Chapter
1

Preformulation

DEFINITION

Preformulation (pre + formulation, i.e. prior to formulation) as the name depicts before initiating formulation development, preformulation is the step to understand physiochemical properties (physical and chemical properties) of drug substance. Preformulation studies help the formulation scientist to develop in-depth understanding about physiochemical parameters of drug substance, which leads to design optimum drug delivery system without significant barriers during development. In other words, preformulation studies describes as the process of optimizing the delivery of drug through determination of physiochemical properties of the new compound that could affect drug performance and development of an efficacious, stable and safe dosage form.

"It is defined as phase of research and development in which preformulation scientist characterize physical and chemical properties of new drug molecule in order to develop safe, effective and stable dosage form."

Before beginning the formal preformulation programs the preformulation scientist must consider the following factors:

i. The amount of drug available.
ii. The physicochemical properties of the drug already known.
iii. Therapeutic category and anticipated dose of compound.

Objectives of Preformulation Studies

The primary objectives of preformulation studies are as follows:

i. Establish the identity and physiochemical parameters of a new drug substance.
ii. Establish chemical stability profile of drug substance.
iii. Bulk characterization
iv. Establish drug substance compatibility with common excipients
v. To establish relation between physiochemical properties of drug substance and formulation stability. Preformulation studies give preliminary idea about selection of excipients, which makes the formulation stable.
vi. Establish relation between physiochemical properties of drug substance and bioavailability. Preformulation studies give preliminary idea about selection of excipients, selection of particle size and morph of drug substance, which can affect bioavailability of drug.

vii. Preformulation studies give preliminary idea about selection of manufacturing process. For example, if drug substance has fine particle size than it is advisable to use granulation process instead of direct compression because there is possibility of blend non-uniformity, if direct compression method is used.

PHYSICAL PROPERTIES

Crystallinity and Polymorphism

Crystal habit and internal structure of a drug can affect bulk and physiochemical properties which ranged from flowability to chemical stability.

A single compound can have several different crystal habits depending upon the environment for growing crystals. Changes with internal structure usually alter the crystal habit, for example, conversion of a sodium salt to its free acid form produce both a change in internal structure and crystal habit (Table 1.1 and Fig. 1.1).

Polymorphism

When a substance exists in more than one crystalline form, the different forms are designated as polymorphs and the phenomenon as polymorphism. Polymorphism also influences biopharmaceutical behavior of drug. A pure more soluble B form of chloramphenicol palmitate is more bioavailable after oral administration as compared to less soluble pure A form and their mixture.

Amorphous (from the Greek, A: without, morphé: shape, form) solid is any non-crystalline solid in which the atoms and molecules are not organized in a definite lattice pattern. Amorphous forms are typically prepared by rapid precipitation, lyophilization or rapid cooling of liquid melts. Since amorphous forms are usually of higher thermodynamic energy than corresponding crystalline forms, solubility as well as dissolution rates are generally greater. Polymorphisms are of two types:

1. **Enantiotropic:** It is the one which can be reversibly change into another form by altering the temperature or pressure, e.g. sulphur.
2. **Monotropic:** It is the one in which transition from one morph to another form is irreversible.

Depending upon their relative stability, one of the several polymorphic forms will be physically more stable than others. Stable morph represents lowest energy state, has highest melting point and least aqueous solubility. The remaining polymorphs

TABLE 1.1: Various types of crystal system

Sr. No.	*Crystal system*	*Angle of axis*	*Length of axis*	*Example*
1	Cubic (regular system)	$\alpha = \beta = \gamma = 90°$	$a = b = c$	NaCl
2	Tetragonal	$\alpha = \beta = \gamma = 90°$	$a = b \neq c$	Nickel sulfide
3	Orthorhombic	$\alpha = \beta = \gamma = 90°$	$a \neq b \neq c$	$KMnO_4$
4	Monoclonic	$\alpha = \gamma = 90°$ and $\beta \neq 90°$	$a \neq b \neq c$	Sucrose
5	Triclinic (asymmetric)	$\alpha \neq \beta \neq \gamma \neq 90°$	$a \neq b \neq c$	$CuSO_4$
6	Trigonal (rhombohedral)	$\alpha = \beta = \gamma \neq 90°$	$a = b = c$	Sodium nitrate
7	Hexagonal	$\alpha = \beta = 90°$ and $\gamma = 120°$	$a = b \neq c$	$AgNO_3$

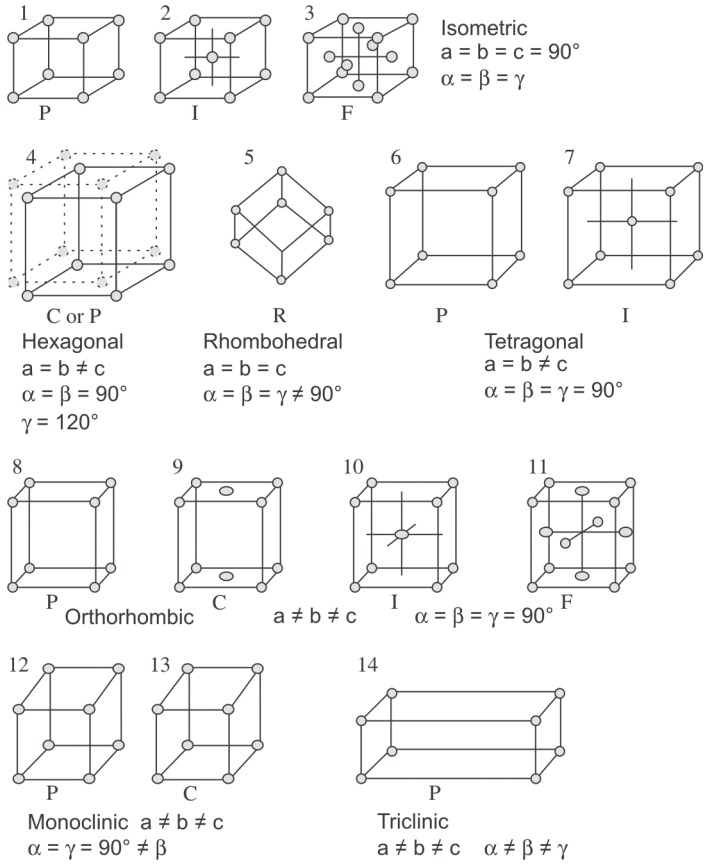

Fig. 1.1: Different types of crystal systems

are called metastable forms, which represent the higher energy state, have low melting point and high aqueous solubility. Because of their higher energy state, the metastable forms have a thermodynamic tendency to convert to the stable form.

Order of dissolution: Amorphous > Metastable > Stable

Detection: Morph compound can be detected by following techniques

- Optical crystallography
- X-ray diffractions
- Differential scanning calorimetry

Pseudopolymorphism

A crystalline compound may contain either a stoichiometric or non-stoichiometric amount of crystallization solvent.

Non-stoichiometric adducts such as inclusion or catharses, involve entrapped solvent molecules within the crystal lattice. Usually this adduct is undesirable, owing to its lack of reproducibility and should be avoided for development.

Stoichiometric adducts, commonly referred as solvates is a molecular complex that has incorporated the crystallizing solvent molecules into specific sites within the crystal

lattice. The solvate can exist in different crystalline form called pseudopolymorph and phenomenon called pseudopolymorphism.

When the incorporated solvent is water, the complex is called a hydrate and the term hemihydrate, monohydrate and dihydrate describes various hydrate forms. A compound not containing any water is called anhydrous compound. Generally the anhydrous form of a drug has a greater aqueous solubility than the hydrates. This is because the hydrates are already in interaction with water and, therefore, has less energy for crystal break-up in comparison to anhydrates (thermodynamically higher energy state) for further interaction of water, e.g. ampicillin and theophylline anhydrous form have more aqueous solubility. Pseudopolymorphs should be identified since most polymorphs can be obtained by changing the recrystallizing solvent. Solvents inducing polymorphic changes are water, methanol, ethanol, acetone, chloroform, n-propanolol, isopropyl alcohol, n-butanol, n-pentanol, benzene and toluene.

These hydrates water and solvates (e.g. methanolate, ethanolate) have been confused with the true polymorphism. The distinction between these false forms and true polymorphs can be obtained by observing the melting behaviour of the compound dispersed in silicon oil using hot stage microscopy. Pseudopolymorphs will evolve a gas (steam or solvent vapour) causing bubbling of the oil while, true polymorphs merely melt. The temperature at which the solvent volatilizes will be close to the boiling point of the solvent and can be used for identification.

PARTICLE SIZE DISTRIBUTION

Bulk flow, formulation homogeneity, and surface-area controlled processes such as dissolution and chemical reactivity are directly affected by size, shape and surface morphology of the drug particles. In general, each new drug candidate should be tested for particle size distribution during preformulation.

Various chemical and physical properties of drug substances are affected by their particle size distribution and shapes. The effect is not only on the physical properties of solid drugs but also in some instances, on their biopharmaceutical behavior. It is generally recognized that poorly soluble drugs showing a dissolution-rate limiting step in the absorption process will be more readily bioavailable, when administered in a finely subdivided state rather than as a coarse material. Gibbs Kelvin has explained the relationship between particle size and solubility.

Gibbs Kelvin relation: It is relationship between particle size and apparent solubility of a drug.

$$\log \frac{S_r}{S_\alpha} = \frac{2\gamma_{SL} M}{2.303\ RT\ \rho r}$$

Where, S_r = apparent solubility of a drug
S_α = true equilibrium solubility
γ_{SL} = interfacial energy that exists between solid and liquid
r = radius of particle
M = molecular weight
R = gas constant
T = absolute temperature
ρ = density of the solid

In case of tablets, particle size distribution influences the flow and the mixing efficiency of powders and granules. Particle size distribution can also be a factor in stability; fine materials are relatively more open to attack from atmospheric oxygen, humidity, and interacting excipients.

Methods of Particle Size Determination

1. Simple method—microscopy with help of light microscope and sieving. Sieving method needs comparatively large sample of bulk material, thus this method is less useful at preformulation stage due to lack of bulk material (Table 1.2).
2. Instrument based on light scattering—ROYCO.
3. Instrument based on light blockage—HIAC.
4. Instrument based on blockage of electrical conductivity path—Coulter counter.
5. Based on rate difference of sedimentation of different particle—Anderson pipette method.

POWDER FLOW PROPERTIES

Assessment of flow properties of a drug powder is important to the formulator. When limited amount of drug are available then can be evaluated simply by measurement of:

a. Bulk density; and
b. Angle of repose.

Bulk Density

Bulk density of the drug substance is very useful in having some idea as to the size of final dosage form. Carr's compressibility index and Hausner Index can be used to predict the flow property based on density measurement (Tables 1.3 and 1.4).

TABLE 1.2: Common techniques for measurement of particle size

S. No.	*Technique*	*Particle size (um)*
1	Microscopic	1–100
2	Sieve	>50
3	Sedimentation	>1
4	Permeability	>1
5	Centrifugal	<50
6	Light scattering	0.5–50

TABLE 1.3: Grading of the powder for their flow properties, according to Carr's index

Consolidation index (Carr index in %)	*Flow*
5–15	Excellent
12–16	Good
18–21	Fair to passable
23–35	Poor
33–38	Very poor
>40	Very very poor

TABLE 1.4: Compressibility and flow ability of pharmaceutical excipients

Material	*% compressibility*	*Flow*
Celutab	11	Excellent
Emcompress (DCP)	15	Excellent
Starch-1500	19	Fair passable
Lactose monohydrate	19	Fair passable
Maize starch	26–27	Poor
Dicalcium phosphate dihydrate (coarse)	27	Poor
Magnesium sterate	31	Poor
Titanium dioxide	34	Very poor
Dicalcium phosphate dihydrate (fine)	41	Very poor

Carr's index (%) or consolidation index

$$= \frac{\text{Tapped density} - \text{Poured density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner index} = \frac{\text{Tapped density}}{\text{Poured density}}$$

Fluff (poured) density is the ratio of mass of powder to the fluff volume. Fluff volume is the volume occupied by a certain mass, when gently poured into a measuring cylinder.

Tapped density is the ratio of mass of powder to the tapped volume. Tapped volume is the volume occupied by the same mass of powder after a standard tapping of a measure.

Angle of Repose

Angle of repose is defined as the maximum angle possible between the surface of pile of the powder and the horizontal plane (Fig. 1.2).

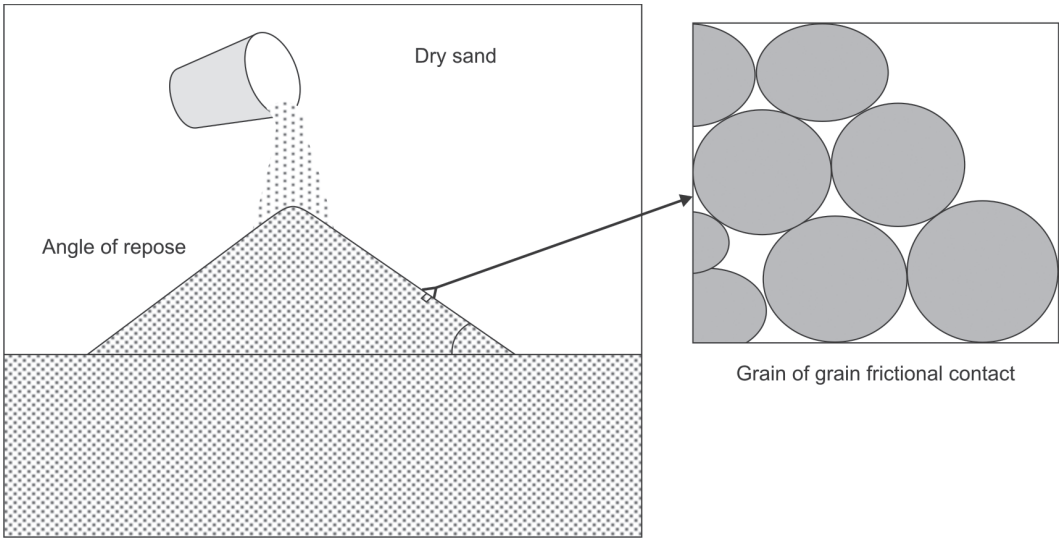

Fig. 1.2: Angle of repose of dry sand

$$\tan \theta = \frac{h}{r}$$

$$\theta = \tan^{-1} \frac{h}{r}$$

where, θ = angle of repose, h = height of pile, and r = radius of the base of pile.

The lower the angle of repose, the better the flow property. Certain observations are made (Tables 1.5 and 1.6)

i. Decrease in particle size leads to a higher angle of repose.
ii. Lubricant at low concentration decreases the angle of repose. At high concentration, this enhances the angle of repose.
iii. Fines (passed through 100 meshes) increase the angle of repose.

SOLUBILITY

Solid drugs administered orally for systemic activity must dissolve in the gastrointestinal fluids prior to their absorption. Thus the solubility and rate of dissolution of drugs in GIT fluids could influence the rate and extent of their absorptions (Fig. 1.3).

A compound having good aqueous solubility over the pH range 1–7 at 37°C does not usually exhibit bioabsorption problem.

Analytical methods useful for solubility measurements include HPLC, UV spectroscopy, fluorescence spectroscopy and gas chromatography. For most drugs, reverse phase HPLC offers an efficient and accurate means of collecting solubility data. Its major advantages are direct analysis of aqueous samples, high sensitivity and specific

TABLE 1.5: Relationship between angle of repose (θ) and powder flow

Angle of repose (θ degrees)	*Flow*
<25	Excellent
25–30	Good
30–40	Passable
>40	Very poor

TABLE 1.6: Angle of repose of some pharmaceutical excipients

Substance	*Angle of repose (θ°)*
Calcium state NF	10
Dextrose	25
Lactose USP (granular grade)	15
Lactose (spray dried) USP	20
Magnesium oxide	20
Microcrystalline cellulose USP (granular grade)	15
Starch NF	15
Stearic acid NF	15
Talc USP	15
Sodium bicarbonate USP	20

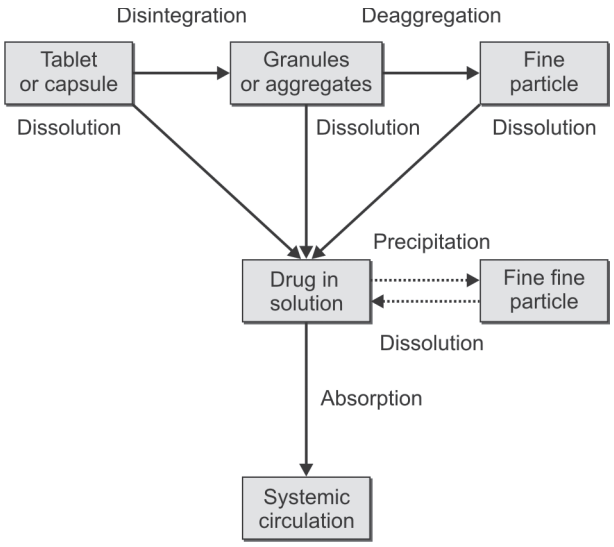

Fig. 1.3: Solubility or dissolution as critical step during absorption

determination of drug concentration due to chromatographic separation of drug from impurities or degradation products.

Intrinsic Solubility (C_0)

An increase in solubility of a new drug in an acidic solution compared with its aqueous solubility suggests that drug is a weak base. However, weak acidic drug has good solubility in alkali solution. An increase in both acidic and alkaline solubility suggests either amphoteric or zwitterion behaviour; in this case there will be two pKa, one acidic and one basic. No change in solubility shows non-ionizable behaviour of molecule with no measurable pKa. Here, solubility cannot be changed with change in pH of the solution.

Intrinsic solubility (true solubility) C_0 is the solubility due to unionized form of drug. When the purity of drug sample can be assured, then the solubility value obtained in acid for a weak acid or alkali for a weak base can be assumed intrinsic solubility.

The solubility should ideally be measured at two temperatures:

a. 4 or 5°C to ensure good physical stability.

b. 37°C to support biopharmaceutical evaluation.

pH

The degree of ionization and therefore the solubility of acidic and basic compound depend upon the pH of the media. The saturation solubility for such compounds at a particular pH is sum total of solubility of ionized and unionized forms.

$$S_t = [BH^+] + [B]$$

BH^+ is protonated species (salt species), B is free base, S_t = total molar solubility.

The pH at which both base and salt species are simultaneously saturated is defined as the pH_{max}

$$S_t, pH = pH_{max} = [BH^+]_S + [B]_S$$

Where the subscript (s) denotes saturation.

For weak bases in the pH region, where the solubility of protonated form is limiting the molar solubility is

$$S_t,\ pH < pH_{max} = [BH^+]_S + [B]$$

Similarly, the solubility in pH region where free base is limiting is expressed as:

$$S_t,\ pH > pH_{max} = [BH^+] + [B]_S$$

Corresponding equation for acidic compound:

$$S_t\ pH < pH_{max} = [AH]_S + A^-$$

$$S_t\ pH > pH_{max} = [A^-]_{S+}[AH]$$

Since ionizable compounds may be available in free or salt form, one could use either in solubility experiments, e.g. phenazopyridine is available in free base and hydrochloride salt. The solubility behaviour of phenazopyridine free base over pH range 1 to 10 shows that phenazopyridine, with pKa of 5.2, exhibits maximum solubility at pH 3.45 (pH_{max}). If pH of the phenazopyridine solution changed from pH 3.5, solution will become supersaturated and drug will precipitate.

pKa DETERMINATION (DISSOCIATION CONSTANT)

The amount of drug that exists in unionized form is related to dissociation constant (pKa) of drug and pH of the fluid at the absorption site. The dissociation constants of both acidic and basic drugs are expressed by pKa values. Lower the pKa of an acidic drug represents strong acid while, higher the pKa of basic drug represents strong base. Thus, from the knowledge of pKa of the drug and pH at the absorption site (or biological fluid), the relative amount of ionized and unionized drug in solution at a particular pH and the percent of drug ionized at this pH can be determined by **Henderson–Hasselbalch equation**

For weak acid:

$$pH = pKa + \log \frac{\text{Ionized drug concentration}}{\text{Unionized drug concentration}}$$

$$\% \text{ drug ionized} = \frac{10^{pH-PKa}}{1+10^{pH-PKa}} \times 100$$

For weak base:

$$pH = pK_a + \log \frac{\text{Unionized drug concentration}}{\text{Ionized drug concentration}}$$

$$\% \text{ drug ionized} = \frac{10^{PKa-pH}}{1+10^{PKa-pH}} \times 100$$

Above equations can be used

i. To determine the pKa: pKa is that pH at with ionized and unionized drug concentration is same. Buffer, temperature, ionic strength and co-solvent affect the pKa value and should be controlled during pKa determinations.
ii. To determine solubility at any pH provided that the intrinsic solubility (C_0) and pK_a are known.
iii. To determine percentage ionization.

PARTITION COEFFICIENT

Partition coefficient (oil/water) is a measure of a drug's lipophilicity and an indication of its ability to cross cell membranes. It is defined as the ratio of un-ionized drug distributed between the organic and aqueous phases at equilibrium.

$$P_{o/w} = (C_{oil}/C_{water})_{equilibrium}$$

For drug delivery, the lipophilic/hydrophilic balance has been shown to be a contributing factor for the rate and extent of drug absorption. Although partition coefficient data alone does not provide understanding of *in vivo* absorption, it does provide a means of characterizing the lipophilic/hydrophilic nature of the drug.

Since, biological membranes are lipoidal in nature, the rate of drug transfer for passively absorbed drugs is directly related to the lipophilicity of the molecule. The partition coefficient is commonly determined using an oil phase of octanol or chloroform and water. Drugs having values of P much greater than 1 are classified as lipophilic, whereas those with partition coefficients much less than 1 are indicative of a hydrophilic drug. Although, it appears that the partition coefficient may be the best predictor of absorption rate, the effect of dissolution rate, pKa, and solubility on absorption must not be neglected.

Applications of Partition Coefficient

Partition coefficient (solvent water quotient of drug distribution) has a number of applications which are relevant to preformulation.

i. Solubility both in aqueous and in mixed solvent.
ii. Drug absorption *in vivo.*
iii. Partition chromatographic (like HPLC and TLC) analytical method development.
iv. Extraction of crude drugs.
v. Recovery of antibiotics from fermentation broth.

Dissolution

The dissolution rate of the drug is only important where it is rate limiting step in the absorption process.

An equation which describes the process of drug dissolution is the Noyes–Whitney equation.

$$\frac{dC}{dt} = KS(C_s - C_t)$$

where $\frac{dC}{dt}$ = rate of dissolution,

K = dissolution rate constant,

S = surface area of the dissolved solid,

C_t = concentration at time 't', and

C_s = saturation solubility.

The constant 'K' has been shown to be equal to D/h, where D is the diffusion coefficient of the dissolving solid and 'h' is the thickness of the diffusion layer (Fig. 1.4). The diffusion layer is a thin stationary film of solution adjacent to the surface of the solid. The layer is saturated with drug. Thus, the drug concentration in the layer is equal to C_s. The term C_s–C_t represents the concentration gradient between the diffusion layer and the bulk solution. In dissolution rate limited absorption C_t is negligible, then equation becomes:

$$\frac{dC}{dt} = \frac{DSC_s}{h}$$

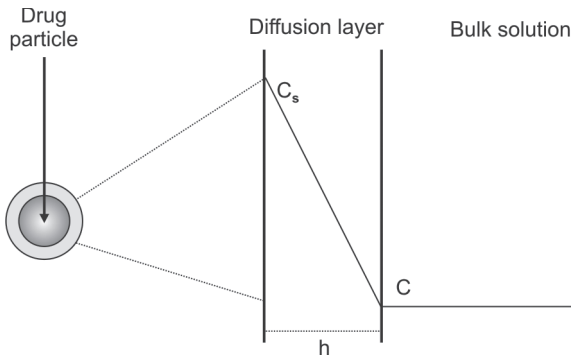

Fig. 1.4: Dissolution of drug particle according to Noyes–Whitney equation

CHEMICAL PROPERTIES

Chemical instability normally results from either of the following reaction:

i. Hydrolysis
ii. Oxidation
iii. Photolysis
iv. Racemization
v. Polymerization

Hydrolysis

The most likely cause of drug instability is hydrolysis, partially in solid dosage form. Hydrolysis may be defined as the reaction of a compound with water. It is of two type ionic and molecular form of hydrolysis.

***Ionic hydrolysis*:** It occurs with salt of weak acids, e.g. potassium acetate and bases of codeine phosphate interact with water to give alkaline and acidic solution respectively.

***Molecular hydrolysis*:** Slower irreversible process involving cleavage of the drug molecule. This form of hydrolysis is mainly responsible for the decomposition of pharmaceutical products ester, e.g. the local anesthetic amethocaine and benzocaine and amines.

It involves nucleophilic attack of labile groups, e.g. Lactam > Ester > Amide > Imide. When this attack is by a solvent other than water then it is known as **solvolysis.** It generally follows second order kinetics as there are two reacting species, water and API. In aqueous solution, water is in excess, the reaction is first order. A number of conditions catalyzes the breakdown.

i. Presence of OH^- ion
ii. Presence of H_3O^+ ion
iii. Presence of divalent metal ion is quicker than molecular ion
iv. Ionic hydrolysis (protolysis)
v. Heat
vi. Light
vii. Solution polarity and ionic strength
viii. High drug concentration

Prevention of Hydrolysis

1. **pH adjustment:** Most of the potent drugs are weakly acidic or weakly basic,which are more soluble when ionized so their instability will increase.

Remedy: The hydrolysis can be prevented by

i. Formulating the drug solution close to its pH of optimum stability.
ii. Addition of water miscible solvent in formulation.
iii. Optimum buffer concentration to suppress ionization.

2. **Addition of surfactant:** Nonionic, cationic and anionic surfactant stabilizes the drug against base catalysis.
3. **Salts and esters:** The solubility of pharmaceuticals undergoing ester hydrolysis can be reduced by forming less soluble salts or ester of drug, e.g. phosphate ester of clindamycin.
4. **Store with desiccants.**
5. **By use ofcomplexing agent.**

Oxidation

It is a very common pathway for drug degradation in liquid and solid formulation. Oxidation occurs in two ways:

1. Auto-oxidation.
2. Free radical chain process.

Auto-oxidation

It is defined as a reaction of any material with molecular oxygen which produces free radicals. These radicals are highly unsaturated and readily take electron from other substance causing oxidation. For auto-oxidation to occur in solid molecular oxygen must be able to diffuse through the crystal llattice to liable sites. Hence crystal morphology and packing are important parameters for determining oxidation kinetics.

Free Radical Chain Process

a. Initiation

$$\text{Activation RH X} \xrightarrow{\text{Light, Heat}} R^{\cdot} + H^{\cdot}$$

b. Propagation

$$R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot}$$

$$RO_2^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$$

c. Decomposition

$$ROOH \longrightarrow RO^{\cdot} + OH^{\cdot}$$

d. Termination

$$RO_2^{\cdot} + X^{\cdot} \longrightarrow \text{Inactive product}$$

$$RO_2^{\cdot} + RO_2^{\cdot} \longrightarrow \text{Inactive product}$$

Functional groups prone to oxidation are:

i. Alkenes
ii. Substituted aromatic groups (toluene, phenols, anisole)
iii. Ethers
iv. Thioethers
v. Amines.

Factors affecting oxidation process

i. Oxygen concentration
ii. Light
iii. Heavy metals particularly those having two or more valence state, (e.g. copper, iron, nickel, cobalt)
iv. Hydrogen and hydroxyl ion
v. Temperature.

Prevention of oxidation

1. Reducing oxygen content: Oxidative degradation of drug takes place in an aqueous solution, so the oxygen content can be decreased by boiling water.
2. Storage in a dark and cool condition.
3. Addition of an antioxidant: They act by acting as reducing agent and chain inhibitors of radical induced decomposition (Table 1.7).
4. Addition of chelating agent: It forms complexes with trace amount of heavy metal ion and inactivate their catalyzing activity, e.g. EDTA, citric acid, tartaric acid.
5. Adjustment of pH: To optimum stability in order to reduce oxidation potential of the system.
6. Changing solvent: Solvent other than water may have catalyzing effect on oxidation reaction when used in combination with water or alone, e.g. aldehydes, ethers, ketones may influence free radical reaction.

Photolysis

Photolysis catalyze oxidation and to some extent hydrolysis. This energy associated with the radiation increases as its wavelength decreases, so that the energy of UV > visible > IR and is independent of temperature (Table 1.8).

TABLE 1.7: List of antioxidants along with their examples

Antioxidant	
Oil soluble	*Water soluble*
Free radical acceptor and inhibit free radical chain	Oxidized itself and prevent Oxidation of drug
Examples	
Hydroquinone	Sodium meta bisulphate
Propyl gallate	Sodium bisulphite
Butylated hydroxy anisole (BHA)	Acetyl cysteine, ascorbic acid
Butylated hydroxy toluene (BHT)	Sodium thiosulfate, sulphur dioxide
Lecithin	Thioglycolic acid
α-tocopherol	Thioglycerol

TABLE 1.8: Energy associated with different radiations

Types of radiation	*Wavelength*	*Energy (Kcal mol^{-1})*
UV	50–400	287–72
Visible	400–750	72–36
IR	750–10,000	36–1

When molecules are exposed to electromagnetic radiations they absorb light (photons) at characteristic wavelengths which causes an increase in the energy state of the compound. This energy can:

i. Cause decomposition
ii. Be retained or transferred
iii. Converted to heat
iv. Result in emission of light at a new wavelength (fluorescence, phosphorescence)
v. Natural sunlight lies in the wavelength range 290–780 nm of which only the higher energy (UV) (290–320 nm) cause photo degradation of drugs.

These photolysis can be prevented by suitable packing, i.e. using amber coloured glass bottles, card board outers and aluminium foil overwraps and blisters.

Racemization

The inter conversion from one isomer to another can lead to different pharmacokinetic properties (ADME) as well as different pharmacological and toxicological effect, e.g. L-epinephrineis 15 to 20 times more active than D-form, while activity of racemic mixture is just one half of the L-form. It follows first order kinetics. It depends on temperature, solvent, catalyst and presence or absence of light.

Polymerization

It is a continuous reaction between molecules. More than one monomer reacts to form a polymer For examples:

- Darkening of glucose solution is attributed to polymerization of breakdown product [5-(hydroxyl methyl) furfural].
- Polymerization of HCHO to para-HCHO which crystallizes out from the solution.

BIOPHARMACEUTICAL CLASSIFICATION SYSTEM

BCS as a tool is used in drug product development. BCS classifies API in one of four categories depending on its solubility and permeability as they pertain to oral dosing. The drugs are classified in BCS on the basis of following parameters:

1. Solubility
2. Permeability
3. Dissolution

1. **Solubility:** It is based on the highest dose strength of an immediate release product. A drug is considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1 to 6.8. The volume estimate of 250 mL is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with a glass of water.
2. **Permeability:** It is based indirectly on the extent of absorption of a drug substance in humans and directly on the measurement of rates of mass transfer across human intestinal membrane. A drug substance is considered highly permeable when the extent of absorption in humans is determined to be 85% or more of the administered dose based on a mass-balance determination or in comparison to an intravenous dose.

3. **Dissolution:** An immediate release product is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 15 minutes using USP Dissolution apparatus 1 at 100 RPM or Apparatus 2 at 50 RPM in a volume of 900 mL or less in the following media: 0.1 N Hydrochloric acid simulated gastric fluid, pH 4.5 buffer and pH 6.8 buffer or simulated intestinal fluid. According to the biopharmaceutics classification system, drug substances (Fig. 1.5) are classified as follows:

Class I. High permeability, high solubility: Example is metoprolol. These drugs are well absorbed and their absorption rate is usually higher.

Class II. High permeability, low solubility: Example is glibenclamide, bicalutamide, The bioavailability of those products is limited by their solubilization rate, i.e. solubility is less.

Class III. Low permeability, high solubility: Example is cimetidine. The absorption is limited by the permeation rate but the drug is solubilize very fast.

Class IV. Low permeability, low solubility: Example is hydrochlorothiazide. Those compounds have a poor bioavailability. Generally they are not well absorbed and have low solubility.

STABILITY STUDIES

The stability studies are done to determine shelf-life of the product, excipient-API compatibility and API vulnerability to degrade by oxidation, hydrolysis, isomerisation, polymerization, decarboxylation, moisture, heat and light. Properly conducted stability studies must also include an examination of specific decomposition products by appropriate techniques to establish identity and relative toxicity of the decomposition products and the concentrations in which they are formed. Stability studies should not only take the account of the physical state in which the compound is likely to be used, but also the immediate biological environment likely to be met on administration. The substance for tablet, encapsulation and preparation of suspension, should be examined primarily in solid state. Substances for injection, which must be subjected to some form of sterilization procedure, must be examined particularly for

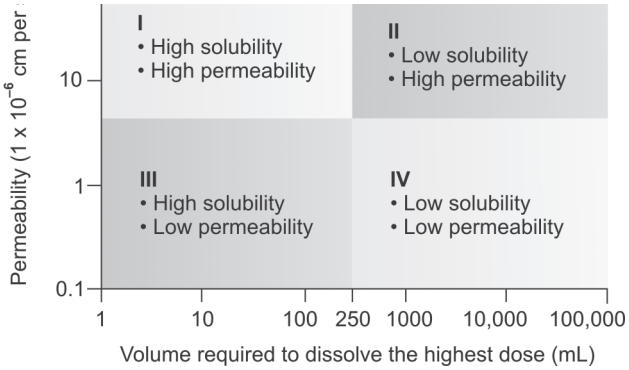

Fig. 1.5: Biopharmaceutical classification system; classes I, II, III and IV

stability at elevated temperature for possible hydrolysis or rearrangement in aqueous media and effects of exposure to CO_2 and light. Similarly, all substances intended for oral administration must be chemically stable to the pH and enzymatic conditions likely to be met in the gastrointestinal tract.

Hence, stability studies must be conducted on the drug substance in the solid state over a range of temperature, at varying degrees of humidity, and in both light and dark. Also, if a product is to be used in multiple dose form in the tropics with fluctuation in temperature, which should be stored ideally in cool or refrigerated conditions, then the stability tests should include a study of the effects of fluctuating temperature. Stability studies are an integral part of the drug development program and are of the most important area in the registration of pharma products. Stability assessment started with studies on the substance to determine degradation products and degradation pathway. Stability studies can influence the specification, limits and control method for drug.

The physico-chemical parameters, presence of additives and storage conditions, which may affect the stability of drugs, have received considerable attention in the field of pharmaceutics. The formulation of a stable dosage form is essential for the patient's safety and drug efficacy.

In the ICH harmonized tripartite guidelines on stability testing of new drug substances and products fundamental recommendation are summarized. According to the ICH guideline, long term and accelerated stability studies (least 6 months) have to be carried out (Table 1.9).

Preformulation stability studies are usually the first quantitative assessment of chemical stability of a new drug. These studies include both solution and solid state experiments under conditions typical for the handling, formulation, storage and administration of a drug candidate.

Stability analysis can be done by:

i. UV spectroscopy
ii. Thin layer chromatography (TLC)
iii. High performance liquid chromatography (HPLC)
iv. Differential scanning calorimetry (DSC)

TABLE 1.9: Long term, accelerated and where appropriate, intermediate storage conditions for the drug substances

Study	*Storage conditions*	*Time period*
Long term*	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	Long term stability will decide shelf life of the product
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	12 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

* Long term stability studies are performed at 25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH.

** If 30°C ± 2°C/65% RH ± 5% RH is the long-term condition, there is no intermediate condition. Intermediate stability sample analysis is to be conducted, if accelerated stability study fails. 12 months of intermediate stability data (with no significant impact in long term) gives confidence for extension of shelf life uptill 18 months.

At least 6 months of accelerated data or 6 months intermediate data and 12 months of long term data required for filing (submission) of the product for US and Europe market.

DRUG EXCIPIENT COMPATIBILITY STUDIES

The successful formulation of a stable and effective solid dosage forms depend on the careful selection of the excipients which are added to facilitate administration, promote the consistent release and bioavailability of drug and protect it from degradation. Incompatability between excipient and drug substance can be detected by:

1. Differential scanning chromatography.
2. By evaluating organoleptic characterstics like change in color.
3. By checking purity of drug substance using HPLC method.

1. **Differential scanning chromatography:** Differential scanning chromatography can be used to investigate and predict any physiochemical interaction between components in a formulation and therefore can be applied to the selection of suitable chemically compatible excipients. For example, in one of the studies, compatibility of Oxcarbazepine (OXC) with excipients was done using DSC analysis. DSC compatibility studies were carried out by comparing the thermal curve of pure OXC with the curves obtained from pure OXC at 1:1 w/w individual mixtures with each excipient under consideration.

   The DSC curve of OXC was typical of a pure crystalline substance, showing a sharp endothermic peak at its melting point, with an onset temperature of 221.13ºC. No significant degradation was seen to occur before 240ºC.

   Inference: OXC was found to be compatible with microcrystalline cellulose, starch and talc. Interaction between OXC and mannitol, monohydrate lactose, colloidal silica, magnesium stearate and sodium lauryl sulfate (SLS) was observed and the extent of interaction varied from only a shift in the OXC melting endotherm to total abolition of the peak (Table 1.10).

2. **By evaluating physical characteristics like change in color:** This is the simplest method, where mixtures of drug substance and excipients are charged for 2 and 4 weeks at accelerated condition (40°C/75% RH) under fixed ratios and change in color is evaluated. For example, in one of the studies, physical compatibility study was done with cephalaexin, observation tabulated below.

   Inference: Based on physical compatibility study, cephalexin seems to be compatible with excipients listed in Table 1.11.

3. **By checking purity of drug substance using HPLC method:** Formulation scientist can use HPLC method to detect impurities generated because of incompatibility between excipients and drug substance. This is the most effective method for

TABLE 1.10: DSC data on drug and excipients

Sample	*Melting endotherm onset (°C)*
OXC	221.13
OXC/microcrystalline cellulose	223.66
OXC/Starch	218.81
OXC/talc	224.50
OXC/mannitol	208.63
OXC/Colloidal silica	206.11
OXC/lactose	199.40
OXC/magnesium stearate	206.64

TABLE 1.11: Physical compatibility studies

Composition	*Description*		
	Initial	*2 weeks*	*4 weeks*
Cephalexin	White to off white powder	No color change	No color change
Cephalexin + microcrystalline cellulose	White to off white powder	No color change	No color change
Cephalexin + lactose	White to off white powder	No color change	No color change
Cephalexin + HPMC15cps	White to off white powder	No color change	No color change
Cephalexin + HPMC K4M	White to off white powder	No color change	No color change
Cephalexin + HPMC K100M	White to off white powder	No color change	No color change
Cephalexin + HPMC K15M	White to off white powder	No color change	No color change
Cephalexin + colloidal silicon dioxide	White to off white powder	No color change	No color change
Cephalexin + magnesium stearate	White to off white powder	No color change	No color change

evaluating incompatibility. Similar to physical compatibility studies, mixtures of drug substance and excipients are charged for 2 and 4 weeks at accelerated condition (40°C/75% RH) under fixed ratios. After two and four weeks, mixtures are evaluated with respect to impurities generated because of interaction between excipients and drug substance.

Conclusion

Preformulation studies properly carried out have a significant part to play in anticipating formulation problem and identifying logical paths in both liquid and solid dosage form technology. The need for adequate drug solubility cannot be overemphasized. The availability of sufficient solubility data should allow the selection of the most appropriate salt for development.

Stabilities studies in solution will indicate the feasibility of parenteral or other liquid dosage forms and can identify methods of stabilization. In parallel, solid state stability by DSC, TLC and HPLC and in the presence of tablet and capsule excipient will indicate the most acceptable vehicles for solid dosage formulations.

Finally by physiochemical property of drug the scientist can assist the synthetic chemist to identify the optimum molecule, provides the biologists with suitable vehicle to elicit pharmacological response and advise the bulk chemist about the selection and production of the best salt with appropriate particle size and morphology for subsequent processing.

ISOLATED KEY POINTS

- Preformulation studies begin when a new chemical entity shows sufficient pharmacological promise and it may be a viable candidate for studies in man
- Aim: To establish the physicochemical properties of a new drug (API)
  To establish the data on drug–excipient compatibility
  To establish its (API) kinetic rate profile

- Some commonly evaluated parameters in preformulation: Organoleptic properties, purity, particle size, particle shape, particle surface area, solubility, dissolution, partition coefficient, ionization constant, crystal properties, polymorphism, density, hygroscopicity, flowability, wettability and stability.
- Purity studies are essential during preformulation. Techniques used for characterizing purity—thin layer chromatography (TLC), high pressure liquid chromatography and gas chromatography (GC).
- Various chemical and physical properties of drug substances are affected by their particle size distribution and shapes. Size and shape also influences the dissolution of poorly soluble drugs, which in turn influences bioavailability.
- Microscopy is the simplest technique of estimating size ranges and shapes, e.g. light microscope and electron microscope. Anderson pipette methodis based on the rate difference of sedimentation of different particles, but techniques like this are seldom used due to their tedious nature. Seiving methods are also used to measure particle size. Instruments based on light scattering (Royco), light blockage (HIAC) and blockage of electrical conductivity path (coulter counter) are also available.
- Common techniques for measuring fine particles of various sizes (in μm) are:
  Microscopic: 1–100 μm, sieve >50 μm, sedimentation >1 μm, elutriation 1– 50 μm,
  Centrifugal <50 μm, permeability >1 μm, light scattering micrometer 0.5–50 μm.
- The solubility of drug is an important physicochemical property because it effects the rate of drug release into dissolution medium and consequently, the bioavailabilty of the drug and therapeutic efficiency of the pharmaceutical product.
- Common solvents used for solubility determination are benzyl alcohol, isopropyl alcohol, surfactant (like tween and sodium lauryl sulfate) aqueous solution, castor oil. Peanut oil, sesame oil and buffer at various pHs, polyethylene glycols, propylene glycol, glycerin, sorbitol, ethyl alcohol and methanol.
- Intrinsic solubility: The solubility should ideally be measured at two temperatures, i.e. 4°C and 37°C. The minimum density of water occurs at 4°C, this leads to a minimum aqueous solubility and at 37°C to support biopharmaceutical evaluation.
- pKa determination: It is the negative logarithm of dissociation constant. It describes about the chemical nature of API. It is mainly determined by using the **Henderson–Hasselbalch equation.**
  For acidic compounds,
  $$pH = pKa + \log (\text{unionized drug})/(\text{ionized drug})$$
  For basic compounds,
  $$pH = pKb + \log (\text{ionized drug})/(\text{unionized drug})$$
- Partition co-efficient: It is defined as the ratio of unionized drug distributed between the organic and aqueous phases at equilibrium. P o/w = (C oil/C water) equilibrium. Partition coefficient (oil/water) is a measure of a drug's lipophilicity and an indication of its ability to cross cell membranes. If P much greater than 1 are classified as lipophilic, whereas those with partition coefficient much less than 1 are indicative of a hydrophilic drug.
- Many drug substance can exist in more than one crystalline from with different space lattice arrangements. This property is known as polymorphism. Polymorphs generally have different melting points, X-ray diffraction patterns and solubility even though they are chemically identical.

- Powder flow properties can be evaluated by measurements of bulk density and angle of repose. Changes in particles size and shape are generally very important.
- Knowledge of absolute and bulk density of the drug substance is very useful in having some idea on the size of final dosage form, the density of solids also of affects their flow properties. Carr's compressibility index can be used to predict the flow properties based on density measurement.

$$\text{Carr's index (\%) or consolidation index} = \frac{\text{Tapped density} - \text{Poured density}}{\text{Tapped density}} \times 100$$

- Angle of repose: The maximum angle which is formed between the surface of a pile of powder and horizontal surface is called the angle of repose.
- Elevated temperature studies: The elevated temperatures commonly used are 40ºC, 50ºC and 60ºC with ambient humidity. The samples are stored at highest temperature are observed weekly for physical and chemical changes and compared to an appropriate control. If a substantial change is seen, samples stored at lower temperature are examined. If no changes are seen after 30 days at 60ºC, the stability prospects is excellent.
- Photolytic stability: Many drugs fade on light exposure. Though the extent of degradations may be small and limited to the exposed surface area, it leads to aesthetic problem. Exposure of drug to 400 and 900 foot-candles of illumination for 4 and 2 week periods respectively is adequate to provide some idea of photosensitivity. Resulting data may be useful in determining if an amber colored container is required or if color masking dye should be used in the formulation.
- Stability to oxidation: Drug's sensitivity to oxidation can be examined by exposing it to atmosphere of high oxygen tension. Usually a 40% oxygen atmosphere is used for rapid evaluation. Results may be useful in predicting whether antioxidant is required in the formulation or final product should be packaged under inert atmospheric conditions.
- Compatibility studies: The knowledge of drug excipients interaction is useful for the formulation to select appropriate excipients.

PRACTICE QUESTIONS

Long Answer Type Questions

1. What do you understand by the term preformulation? Discuss in brief about the objectives of preformulation studies.

2. Enumerate the various factors involved in preformulation studies? Discuss in detail about organoleptic properties?

3. Write short notes on following in context to preformulation studies.

a. Bulk characterization

b. Gibbs Kelvin relation

c. Crystallinity and polymorphism

4. Discuss in detail about physiochemical parameters related to preformulation.

5. How solubility and pKa play role in preformulation studies?
6. Discuss in brief the significance of pKa determination and dissociation constant in preformulation studies?
7. Make a chart for drug X, which is to be formulated in the form of tablet and carry out its preformulation studies?
8. What are various methods of drug excipient compatibility studies? Give brief explanation of each method.
9. Write in brief note on stability studies?
10. Write short notes on: (a) Contact angle, (b) surface area, (c) young's equation.

Objective Type Questions

1. Gibbs–Kelvinis relationship exists between _____ and apparent solubility of a drug.
2. When a substance exists in more than one crystalline form than the phenomenon is called _____ .
3. Preformulation studies includes all the studies except the following factor.
   a. The amount of drug available.
   b. The physicochemical properties of the drug already known.
   c. Therapeutic category and anticipated dose of compound.
   d. Route of drug administration
4. The primary objectives of preformulation studies are as follows:
   a. Establish the identity and physiochemical parameters of a new drug substance.
   b. Establish chemical stability profile of drug substance.
   c. Establish drug substance compatibility with common excipients
   d. Preformulation studies give preliminary idea about selection of excipients, which makes the formulation stable.
   e. All of the above
5. Stability analysis can be done by:
   a. IR
   b. High performance liquid chromatography (HPLC)
   c. Differential scanning calorimetry (DSC)
   d. All
6. Match the following based on particle size determination:

S. No.	*Instrument*		*Principle*
1	Anderson pipette	a	Based on light scattering
2	ROYCO	b	Based on rate difference of sedimentation of different particle
3	HIAC	c	Instrument based on blockage of electrical conductivity path
4	Coulter counter	d	Based on light blockage

7. Match the following based on grading of the powder for their flow properties and Carr index

S. No.	*Carr index*		*Flow*
1	5–15	a	Poor
2	18–21	b	Very poor
3	23–35	c	Excellent
4	33–38	d	Good

8. Match the following between angle of repose (θ) and powder flow

S. No.	*Angel of repose (θ) (degrees)*		*Flow*
1	< 25	a	Very poor
2	25–30	b	Excellent
3	30–40*	c	Good
4	> 40	d	Poor

ANSWERS

1. Particle size **2.** Polymorphism **3.** d
4. e **5.** d **6.** 1—b, 2—a, 3—d, 4—c
7. 1—c, 2—d, 3—a, 4—b **8.** 1—b, 2—c, 3—d, 4—a