of diffusant.

- If diffusion is the rate determining step, then we can use Fick's first law of diffusion to describe the overall process.

OR

Fick's I law

- Fick's first law states that the flux is directly proportional to the concentration gradient

$$J \equiv atoms / area / time \propto concentration\ gradient$$

$$J \propto \frac{dc}{dx} \text{ OR } J = -D\frac{dc}{dx} \quad ...(2)$$

flux in steady state flow

Negative sign indicates a decrease in concentration
But flux is positive quantity

dc=change in conc. of material g/cm^3.
D=diffusion coefficient of a penetrant, cm/sec^2.
Dx=change in the distance, cm.

Flicks second law of diffusion:

Fick's Second Law

It states the relation between the change in concentration gradient of the particles and time

$$\frac{d\varphi}{dt} = D\frac{d^2\varphi}{dx^2}$$

$d\varphi$ = change in concentration of the particle

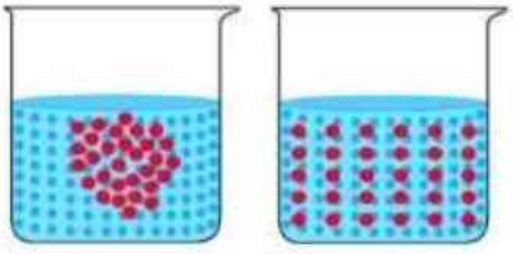
dt = change in time

dx = change in position

D = diffusion coefficient or diffusivity

$\frac{d\varphi}{dt}$ = change in concentration with time

$\frac{d^2\varphi}{dx^2}$ = the second derivative of $\frac{d\varphi}{dt}$



Concentration becoming uniform over time

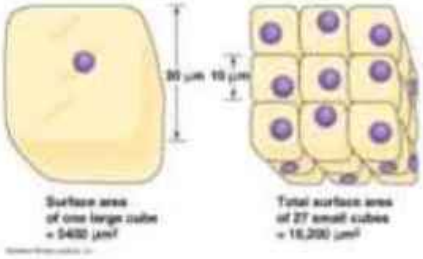
Driving forces that facilitate diffusion-

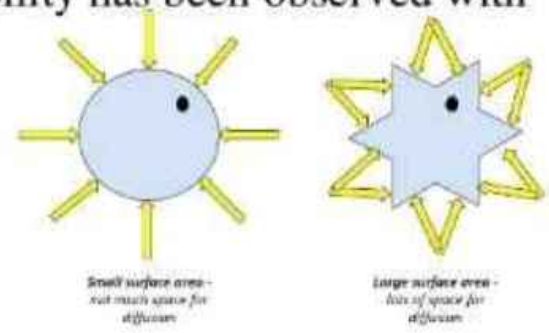
Driving force	Example
Concentration	Passive diffusion
Pressure	Osmotic drug release
Temperature	lyophilization
Electric potential	electrophoresis

Parameters in the Diffusion process-

1. Surface area, A:

- The surface area per gram (or per dose) of a solid drug can be changed by altering the particle size. For example, a cube 3 cm on each side has a surface area of 54 cm^2. If this cube is broken into cubes with sides of 1 cm, the total surface area is 162 cm^2. Actually if we break up the particles by grinding we will have irregular shapes and even larger surface areas. Generally as A increases the dissolution rate will also increase. Improved bioavailability has been observed with griseofulvin, digoxin, etc.





2. Diffusion layer thickness, h:

- This thickness is determined by the agitation in the bulk solution. *In vivo* we usually have very little control over this parameter. It is important though when we perform *in vitro* dissolution studies because we have to control the agitation rate so that we get similar results *in vitro* as we would *in vivo*.

- The apparent thickness of the stagnant layer can be reduced when the drug dissolves into a reactive medium. For example, with a weakly basic drug in an acidic medium, the drug will react (ionize) with the diffusing proton (H^+) and this will result in an effective decrease in the thickness of the stagnant layer.

- The effective thickness is now h' not h. Also the bulk concentration of the drug is effectively zero. For this reason weak bases will dissolve more quickly in the stomach.

3. Diffusion coefficient, D:

- The value of D depends on the size of the molecule and the viscosity of the dissolution medium. Increasing the viscosity will decrease the diffusion coefficient and thus the dissolution rate. This could be used to produce a sustained release effect by including a larger proportion of something like sucrose or acacia in a tablet formulation.

4.Drug solubility, Cs:

- Solubility is another determinant of dissolution rate. As Cs increases so does the dissolution rate. We can look at ways of changing the solubility of a drug.

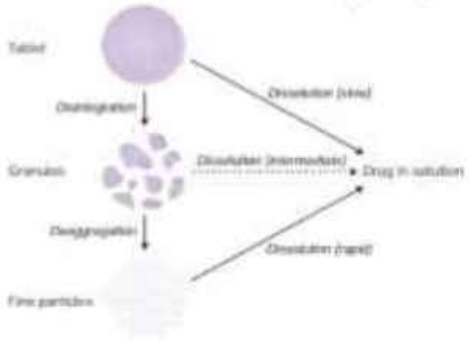
Additional parameters related to diffusion in DRUG RELEASE-

1. Considering the sink condition, the factor affecting the apparent rate of the release of the core molecule.
2. The diffusion path length.
3. Molecular collision radius of the diffusing substance.
4. Viscosity of the diffusing environment.
5. Surface area of the dosage form in contact.
6. Concentration difference between the start of the molecular diffusion and sink condition.

DISSOLUTION PARAMETER

Defination:

The transfer of molecules or ions from a solid state into solution is known as dissolution. Dissolution and then diffusion is a Pre-requisite for the drug absorption. Physicochemically , "Dissolution is the process by which a solid substance enters the solvent phase to yield a solution". Dissolution (release of the drug from the dosage form) is of primary importance for all conventionally constructed, solid oral dosage forms in general, and for modified-release dosage forms in particular, and can be the rate limiting step for the absorption of drugs administered orally.



Dissolution Rate: Dissolution rate may be defined as amount of drug substance that goes in the solution per unit time under standard conditions of liquid/solid interface, temperature and solvent composition. The rate of dissolution quantifies the speed of the dissolution process.

Importance & need for dissolution testing:

Dissolution testing is of utmost importance in following aspects-

- As **reliable Predictor** of in vivo dissolution performance of drug.
- A **Rate limiting factor** in determining the physiological availability of drug.
- As **Quality control tool** for monitoring the uniformity and reproducibility of production batches.
- As **Research tool** in optimizing parameters and ingredients in new drug formulation.
- It is **widely accepted** as animal experimentation has been restricted under the Act of Prevention of cruelty of animals.

Parameters in the Dissolution process-

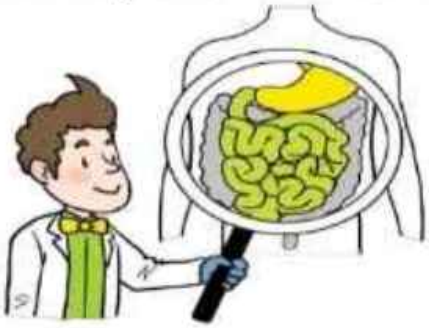
1. Effects of agitation.
2. Effect of dissolution fluid.
3. Influence of PH of dissolution fluid.
4. Effect of surface tension of the dissolution medium.
5. Effect of viscosity of the dissolution medium.
6. Effect of the presence of unreactive & reactive additives in the dissolution medium.
7. Volume of dissolution medium & sink conditions.
8. Deaeration of the dissolution medium.
9. Effects of temperature of the dissolution medium.

1. Effects of agitation

- The relationship between the intensity of agitation & the rate of dissolution varies considerably according to the type of agitation used, degree of laminar & turbulent flow in the system , the shape & design of the stirrer & the physiochemical properties of the solid.
- Dissolution test used high speed agitation may lack discriminative value & can yield misleading results.
- Accordingly, the compendial methods in general, are conducted under relatively low agitation.
- For the basket method, 100 rpm usually is utilized, while for the paddle procedure, a 50-75 rpm is recommended.

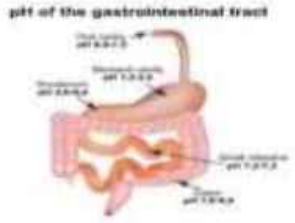
2. Effect of dissolution fluid

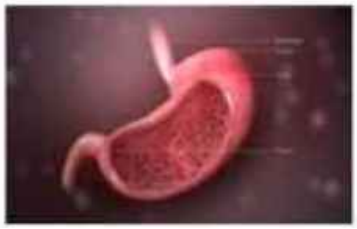
- Selection of proper medium for dissolution testing depends largely on the physicochemical properties of the drug.
- In the early 1960s, when dissolution was still in its infancy, great effort was spent on emulating the in vivo conditions in the gastrointestinal tract, especially pH, surface tension, viscosity and sink conditions.



3. Influence of PH of dissolution fluids

- In 1949, a committee assigned by the American drug and pharmaceutical manufacturers associations recommended the use of distilled water as the test medium for the disintegration test.
- It was observed that in many instances water and dilute acid gave closely comparable results.
- USP V included simulated gastric fluid as the test medium for tablets containing ingredients which reacted more readily in acid solution than in water (e.g., calcium carbonate).
- The medium again was changed to water in the USP VII.





- Changes in pH exert the greatest effect in terms of drug solubility.
- For weak acids, the dissolution rate increases with increasing pH, whereas, for weak bases, the dissolution rate increases with decreasing pH.
- [average pH of stomach in men is 1.9 and 2.5 in women].
- Therefore for acetylsalicylic acid (pKa =3.5) tablets and capsules, the dissolution rate would be expected to increase if the pH of the dissolution medium was higher than 3.
- For tablets containing active ingredients, whose solubilities are independent of pH, the dissolution rate does not vary significantly with changes in pH of the dissolution medium unless they contain certain excipients that are influenced by pH.
- For example, Tablets that are formulated with carbon dioxide producing compounds, such as sodium bicarbonate, magnesium carbonate or calcium carbonate, tend to have slightly faster dissolution rate in acid medium than in water because rapid disintegration increases the effective surface area.

4. Effect of surface tension of the dissolution medium

- According to the diffusion film theory, dissolution of the drug is governed by the interplay between two processes, the release of the drug from the solid surface and its transfer throughout the bulk of the dissolution medium.
- If the drug is hydrophobic the dissolution rate is influenced primarily by the release processes, whereas, for hydrophilic drugs the transfer process is more likely to be the rate limiting step.
- Incorporation of surface active agents in the dissolution medium, is expected to enhance the dissolution rate of a poorly soluble drug in solid dosage forms by reducing the interfacial tension and micelle formation.
- Addition of surfactant below the Critical micelle concentration (CMC) can increase significantly the dissolution rate because of better penetration of the solvent into the tablet resulting in greater availability if drug surface.

5. Effect of viscosity of the dissolution medium

- If the interaction at the interfaces, occurs much faster than the rate of transport, such as in the case of diffusion controlled dissolution processes, it would be expected that the dissolution rate decreases with an increase in viscosity.
- The rate of dissolution of zinc in HCl solution containing sucrose was inversely proportional to the viscosity of solution.
- The stokes-einstein equation expresses the diffusion coefficient as a function of viscosity, as can be seen from the following treatment.

$$D = \mu kT$$

- μ = mobility (velocity at a force of one dyne)
- k = boltzmann constant ($1.38 \times 10 - 16$)

6. Effect of the presence of unreactive & reactive additives in the dissolution medium.

- When neutral ionic compounds, such as sodium chloride and sodium sulfate, or non ionic organic compounds, such as dextrose, were added to the dissolution medium the dissolution of benzoic acid was dependent linearly upon its solubility in the particular solvent.
- When certain buffers or bases were added to the aqueous solvent , an increase in the dissolution rate was observed.

7. Volume of dissolution medium and sink conditions

- The proper volume of the dissolution medium depends mainly on the solubility of the drug in the selected fluid.
- If the drug is poorly soluble in water, a relatively large amount of fluid should be used if complete dissolution is to be expected.
- In order to maintain the effect of the concentration gradient and maintain sink conditions, the concentration of the drug should not exceed 10 – 15% of its maximum solubility in the dissolution medium selected.

8. Deaeration of the dissolution medium

- Presence of dissolved air or other gases in the dissolution medium may influence the dissolution rate of certain formulations and lead to variable and unreliable results.
- Example, the dissolved air in distilled water could significantly lower its pH and consequently affect the dissolution rate of drugs that are sensitive to pH changes, e.g., weak acids.
- Another serious effect is the tendency of the dissolved air to be released from the medium in the form of tiny air bubbles that circulate at random and invariably affect the of the hydrodynamic flow pattern generated by the stirring mechanism.
- The gathering of air bubbles on the solid surface could also lead to a reduction in the specific gravity to the point where the tablet, or its disintegrating powder bed, float to the top of he basket in the liquid medium with a minimum chance of being wetted efficiently.

9. Effect of temperature of the dissolution medium

- Drug solubility is temperature dependent, therefore careful temperature control during the dissolution process is extremely important.
- Generally a temperature of 37°±0.5 is maintained during dissolution determination of oral dosage forms and suppositories.
- For topical preparations as low as 30° and 25°have been used.
- The effect of temperature variations of the dissolution medium depends on the temperature/ solubility curves of the drug and the excipients in the formulation.
- Carstetensen pointed out that for a diffusion coefficient D is dependent upon the temperature according to equation $D = UkT$

• U = mobility (defined as the velocity when exposed to a force of one dyne)

• k = boltzmann constant

• T = absolute temperature

Pharmacokinetics parameters

- Pharmacokinetics is defined as the kinetics of drug absorption, distribution, metabolism, and excretion and their relationship with pharmacologic, therapeutic or toxicologic response in mans and animals.

Plasma drug concentration-time profile

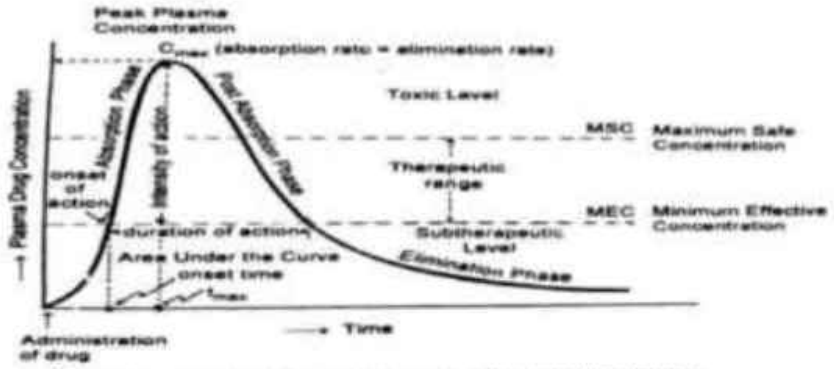
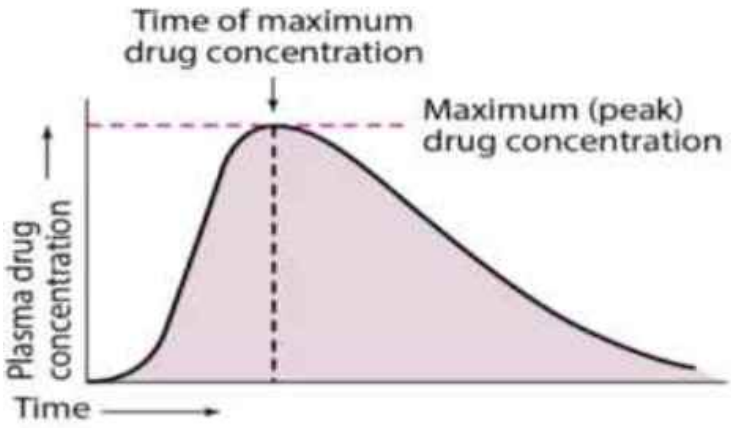
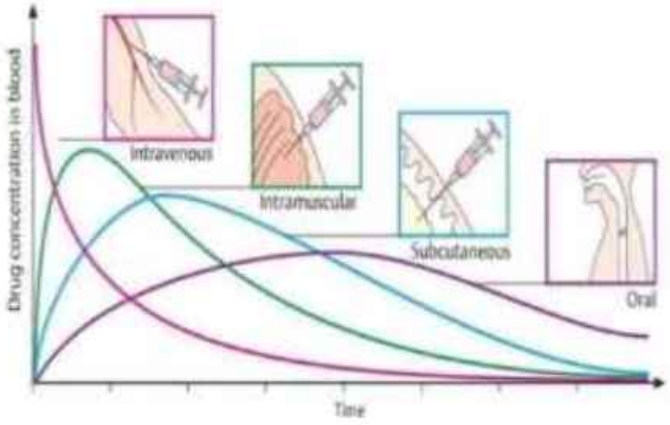


Fig. 9.1 A typical plasma concentration-time profile showing pharmacokinetic and pharmacodynamic parameters obtained after oral administration of single dose of a drug

Three important pharmacokinetic parameters:

1. Peak plasma concentration (Cmax)
2. Time of peak concentration (tmax)
3. Area under the curve (AUC)





1. Peak plasma concentration (Cmax)

- The point of maximum concentration of a drug in plasma is called as peak and the concentration of drug at peak is known as peak plasma concentration.
- It is also called as peak height concentration and maximum drug concentration.
- Cmax is expressed in mcg/ml.

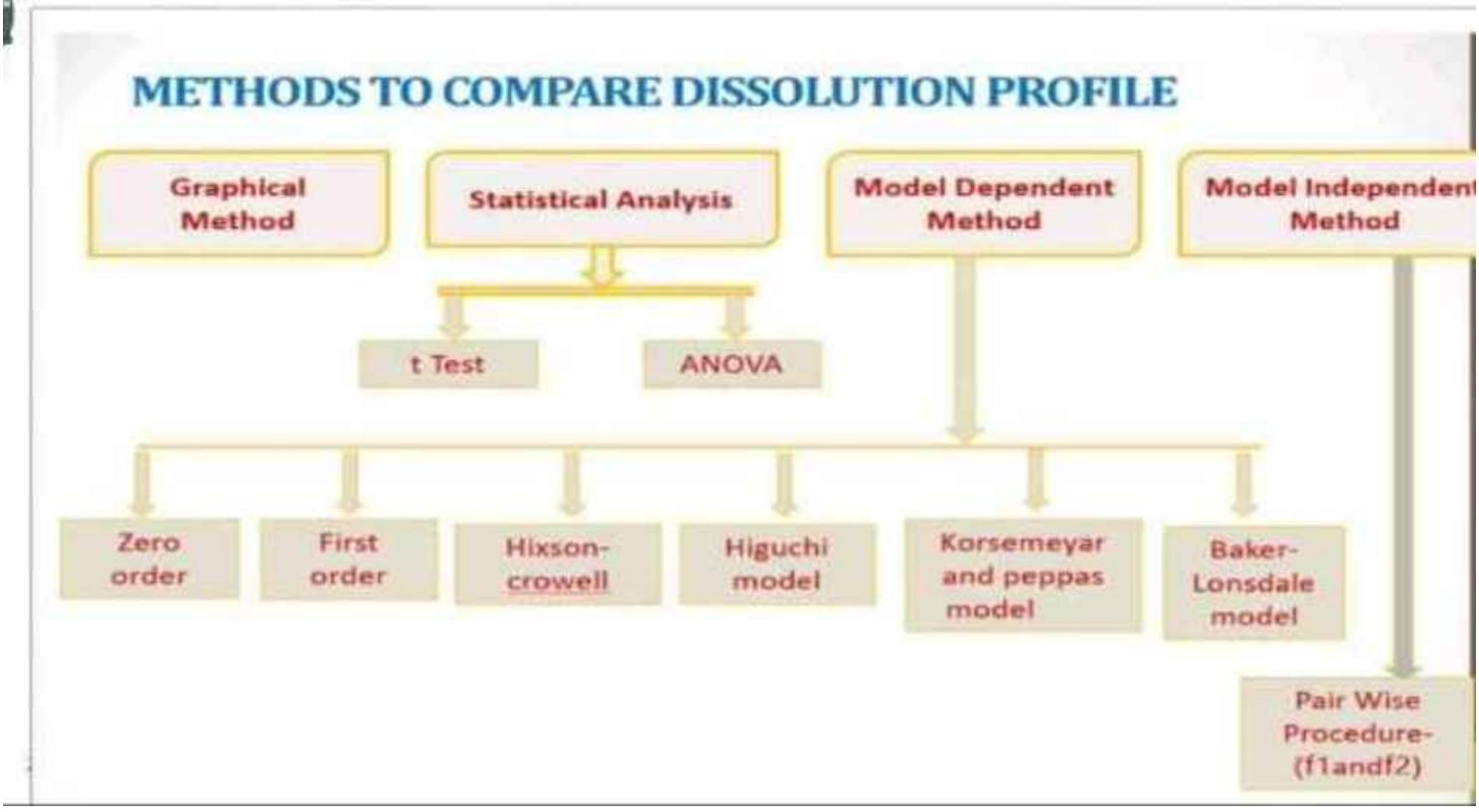
2. Time of peak concentration (Tmax)

- The time for drug to reach peak concentration in plasma (after extravascular administration) is called the time of peak concentration.
- It is expressed in hours.
- Onset time and onset of action is dependent upon tmax.
- The parameter is of particular importance in assessing the efficacy of drugs used to treat acute conditions like pain and insomnia.

3.Area under the curve (AUC)

- It represents the total integrated area under the plasma level-time profile and expresses the total amount of drug that comes into the systemic circulation after its administration.
- AUC is expressed in mcg/ml X HRS.
- It is important for the drugs that are administered repetitively for the treatment of chronic conditions like asthma or epilepsy.

Similarity factors- f2 and f1



DIFFERENCE FACTOR (f1) & SIMILARITY FACTOR(f2)

DIFFERENCE FACTOR (f1)

- The difference factor (f1) as defined by FDA calculates the % difference between 2 curves at each time point and is a measurement of the relative error between 2 curves.

$$f_1 = \left\{ \frac{\left\{ \sum_{t=1}^{n} |Rt - Tt| \right\}}{\sum_{t=1}^{n} Rt} \right\} \times 100$$

where, n = number of time points

Rt = % dissolved at time t of reference product (pre change)

Tt = % dissolved at time t of test product (post change)

SIMILARITY FACTOR(f2)

The similarity factor (f2) as defined by FDA is logarithmic reciprocal square root transformation of sum of squared error and is a measurement of the similarity in the percentage (%) dissolution between the two curves

$$f2 = 50 \times \log\left[\left\{\left[1+\frac{1}{n}\sum_{r=1}^{n} wt(Rt-Tt)\right\}^{-0.5} \times 100\right]\right]$$

Limits for similarity and Difference factors

Difference factor	Similarity factor	inference
0	100	Dissolutions profile are similar
≤15	≥50	Similarity or equivalence of two profiles

Advantages:

1. They are easy to produce.

They provide single number to describe the comparision of dissolution profile data.

Disadvantages:

1. The values of f1 & f2 are sensitive to the number of dissolution time point used.
2. If the test & reference formulation are interchanged , f2 is unchanged but f1 is not, yet difference between two mean profile remains same.
3. The basis of criteria for deciding the difference or similarity between dissolution profile is unclear.

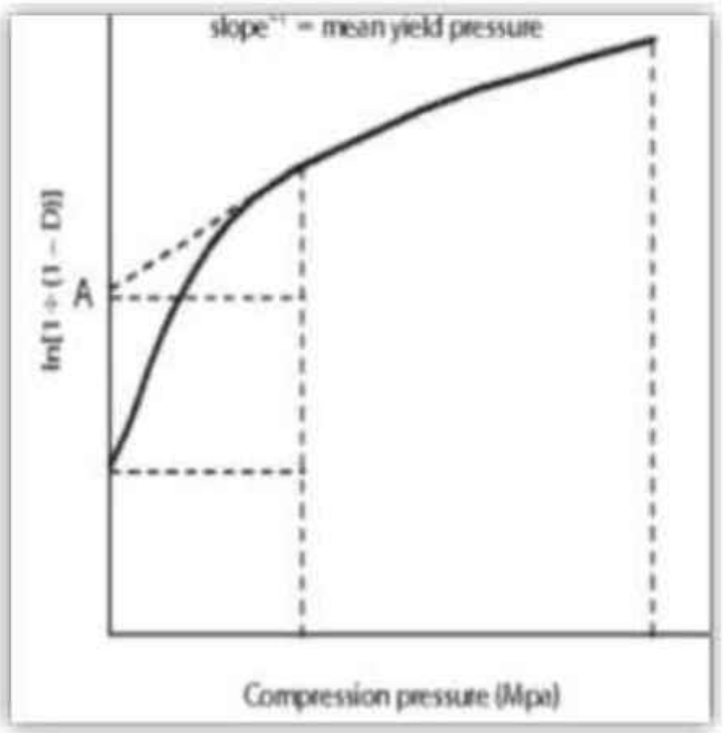
The evaluation of similarity between dissolution profile is based on following conditions:

- Minimum of three dissolution time points are measured.
- Number of drug products tested for dissolution is 12 for both test & reference.
- Not more than one mean value of >85% dissolved for each product.
- Standard deviation of mean of any product should not be more than 10% from 2nd to last dissolution time point.

Heckel plot

- In 1961, Heckel postulated a linear relationship **between the relative porosity (Inverse density) of a powder and the applied pressure.**
- Plotting the value of [1/(1-D)] against **applied pressure, P,** yields a linear graph having **slope, K** and **intercept, A**.
- **Reciprocal of K i.e: Heckel Constant,** yields a material-dependent constant known as **Yield Pressure**.
- The **slope** of the linear regression is **Heckel constant**.
- **Large** value of Heckel constant indicate **susceptibility to plastic deformation at low pressure.**

- **Yield Pressure** is inversely relatable to the ability of the material to deform plastically under pressure.
- **Low** values of yield pressure indicate a **faster onset of plastic deformation.**
- Intercept of the line indicates **degree of densification** by rearrangement.
- Heckel plot allows for **interpretation** of the **mechanism of bonding**.


slope^{-1} = mean yield pressure
ln[1 ÷ (1 – D)]
A
Compression pressure (Mpa)

HIGUCHI EQUATION (DIFFUSION MATRIX FORMULATION)

- Higuchi proposed this model in 1961 to describe drug release from matrix system. It is developed to study the water soluble and low soluble drugs incorporated in semisolids and solid matrices.
- It is based on the hypothesis that;

1. Initial drug concentration in the matrix is much higher than drug solubility.
2. Drug diffusion takes place in only one direction (edge effect must be negligible).
3. Drug particles are much smaller than system thickness.
4. Matrix swelling and dissolution are negligible.
5. Drug diffusivity is constant.
6. Perfect sink conditions are always attained in the release environment.

- Higuchi model is given by

$$Q=K_H.\sqrt{T}\ (or)\ Q=A\sqrt{D(2C-Cs)Cs.\ T}$$

where,

Q= Amount of drug released in time 't' per unit area.

K= Higuchi constant.

T= Time (in hours).

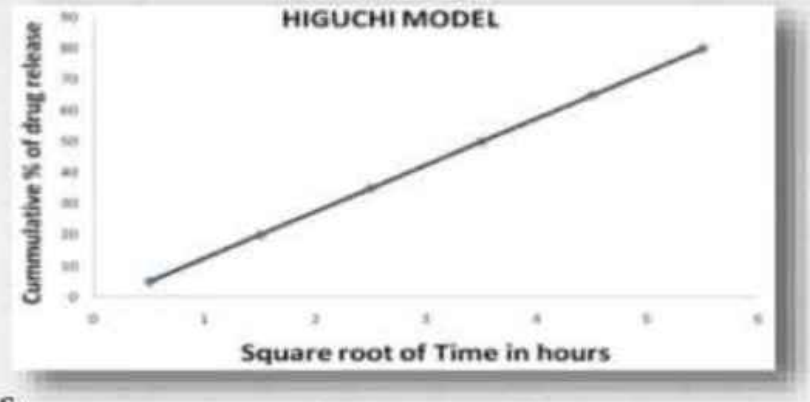
C= Initial frug concentration

Cs= Drug solubility in matrix media.

D= Diffusivity of drug molecules in solvent.

PLOT:

- The data obtained were plotted as cumulative percentage drug release Vs square root of time.



APPLICATIONS:

- Modified release pharmaceutical dosage forms , transdermal systems and matrix tablets with water soluble drugs.

KORSMEYER - PEPPAS PLOT

- The KORSEMEYER AND PEPPAS described this method.
- It is given by the equation :

$$Mt / M_{\infty} = Kt^{n}$$

Where,

Mt / M_{∞} is fraction of drug released

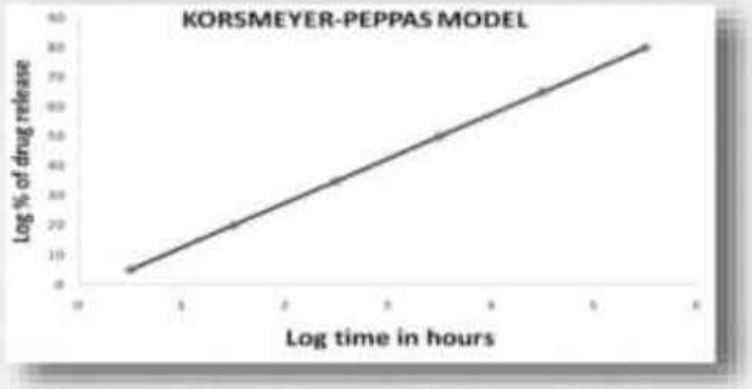
t = time K=constant includes structural and geometrical characteristics of the dosage form

n= release component which is indicative of drug release mechanism where , n is diffusion exponent.

- If n= 1 , the release is zero order, n = 0.5 the release is best described by the Fickian diffusion $0.5 < n < 1$ then release is through anomalous diffusion or case two diffusion.
- In this model a plot of percent drug release versus time is linear.

PLOT:

- The data obtained were plotted as log cumulative percentage drug release Vs log time.



APPLICATIONS:

- It is applicable to linearization of release data from microcapsules and microspheres.

LINEARITY CONCEPT OF SIGNIFICANCE

- Tests of statistical significance are invariably applied now-a-days by research scientists. Good medical journals refuse to accept papers for publication if the authors have not used the philosophy of significance testing in evaluating their results.
- It is not adequate to mechanically undertake significance tests , the scientists/experimental workers must fully understand the basic concepts underlying a significance test, the assumptions involved and the limitations, for making the proper interpretations.
- Generally, the existence of statistical significance difference is regarded as a proof of the existence of an important difference between two sample results. Similarly, the non-significant differences are regarded as proof of no differences in two sample results.

STANDARD DEVIATION

- Standard Deviation is a number that describes how much data vary or spread out.
- It uses difference of each data value from the mean.
- The higher the number, more data is spread out.
- Finding Standard Deviation

1. Find the mean, $\bar{x}$
2. Find the difference of each data value from the mean,

$$x - \bar{x},$$

3. Square each difference

$$(x_i - \bar{x})^2$$

4. Sum all the squared values
5. Divide by the number of data taken by the sample minus 1.
6. Take the square root

$$\sigma = \sqrt{\frac{\sum_{i}^{n} (x_i - \bar{x})^2}{n - 1}}$$

CHI-SQUARE TEST

- The chi-square test is an important test amongst the several tests of significance developed by statisticians.
- It was developed by Karl Pearson in 1900.
- CHI SQUARE TEST is a non parametric test not based on any assumption or distribution of any variable.
- This statistical test follows a specific distribution known as chi square distribution.
- In general, the test we use to measure the differences between what is observed and what is expected according to an assumed hypothesis is called the chi-square test.

Chi-Square as a Parametric Test

Test for comparing variance

$$\chi^2 = \frac{\sigma_s^2}{\sigma_p^2}(n-1)$$

Chi-Square as a Non-Parametric Test

- Test of Goodness of Fit
- Test of Independence

$$\chi = \sum \left[\frac{(O-E)^2}{E} \right]$$

Where, O= observed frequencies

E= expected frequency

AS A TEST OF GOODNESS OF FIT

- It enables us to see how well does the assumed theoretical distribution (such as Binomial distribution, Poisson distribution or Normal distribution) fit to the observed data. When the calculated value of $\chi 2$ is less than the table value at certain level of significance, the fit is considered to be good one and if the calculated value is greater than the table value, the fit is not considered to be good.

AS A TEST OF INDEPENDENCE

- $\chi 2$ test enables us to explain whether or not two attributes are associated. Testing independence determines whether two or more observations across two populations are dependent on each other (i.e., whether one variable helps to estimate the other). If the calculated value is less than the table value at certain level of significance for a given degree of freedom, we conclude that null hypotheses stands which means that two attributes are independent or not associated. If calculated value is greater than the table value, we reject the null hypotheses.

Observed frequency	Expected frequency	(O-E)	(O-E)2	(O-E)2 / E
63	60	3	9	0.15
45	60	-15	225	3.75
72	60	12	144	2.4
				6.3

Steps involved are:

1. Determine The Hypothesis:

- Ho : The two variables are independent
- Ha : The two variables are associated

2. Calculate Expected frequency

$$E = \frac{\text{(Row total) (Column total)}}{\text{Grant total}}$$

3. Calculate test statistic

$$\chi^2 = \sum \left[\frac{(O-E)^2}{E} \right]$$

4. Determine degree of Freedom

$$Df = (R-1)(C-1)$$

Where, R= number of levels in row variable and C= number of levels in column variable.

5. Compare computed test statistic against a tabled/critical value

The computed value of the Pearson chi-square statistic is compared with the critical value to determine if the computed value is improbable The critical tabled values are based on sampling distributions of the Pearson chi-square statistic. If calculated $\chi 2$ is greater than the $\chi 2$ table value, reject Ho.

STUDENT'S t- test

- The test is used to compare samples from two different batches.
- It is usually used with small (<30) samples that are normally distributed.
- Types of "t" test are:
- Single sample t test – we have only 1 group; want to test against a hypothetical mean.
- Independent samples t test – we have 2 means, 2 groups; no relation between groups, E.g.: When we want to compare the mean of T/T group with Placebo group.
- Paired t test – It consists of samples of matched pairs of similar units or one group of units tested twice. E.g.: Difference of mean pre & post drug intervention.

- **Equation for a one-sample t-test**

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}}$$

Where, t= the t statistic

$\bar{x}$= the mean of the sample

μ= the comparison mean

s= the sample standard deviation

n= the sample size

- **Equation for the Independent samples t-test**

The independent samples t-test procedure compares means for two groups of cases.

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\left[\frac{SS_1 + SS_2}{n_1 + n_2 - 2}\right]\left[\frac{1}{n_1} + \frac{1}{n_2}\right]}}$$

Here,

$\overline{X}_1$ and $\overline{X}_2$ are the means of the two different groups

$n_1 = n$ of Group 1

$n_2 = n$ of Group 2

SS = sum of squares

Paired t-test

- A paired t-test is used to compare two population means where you have two samples in which observations in one sample can be paired with observations in the other sample.
- A comparison of two different methods of measurement or two different treatments where the measurements/treatments are applied to the same subjects.
- E.g.:

1. pre-test/post-test samples in which a factor is measured before and after an intervention
2. Cross-over trials in which individuals are randomized to two treatments and then the same individuals are crossed-over to the alternative treatment
3. Matched samples, in which individuals are matched on personal characteristics such as age and sex

- Suppose a sample of "n" subjects were given an antihypertensive drug we want to check blood pressure before and after treatment . We want to find out the effectiveness of the treatment by comparing mean pre & post t/t.
- To test the null hypothesis that the true mean difference is zero, the procedure is as follows:

1. Calculate the difference (di = yi − xi) between the two observations on each pair.
2. Calculate the mean difference, d.
3. Calculate the standard error of the mean differences. S.E=S.D/$\sqrt{n}$
4. Calculate the t-statistic, which is given by T = d/S.E, Under the null hypothesis, this statistic follows a t-distribution with n − 1 degrees of freedom.
5. Use tables of the t-distribution to compare your value for T to the t_{n-1} distribution. This will give the p-value for the paired t-test.

ANALYSIS OF VARIANCE (ANOVA)

- The analysis of variance(ANOVA) was developed by R. A. Fisher in 1920.
- It is possible to study the significance of the difference of mean values of a large number of samples at the same time because of ANOVA.
- The ANOVA is classified into two ways:

1. One-way classification
2. Two-way classification

- In one-way classification we take into account the effect of only one variable.
- If there is a two-way classification, the effect of two variables can be studied.

- The basic principle of ANOVA is to test for differences among the means of the populations by examining the amount of variation within each of these samples, relative to the amount of variation between the samples.

One-way (or single factor) ANOVA:

- t is the simplest type of ANOVA, in which only one source of variation, or factor, is investigated.
- It is an extension to three or more samples of the t test procedure for use with two independent samples.
- In another way t test for use with two independent samples is a special case of one-way analysis of variance.

The technique involves the following steps:

1. Obtain the mean of each sample i.e.,

$$\overline{X_1}, \overline{X_2}, \overline{X_3}, \ldots, \overline{X_K}$$

2. Find the mean of the sample means:

$$\overline{\overline{X}} = \frac{\overline{X_1} + \overline{X_2} + \overline{X_3} + \ldots + \overline{X_K}}{No.ofSamples(K)}$$

3. Calculate the sum of squares for variance between the samples (or SS between):

$$SSbetween = n_1\left(\overline{X_1} - \overline{\overline{X}}\right)^2 + n_2\left(\overline{X_2} - \overline{\overline{X}}\right)^2 + \ldots + n_K\left(\overline{X_K} - \overline{\overline{X}}\right)^2$$

4. Calculate Mean Square (MS) between:

$$\text{MS Between} = \frac{\text{SS between}}{(K-1)}$$

5. Calculate the sum of squares for variance within the samples (or SS within):

$$\text{SS within} = \sum(x_{1i} - \bar{x}_1)^2 + \sum(x_{2i} - \bar{x}_2)^2 + \ldots + \sum(x_{ki} - \bar{x}_k)^2$$

6. Calculate Mean Square (MS) within:

$$\text{MS within} = \frac{\text{SS within}}{(n-k)}$$

7. Calculate SS for total variance:

$$SS \text{ for total variance} = \sum \left(X_{ij} - \overline{\overline{X}} \right)^2$$

- SS for total variance = SS between + SS within
- The degrees of freedom for between and within must add up to the degrees of freedom for total variance i.e.,

$$(n-1) = (k-1) + (n-k)$$

8. Finally, F-ratio may be worked out as under:

$$F\text{-ratio} = \frac{MS \text{ between}}{MS \text{ within}}$$

- This ratio is used to judge whether the difference among several sample means is significant or its just a matter of sampling fluctuations.

Two Way ANOVA

- Two-way ANOVA technique is used when the data are classified on the basis of two factors.
- For example, the agricultural output may be classified on the basis of different varieties of seeds and also on the basis of different varieties of fertilizers used.
- It is a statistical test used to determine the effect of two nominal predictor variables on a continuous outcome variable.
- two-way ANOVA test analyzes the effect of the independent variables on the expected outcome along with their relationship to the outcome itself.
- two-way design may have repeated measurements of each factor or may not have repeated values.

- Types of two-way ANOVA

1. ANOVA technique in context of two-way design when repeated values are not there.
2. ANOVA technique in context of two-way design when repeated values are there.

❑ **ANOVA technique in context of two-way design when repeated values are not there.**

- It includes calculation of residual or error variation by subtraction, once we have calculated the sum of squares for total variance and for variance between varieties of one treatment as also for variance between varieties of the other treatment.

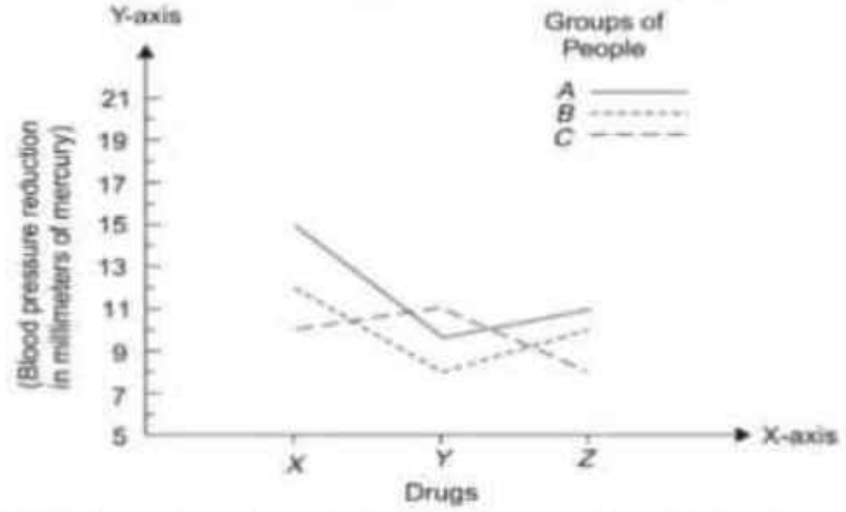
❑ **ANOVA technique in context of two-way design when repeated values are there.**

- We can obtain a separate independent measure of inherent or smallest variations.
- Interaction variation: Interaction is the measure of inter relationship among the two different classifications.

Graphical method for studying interaction in two-way design.

- For graphs we shall select one of the factors to be used as the x-axis.
- Then we plot the averages for all the samples on the graph and connect the averages for each variety of other factor by a distinct line.
- If the connecting lines do not cross over each other, then the graph indicates that there is no interaction.
- But if the lines do cross, they indicate definite interaction or inter-relation between the two factors.

Graph of the averages for amount of blood pressure reduction in millimeters of mercury for different drugs and different groups of people.*



The graph indicates that there is a significant interaction because the different connecting lines for groups of people do cross over each other. We find that A and B are affected very similarly, but C is affected differently.

APPLICATIONS OF ANOVA

- Similar to t-test.
- More versatile than t-test.
- ANOVA is the synthesis of several ideas & it is used for multiple purposes.
- The statistical Analysis depends on the design and discussion of ANOVA therefore includes common statistical designs used in pharmaceutical research.
- In the bioequivalence studies the similarities between the samples will be analyzed with ANOVA only.
- Pharmacokinetic data also will be evaluated using ANOVA.
- Pharmacodynamics (what drugs does to the body) data also will be analyzed with ANOVA only.