PREFORMULATION

Definition:

Preformulation may be described as a phase of the research and development process where the preformulation scientist characterizes the physical, chemical and mechanical properties of a new drug substance, in order to develop stable, safe and effective dosage form.

Objectives:

The preformulation investigations confirm that there are no significant barriers to the compound's development as a marketed drug. The formulation scientist uses these informations to develop dosage forms.

Preformulation is a multidisciplinary development of a drug candidate. See TABLE-1

Principal areas of preformulation

1. Bulk characterization
   - (i) Crystallinity and polymorphism
   - (ii) Hygroscopicity
   - (iii) Fine particle characterization
   - (iv) Powder flow
2. Solubility analysis
   - (i) Ionization constant – pKa
   - (ii) pH solubility profile
   - (iii) Common ion effect – K_{SP}.
   - (iv) Thermal effects
   - (v) Solubilization
   - (vi) Partition coefficient
   - (vii) Dissolution
3. Stability Analysis
   - (i) Stability in toxicology formulation
   - (ii) Solution stability
     – pH stability profile
   - (iii) Solid state stability
     - Bulk stability
     - Compatibility

1. Bulk characterization

When a drug molecule is discovered all the solid-forms are hardly identified. So during bulk characterization the following characteristics are studied.

(i) Crystallinity and polymorphism

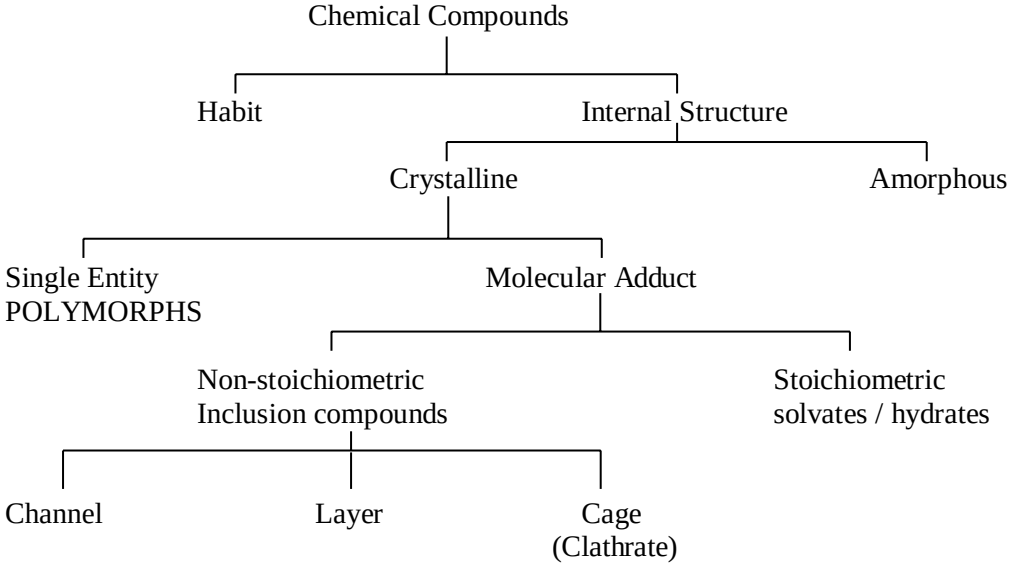
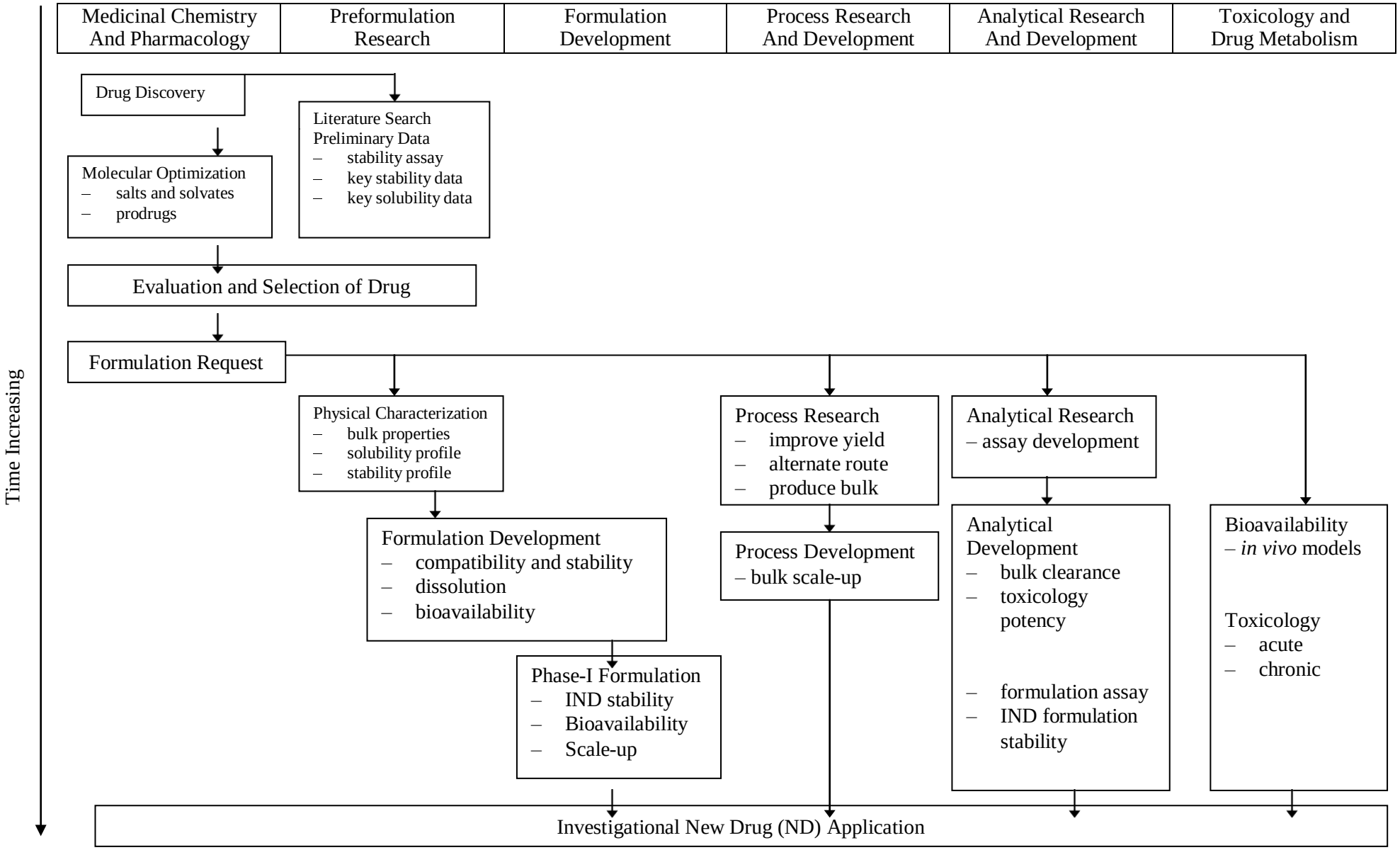


TABLE –1


Medicinal Chemistry And Pharmacology
Preformulation Research
Formulation Development
Process Research And Development
Analytical Research And Development
Toxicology and Drug Metabolism
Time Increasing
Drug Discovery
Molecular Optimization
– salts and solvates
– prodrugs
Literature Search
Preliminary Data
– stability assay
– key stability data
– key solubility data
Evaluation and Selection of Drug
Formulation Request
Physical Characterization
– bulk properties
– solubility profile
– stability profile
Formulation Development
– compatibility and stability
– dissolution
– bioavailability
Phase-I Formulation
– IND stability
– Bioavailability
– Scale-up
Process Research
– improve yield
– alternate route
– produce bulk
Process Development
– bulk scale-up
Analytical Research
– assay development
Analytical Development
– bulk clearance
– toxicology potency
– formulation assay
– IND formulation stability
Bioavailability
– *in vivo* models
Toxicology
– acute
– chronic
Investigational New Drug (ND) Application

- Flowability of powder and chemical stability depends on the habit and internal structure of a drug.

Habit is the description of the outer appearance of a crystal. A single internal-structure for a compound can have several different habits, depending on the environment for growing crystals. Different habits of crystals are given below.

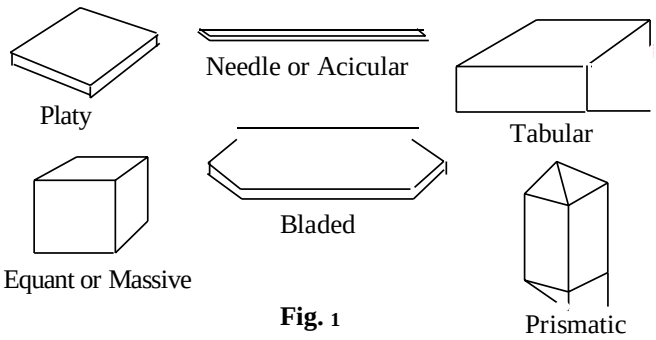


Fig. 1

Internal Structure

Crystalline state

In this state of matter atoms or molecules are arranged in highly ordered form and is associated with three-dimensional periodicity.

[N.B. Atoms or molecules tend to organize themselves into their most favorable thermodynamic state, which under certain conditions results in their appearance as crystals.
N.B. The repeating three-dimensional patterns are called crystal lattices. The crystal lattice can be analyzed from its X-ray diffraction pattern.]

Amorphous forms

In this forms the solids do not have any fixed internal structure. They have atoms or molecules randomly placed as in a liquid.
e.g. Amorphous Novobiocin
[N.B. Amorphous forms are prepared by rapid precipitation, lyophillization or rapid cooling of molten liquids e.g. glass]

Difference between crystalline and amorphous form

Crystalline forms	Amorphous forms
(i) Crystalline forms have fixed internal structure	(i) Amorphous forms do not have any fixed internal structure
(ii) Crystalline forms are more stable than its amorphous forms.	(ii) Amorphous form has higher thermodynamic energy than its crystalline form.
(iii) Crystalline forms are more stable than its amorphous forms.	(iii) Amorphous forms are less stable than its crystalline forms.
(iv) Crystalline form has lesser solubility than its amorphous form.	(iv) Amorphous forms have greater solubility than its crystalline forms.
(v) Crystalline form has lesser tendency to change its form during storage.	(v) Amorphous tend to revert to more stable forms during storage.

Polymorphs

When a substance exists in more than one crystalline form, the various forms are called Polymorphs and the phenomenon as polymorphism . e.g . Chloramphenicol palmitate has three polymorphs A, B and C .

[N.B. various polymorphs can be prepared by crystallizing the drug from different drugs under diverse conditions . Depending on their relative stability, one of the several polymorphic forms will be physically more stable than the others. Such a stable polymorph represents the lowest energy state, has highest melting point and least solubility. The representing polymorphs are called metastable forms which represents higher energy state, the metastable forms have a thermodynamic tendency to convert to the stable form . A metastable form cannot be called unstable because if it is kept dry, it will remain stable for years.]

Molecular Adducts

During the process of crystallization, some compounds have a tendency to trap the solvent molecules.

1. Non-Stoichiometric inclusion compounds (or adducts)

In these crystals solvent molecules are entrapped within the crystal lattice and the number of solvent molecules are not included in stoichiometric number. Depending on the shape they are of three types :-

(1) Channel

When the crystal contains continuous channels in which the solvent molecule can be included. e.g . Urea forms channel.

(2) ***Layers***:- Here solvent molecules are entrapped in between layers of crystals.

(3) ***Clathrates***(**Cage**):- Solvent molecules are entrapped within the cavity of the crystal from all sides.

2. Stoichiometric inclusion compounds (or stoichiometric adducts)

This molecular complex has incorporated the crystallizing solvent molecules into specific sites within the crystal lattice and has stoichiometric number of solvent molecules complexed.

When the incorporated solvent is water, the complex is called hydrates and when the solvent is other than water, the complex is called solvates. Depending on the ratio of water molecules within a complex the following nomenclature is followed.

(i) *Anhydrous* : 1 mole compound + 0 mole water
(ii) *Hemihydrate*: 1 mole compound + ½ mole water
(iii) *Monohydrate:* 1 mole compound + 1 mole water
(iv) *Dihydrate* : 1 mole compound + 2 moles water

Properties of solvates / hydrates

(i) Generally, the anhydrous form of a drug has greater aqueous solubility than its hydrates. This is because the hydrates are already in equilibrium with water and therefore have less demand for water. e.g. anhydrous forms of theophyline and ampicillin have higher aqueous solubility than the hydrates.
(ii) Non aqueous solvates have greater aqueous solubility than the non-solvates. E.g. chloroform solvates of griseofulvin are more water soluble than their nonsolvate forms.

ANALYTICAL METHODS FOR CHARACTERIZATION OF SOLID FORMS

Methods of studying solid forms are listed as below:

Method	Material required per sample
Microscopy	1 mg
Hot stage microscopy	1 mg
Differential Scanning Calorimetry (DSC)	2 – 5 mg
Differential Thermal Analysis (DTA)	2 – 5 mg
Thermogravimetric Analysis	10 mg
Infrared Spectroscopy	2 – 20 mg
X-ray Powder Diffraction	500 mg
Scanning Electron Microscopy	2 mg
Dissolution / Solubility Analysis	mg to gm

Microscopy

In this type of microscope light passes through cross-polarizing filters.

Amorphous substances (e.g. super-cooled glass and non-crystalline organic compounds or substances with cubic crystal lattices e.g. NaCl) have single refractive index. Through this type of microscope the amorphous substances do not transmit light, and they appear black. They are called isotropic substances.

Hot-stage microscopy

In this case, the polarizing microscope is fitted with a hot stage to investigate polymorphism, melting points, transition temperatures and rates of transition at controlled rates.
It facilitates in explaining the thermal behavior of a substance from the DSC and TGA curves.

[N.B. A problem often encountered during thermal microscopy is that organic molecules can degrade during the melting process, and recrystallization of the melt may not occur, because of the presence of contaminant degradation products.]

Thermal Analysis

Differential Thermal Analysis

In DTA instrument a record is produced where temperature difference (ΔT) (between the sample and reference material) is plotted against temperature (T) when two specimens are subjected to an identically controlled temperature regime.
The reference material is alumina, keiselguhr.

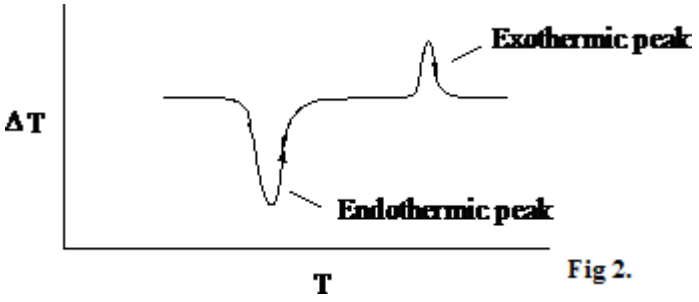


Fig 2.

Differential Scanning Calorimetry

In DSC method the difference in energy inputs (ΔH) into a sample and reference material is measured as a function of temperature as the specimens are subjected to a identically controlled temperature programme.

Samples that may be studied by DSC or DTA are:

Powders, fibres, single crystals, polymer films, semi-solids or liquids.

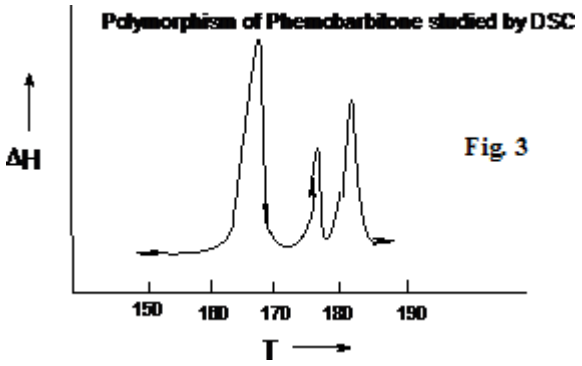


Fig. 3

Applications of DTA / DSC in preformulation studies

1. To determine the purity of a sample
2. To determine the number of polymorphs and to determine the ratio of each polymorph.
3. To determine the heat of solvation
4. To determine the thermal degradation of a drug or excipients.
5. To determine the glass-transition temperature (t_g) of a polymer.

Thermogravimetric Analysis (TGA)

TGA measures the changes in sample weight as a function of time (isothermal changes) or temperature.

Application of TGA in preformulation study

1. Desolvation and decomposition processes are monitored.
2. Comparing TGA and DSC data recorded under identical conditions can greatly help in the explanation of the thermal process.

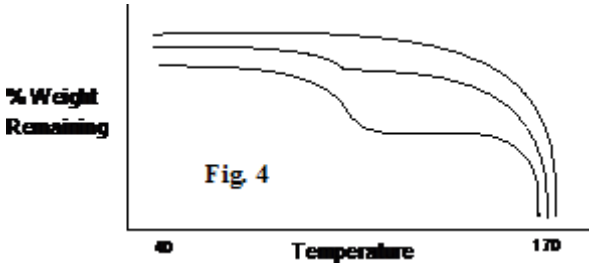


Fig. 4

TGA and DSC analysis of an acetate salt of an organic amine that has two crystalline forms, anhydrous and dihydrate are shown above. Anhydrous / dihydrate (10:1) mixture was prepared by dry blending. Heating rate was 5^0C/min.

From DSC curve, it is evident that the dihydrate form loses two molecules of water via an endothermic transition between 70^0C and 90^0C. The second endotherm at 155^0C corresponds to melting process.

From TA curve, it is evident that at 70 – 90^0C weight-loss was due to the loss two molecules of water and the weight loss at 155^0C was due to vaporization of acetic acid and decomposition.

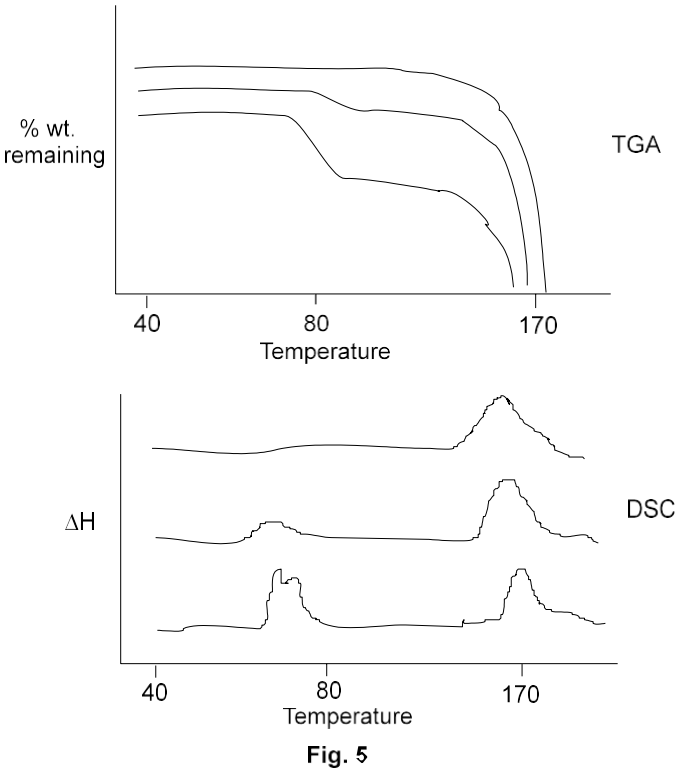


Fig. 5

X-RAY POWDER DIFFRACTION

When a X-ray beam falls on a powder the beam is diffracted. This diffraction in found only in case of crystalline powder. Amorphous forms do not show X-ray diffraction.

Uses:

(i) Each diffraction pattern is characteristic of a specific crystalline lattice for a given compound. So in a mixture different crystalline forms can be analyzed using normalized intensities at specific angles.

(ii) Identification of crystalline materials by using their diffraction pattern as a 'finger print'. First, the powder diffraction photograph or diffractometer trace are taken and matched with a standard photograph. All the lines and peaks must match in position and relative intensity.

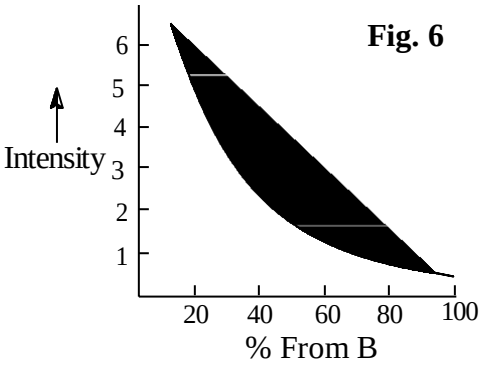


Fig. 6

HYGROSCOPICITY

Definition: Many pharmaceutical materials have a tendency to adsorb atmospheric moisture (especially water-soluble salt forms). They are called hygroscopic materials and this phenomenon is known as hygroscopicity.

Equilibrium moisture content depends upon:

(i) the atmospheric humidity
(ii) temperature
(iii) surface area
(iv) exposure time
(v) mechanism of moisture uptake.

Deliquescent materials:

They absorb sufficient amount of moisture and dissolve completely in it. (e.g. anhydrous calcium chloride).

Tests of hygroscopicity

Procedure
Bulk drug samples are placed in open containers with thin powder bed to assure maximum atmospheric exposure. These samples are then exposed to a range of controlled relative humidity (RH) environments prepared with saturated aqueous salt solutions.

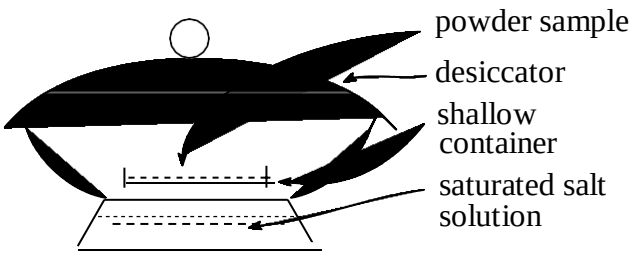


Fig. 7

The amount of moisture adsorbed can be determined by the following methods:
(i) Gravimetry
(ii) Thermogravimetric analysis (TGA)
(iii) Karl-Fischer titration (KF-titration)
(iv) Gas chromatography (GC)

Time of monitoring depends on the purpose:
(i) For the purpose of '*handling'* data points from 0 to 24 hours are taken
(ii) For the purpose of '*storage*' data points from 0 to 12 weeks are taken.

Significance of hygroscopicity test

(a)

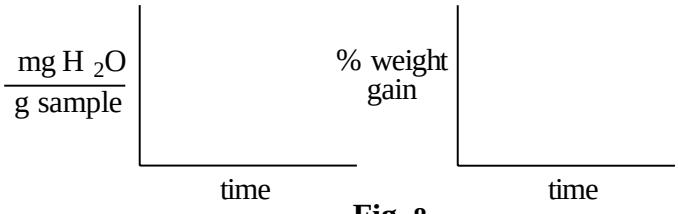


Fig. 8

To decide special handling procedure (with respect to time).

(b)

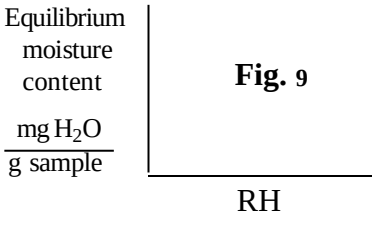


Fig. 9

To decide
(i) the storage condition i.e. at low humidity environment.
(ii) special packaging – e.g. with desiccant.

(c) Moisture level in a powder sample may affect the flowability and compactibility which, are important factors during tableting and capsule filling.

(d) After adsorption of moisture, if hydrates are formed then solubility of that powder may change affecting the dissolution characteristics of the material.
(e) Moisture may degrade some materials. So humidity of a material must be controlled.

FINE PARTICLE CHARACTERIZATION

Parameters those are measured:
(i) particle size and size-distribution
(ii) shape of the particle
(iii) surface morphology of the particles

Instrumental methods of particle size characterization

(i) Light microscope

First a standard graticule (BS 3625) is standardized with a stage micrometer. Then snall number of particles are spread over a glass slide and placed on the stage of the microscope. Particles are focussed and the particle diameters are measured. Several hundred particles are measured and reported as a histogram.

Disadvantage: The procedure is time consuming.

(ii) Stream counting devices

Examples: (a) Coulter counter – electrical sensing zone method
(b) HIAC – counter – optical sensing zone
(c) Malvern particle & droplet sizer – Laser diffraction method.

Procedure:

Samples prepared for analysis are dispersed in a conducting medium (e.g. saline) with the help of ultrasound and a few drops of surfactant (to disperse the particles uniformly). A known volume (0.5 to 2 ml) of this suspension is then drawn into a tube through a small aperture (0.4 to 800 μm diameter) across which a voltage is applied.

As each particle passes through the hole, it is counted and sized according to the resistance generated by displacing that particle's volume of conducting medium.

Size distribution is reported as histogram.

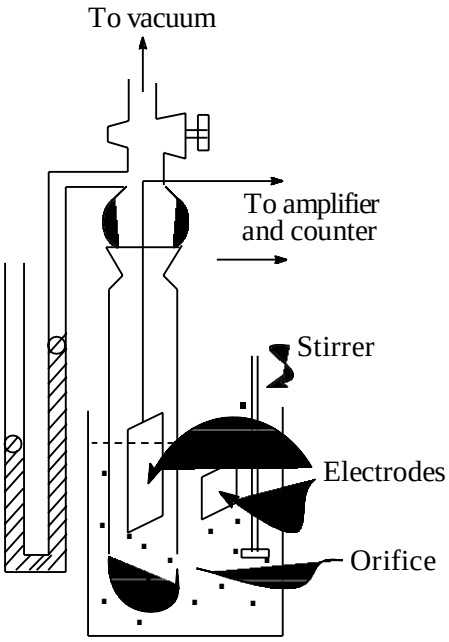


Fig. 10 Coulter counter

(iii) Sieve analysis

A powder sample is passed through a standard sieve set. The particle size is plotted against % weight retained on each sieve.

Use: This method is used generally for large samples.

Instrumental method for determination of specific surface area

Brunauer, Emmett and Teller (BET) nitrogen adsorption method:

A layer of nitrogen molecules is adsorbed to the sample surface at -196^0C. Once the surface is saturated, the sample is heated to room temperature, the nitrogen gas is desorbed, and its volume is measured and converted to the number of adsorbed molecules via the gas law. Since each N_2 molecule occupies an ara of 16 A^2, one may readily compute the surface area per gram of each pre-weighed sample.

Instrumental method for characterization of surface morphology

The scanning electron microscope creates the magnified images by using electrons instead of light waves. The images are black and white.

Procedure

- Biological materials are dried in a special way that prevents them from shrinking.
- Since SEM illuminates them with electrons, they are made conductive by coating with a very thin layer of gold by a machine called *sputter-coater*.
- The sample is placed inside the microscope's vacuum column through an airtight door.
- After the air is pumped out of the column, an electron gun emits a beam of high-energy electrons. This beam travels downward through a series of magnetic lenses designed to focus the electrons to a very fine spot.

- Near the bottom, a set of scanning coils moves the focussed beam back and forth across the specimen, row by row.
- As the electron beam hits each spot on the sample, secondary electrons are knocked loose from its surface. A detector counts these electrons and sends the signals to an amplifier.
- The final image is built up from the number of electrons emitted from each spot on the sample.

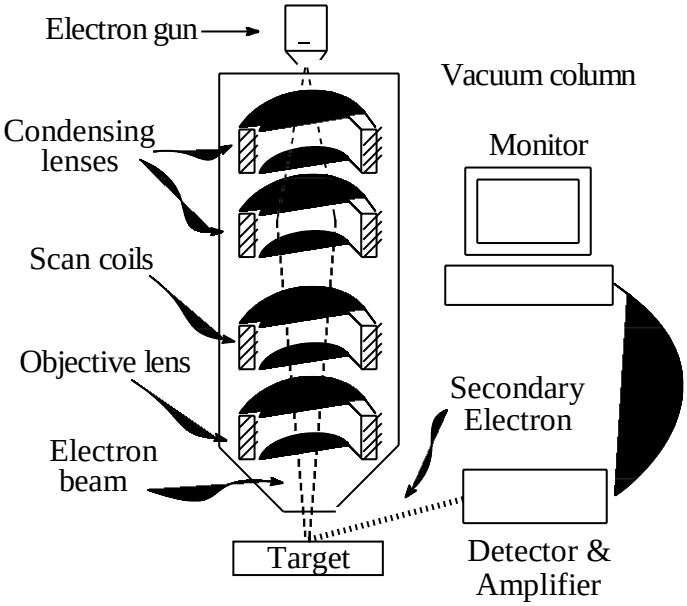


Fig. 11 Scanning Electron Microscope

BULK DENSITY

Apparent Bulk Density (g/cm^3)

Bulk drug powder is sieved through 40 mesh screen. Weight is taken and poured into a graduated cylinder via a large funnel. The volume is called *bulk volume*.

$$Apparent\,Bulk\;\; Density = \frac{Weight\ of\ the\ powder}{Bulk\ Volume}$$

Tapped density (g/cm^3)

Bulk powder is sieved through 40 mesh screen. Weight is taken and poured into a graduated cylinder. The cylinder is tapped 1000 times on a mechanical tapper apparatus. The volume reached a minimum – called *tapped volume*.

$$Tapped\ density = \frac{Weight\ of\ \ the\ \ powder}{Tapped\ volume}$$

True density (g/cm^3)

Solvents of varying densities are selected in which the powder sample is insoluble. Small quantity of surfactant may be mixed with the solvent mixture to enhance wetting and pore penetration. After vigorous agitation, the samples are centrifuged briefly and then left to stand undisturbed until floatation or settling has reached equilibrium.

The samples that remains suspended (i.e. neither suspended not floated) is taken. So the true density of the powder are equal. So the true density of the powder is the density of that solvent. The density of that solvent is determined accurately with a pycnometer.

Source of variation of bulk density

Method of crystallization, milling, formulation.

Methods of correction

By milling, slugging or formulation.

Significance

(i) Bulk density

Bulk density is required during the selection of capsule size for a high dose drug.

In case of low dose drug mixing with excipients is a problem if the bulk densities of the drug and excipients have large difference.

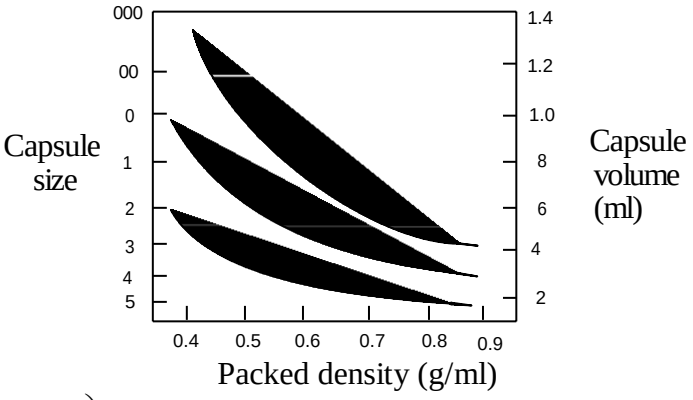
(ii) Tapped density

Knowing the dose and tapped density of the formulation, the capsule size can be determined.

(iii) True density

From bulk density and true density of powder, the void volume or porosity can be measured.

Fig. 12



$$Void\ volume = \left(\frac{m}{\rho_{bulk}} - \frac{m}{\rho_{ture}}\right) = m\left(\frac{1}{\rho_{bulk}} - \frac{1}{\rho_{true}}\right)$$

$$Porosity = \frac{Void\ volume}{Bulk\ volume} = \frac{m\left(\frac{1}{\rho_{bulk}} - \frac{1}{\rho_{true}}\right)}{\frac{m}{\rho_{bulk}}} = 1 - \frac{\rho_{bulk}}{\rho_{true}}$$

Powder flow properties

Powder flow properties depends on

(i) particle size
(ii) density
(iii) shape
(iv) electrostatic charge and adsorbed moisture

that may arise from processing or formulation.

A free-flowing powder may become cohesive during development. This problem may be solved by any of the following ways.

(i) by granulation
(ii) by densification via slugging
(iii) by filling special auger feed equipment (in case of powder)
(iv) by changing the formulation.

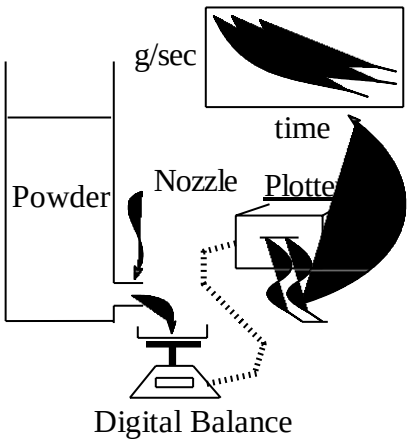
Procedure

For free flowing powder

A simple flow rate apparatus consisting of a grounded metal tube from which drug flows through an orifice onto an electronic balance, which is connected to a strip chart recorder. Several flow rate (g/sec) determinations at various orifice sizes (1/8 to ½ inch) should be carried out.

The grater the standard deviation between multiple flow rate measurements, the greater will be the weight variation of the product (tablets or capsules).

Fig. 13



Compressibility :-

$$\%\ compressibility = \frac{\rho_t - \rho_0}{\rho_t} x100$$

ρ_t = tapped bulk density

ρ_0 = Initial bulk density

Solubility Analysis

Determination of equilibrium solubility of a drug

The drug is dispersed in a solvent. The suspension is agitated at a constant temperature. Samples of the suspension are withdrawn as a function of time, clarified by centrifugation, and assayed to establish a plateau concentration.

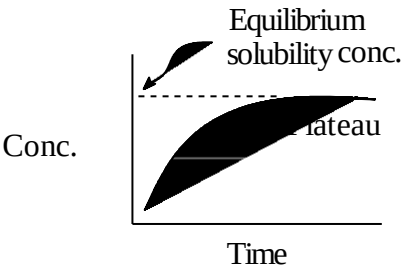


Fig. 14

Solvents taken

(i) 0.9% NaCl at room temperature
(ii) 0.01 M HCl at RT
(iii) 0.1 M HCl at RT
(iv) 0.1 M NaOH at RT
(v) At pH 7.4 buffer at 37^0C

Drug concentration is determined by the following analytical methods

(i) HPLC
(ii) UV –Spectroscopy
(iii) Fluorescence Spectroscopy
(iv) Gas Chromatography

Solubility depends on

(i) pH
(ii) Temperature
(iii) Ionic strength
(iv) Buffer concentration

Significance

(i) A drug for oral administrative should be examined for solubility in an isotonic saline solution and acidic pH. This solubility data may provide the dissolution profile invivo.
(ii) Solubility in various mediums is useful in developing suspension or solution toxicologic and pharmacologic studies.
(iii) Solubility studies identify those drugs with a potential for bioavailability problems. E.g. Drug having limited solubility (7 %) in the fluids of GIT often exhibit poor or erratic absorption unless dosage forms are tailored for the drug.

pK_a Determination

When a weakly acidic or basic drug partially ionizes in GI fluid, generally, the unionized molecules are absorbed quickly.
Handerson-Hasselbach equation provides an estimate of the ionized and unionized drug concentration at a particular pH.
For acidic drug : e.g.

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

Weak acid — Strong base

$$pH = pKa + \log \frac{[ionized]}{[unionized]} = pKa + \log \frac{[A^-]}{[HA]} = pKa + \log \frac{[base]}{[acid]}$$

For basic compounds e.g.

$$B + H_3O^+ \rightleftharpoons BH^+ + H_2O$$

Weak base — Strong acid

$$pH = pKb + \log\frac{[unionized]}{[ionized]} = pKa + \log\frac{[B]}{[BH^+]} = pKa + \log\frac{[base]}{[acid]}$$

Drug	Stomach PH 1.5	Plasma PH = 7.4	Duodenum PH = 5.0
Weak acid e.g. Ibuprofen pKa = 4.4	[HA] = 100 ⇌ [HA] = 100 ⇌ [HA] = 100 ⇅ ⇅ ⇅ $[A^-]$ = 0.13 $[A^-]$ = 100,000 $[A^-]$ = 398.1 [Total] = 100.13 [Total] = 100,100 [Total] = 498.1		
Weak base e.g. Nitrazepam pKa = 3.2	[B] = 100 ⇌ [B] = 100 ⇌ [B] = 100 ⇅ ⇅ ⇅ $[BH^+]$ = 5012 $[BH^+]$ = 0.006 $[BH^+]$ = 1.6 [Total] = 5112 [Total] = 100.006 [Total] = 101.6		

Method of determination of pKa of a drug

(i) Detection of spectral shifts by UV or visible spectroscopy at various pH.
Advantage: Dilute aqueous solutions can be analyzed by this method.

(ii) Potentiometric titration
Advantage: Maximum sensitivity for compounds with pKa in the range of 3 to 10.
Disadvantage: This method is unsuccessful for candidates where precipitation of the unionized forms occurs during titration. To prevent precipitation a co-solvent e.g. methanol or dimethylsulfoxide (DMSO) can be incorporated.

(iii) Variation of solubility at various pH.

Effect of temperature on stability

Heat of solution, ΔH_S represents the heat released or absorbed when a mole of solute is dissolved in a large quantity of solvent.

Significance

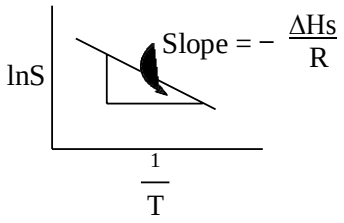
- Most commonly, the solubility process is endothermic, e.g. non-electrolytes, unionized forms of weak acids and bases ⇒ ΔH is positive ⇒ Solubility increases if temperature increases.
- Solutes that are ionized when dissolved releases heat

⇒ the process is exothermic ⇒ ΔH_S is negative ⇒ Solubility increases at lower temperature.

Determination of ΔH_S.
The working equation $\ln S = -\frac{\Delta H_S}{R}\left(\frac{1}{T}\right) + C$ where, S = molar solubility of the drug at T^0K

and R = gas constant

S is detemined at 5^0C, 25^0C, 37^0C and 50^0C.
ΔH_S = – Slope x R



Solubilization
For drug candidates with poor water solubility, preformulation studies should include limited experiments to identify the possible mechanisms for solubilization.
Means of increasing the solubility are:
(i) Addition of a cosolvent to the aqueous system e.g. ethanol, propylene glycol and glycerin.
MOA: These co-solvents disrupt the hydrophobic interactions of water at the non-polar solute / water interfaces.
(ii) Solubilization in micellar solutions such as 0.01 M Tween 20 solution.
(iii) Solubilization by forming molecular complexes e.g. benzoic acid forms complex with caffeine.

Partition coefficient
Partition coefficient is defined, as the ratio of un-ionized drug concentrations between the organic and aqueous phases, at equilibrium.

$K_{O/W} = \left[\frac{C_{oil}}{C_{water}}\right]$ *at equilibrium* Generally, octanol and chloroform are taken as the oil phase.

Significance
Drug molecules having higher $K_{O/W}$ will cross the lipid cell membrane.

Dissolution
The dissolution rate of a drug substance in which surface area is constant during disintegration is described by the modified Noyes-Whitney equation.

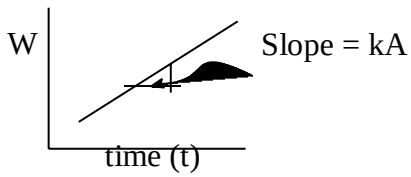
$$\frac{dc}{dt} = \frac{DA}{hV}(C_S - C)$$

where, D = diffusion coefficient of the drug in the dissolution medium
h = thickness of the diffusion layer at the solid/liquid interface
A = surface area of drug exposed to dissolution medium.
V = volume of the medium
C_S = Concentration of saturated solution of the solute in the dissolution medium at the experimental temperature.
C = Concentration of drug in solution at time t.

When A = constant and $C_S >> C$ the equation can be rearranged to

$$\frac{dC}{dt} = \frac{DA}{hV}C_S \quad or, \quad \frac{V\,dC}{dt} = \frac{DA}{h}C_S \quad or, \; W = k\,At$$ where, $k = \frac{D}{h}$

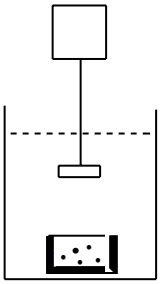
where, W = weight (mg) of drug dissolved at time t
k = intrinsic dissolution rate constant $\left(\frac{mg}{\min cm^2}\right)$



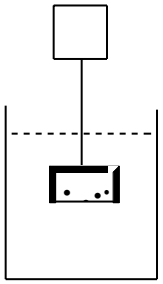
Determination of k

- Pure drug powder is punched in a die and punch apparatus to give a uniform cylindrical shape. The tablet is covered with wax in all sides. One circular face is exposed to the dissolution medium. Thus, as dissolution proceeds, the area, A, remains constant.
- Time to time dissolution medium is taken out and fresh medium added to the chamber.

- With two types of assembly, the experiments can be carried out.



Static disc dissolution aparatus



Rotating disc dissolution apparatus

Stability analysis

Preformulation stability studies are the first quantitative assessment of chemical stability of a new drug. This may involve

1. Stability study in toxicology formulation
2. Stability study in solution state
3. Stability study in solid state.

Stability study in toxicology formulation

A new drug is administered to animals through oral route either by

(i) mixing the drug in the feed
(ii) in the form of solution
(iii) in the form of suspension in aqueous vehicle

- Feed may contain water, vitamin, minerals (metal ions), enzymes and different functional groups that may severely reduce the stability of the new drug. So stability study is should be carried out in the feed and at laboratory temperature.
- For solution and suspension, the chemical stability at different temperature and pH should be checked.
- For suspension-state the drug suspension is occasionally shaken to check dispersibility.

Solution stability

Objective: Identification of conditions necessary to form a stable solution.

Stability of a new drug may depend on:

(i) pH (ii) ionic strength (iii) co-solvent
(iv) light (v) temperature (vi) oxygen.

pH stability study

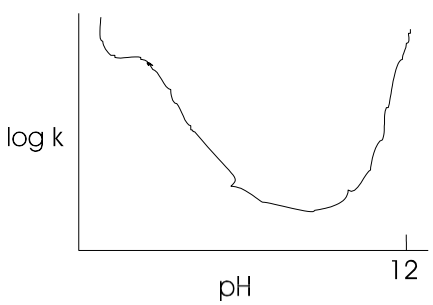
(i) Experiiments to confirm decay at the extremes of pH and temperature. Three stability studies are carried out at the following conditions

(a) 0.1N HCl solution at 90^0C
(b) Solution in water at 90^0C
(c) 0.1 N NaOH solution at 90^0C

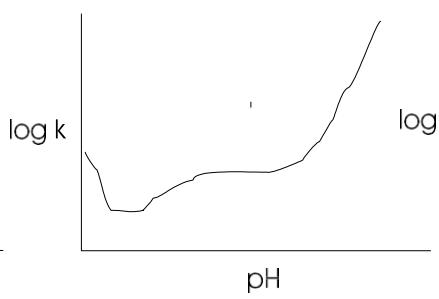
These experiments are intentionally done to confirm the assay specificity and for maximum rates of degradation.

(ii) Now aqueous buffers are used to produce solutions with wide range of pH values but with constant levels of drug concentration, co-solvent and ionic strength.

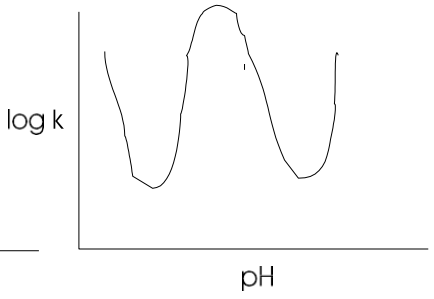
All the rate constants (k) at a single temperature are then plotted as a function of pH.



Ampicillin degradation



Hydrolysis of Aspirin



Hydrolysis of Hydrochlorthiazide

(ii) Ionic strength

Since most pharmaceutical solutions are intended for parenteral routes of administration, the pH-stability studies should be carried out at a constant ionic strength that is compatible with body fluids. The ionic strength (μ) of an isotonic 0.9%w/v sodium chloride solution is 0.15.

Ionic strength for any buffer solution can be calculated by

$$\mu = \frac{1}{2}\sum m_i Z_i^2$$

where, m_i = molar concentration of the ion

Zi = valency of that ion

For computing, μ all the ionic species of the buffer solution and drugs are also taken into calculation.

(iii) Co-solvents

Some drugs are not sufficiently soluble to give concentrations of analytical sensitivity. In those cases co-solvents may be used. However, presence of co-solvents will influence the rate constant. Hence, k values at different co-solvent concentrations are determined and plotted against % of co-solvent. Finally, the line is extrapolated to 0% co-solvent to produce the actual k value (i.e. in pure solvent).

(iv) Light

Drug solutions are kept in

(a) clear glass ampoules
(b) amber color glass container
(c) yellow-green color glass container
(d) container stored in card-board package or wrapped in aluminium foil – this one acts as the control.

Now the stability studies are carried out in the above containers.

(v) Temperature

The rate constant (k) of degradation reaction of a drug varies with temperature according to Arrhenius equation.

$$k = Ae^{-\frac{E_a}{RT}} \qquad \text{or, } \ln = \ln A - \frac{E_a}{R}\left(\frac{1}{T}\right)$$

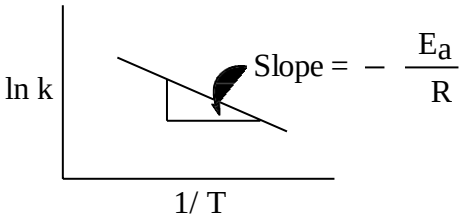
where, k = rate constant

A = frequency factor

E_a = energy of activation

R = gas constant

T = absolute temperature



Procedure

Buffer solutions were prepared and kept at different temperatures. Rate constants are determined at each temperature and the ln k value is plotted against (1/T)).

Inference

- The relationship is linear ⇒ a constant decay mechanism over the temperature range has occurred.
- A broken or non-linear relationship ⇒ a change in the rate-limiting step of the reaction or change in decay mechanism.

Uses

Shelf life of the drug may be calculated.

e.g.

Time	Concentration of drug remaining
0	100 %
$t_{10\%}$	90%

Therefore, $\ln C = \ln C_0 - k_1 t$

$\text{Ln } C/C_0 = -k_1 t$

or, $\ln \frac{90}{100} = -k\, t_{1\ 10\%}$ or, $t_{10\%} = \frac{\ln 0.90}{-k_1} = \frac{0.105}{k_1}$

where, $t_{10\%}$ = time for 10% decay to occur if the reaction follows 1st order kinetics

Conclusion
If the drug is sufficiently stable, liquid formulation development may be started at once.
If the drug is unstable, further investigations may be necessary.

Solid state stability
Objectives
Identification of stable storage conditions for drug in the solid state and identification of compatible excipients for a formulation.
Characteristics
Solid state reactions are much slower, so the rate of appearance of decay product is measured (not the amount of drug remaining unchanged).

- To determine the mechanism of degradation thin layer chromatography (TLC), fluorescence or UV / Visible spectroscopy may be required.
- To study polymorphic changes DSC or IR-spectroscopy is required.
- In case of surface discoloration due to oxidation or reaction with excipients, surface reflectance equipment may be used.

A sample scheme for determining the bulk stability profile of a new drug:

Storage condition	*4 weeks*	*8 weeks*	*12 weeks*
5^0C – Refrigerator			
22^0C – Room Temperature			
37^0C – Ambient humidity			
37^0C / 75% RH (Relative Humidity)			
Light box			
Clear box			
Amber glass			
Yellow-Green glass			
No exposure (Control :- Card-board box or wrapped with aluminium foil)			
50^0C – Ambient Humidity			
– O_2 Head Space			
– N_2 Head Space			
70^0C – Ambient Humidity			
90^0C – Ambient Humidity			

Procedure
1. Weighed samples are placed in open screw-capped vials are exposed to a variety of temperatures, humidities an dlight intnesities. After the desired time samples are taken out and measured by HPLC (5 – 10 mg), DSC (10 to 50mg), IR (2 to 20mg).
2. To test for surface oxidation samples are stored in large (25ml) vials for injection capped with Teflon-lined rubber stopper. The stoppers are penetrated with needles and the headspace is flooded with the desired gas. The resulting needle holes are sealed with wax to prevent degassing.
3. After fixed time those samples are removed and analyzed.

Drug-excipient stability profile
Hypothetical dosage forms are prepared with various excipients and are exposed to various conditions to study the interactions of drug and excipients.