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 effect drug performance and development of an efficacious, stable and safe dosage form.

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

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

OBJECTIVES OF PREFORMULATION STUDIES

The primary objectives of preformulation studies are as follows:

1. Establish the identity and physiochemical parameters of a new drug substance.
2. Establish chemical stability profile of drug substance.
3. Bulk characterization
4. Establish drug substance compatibility with common excipients
5. 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.

6. 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.
7. 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.

STUDIES INVOLVED IN PREFORMULATION

Following studies are conducted as basic preformulation studies; special studies are conducted depending on the type of dosage form and the type of drug molecule:

1. Organoleptic properties
2. Purity
3. Bulk characterization
   - Particle size distribution
   - Surface area
   - Density
   - Wettability
   - Hygroscopicity
   - Compression property
   - Crystallinity and polymorphism
   - Powder flow property
     - Bulk density
     - Angle of repose
4. Physiochemical properties
   - Solubility analysis
   - Intrinsic solubility
   - pH solubility profile
   - Common ion effect (ksp)
   - Solubilization
   - pka determination
   - Solvent

- Partition coefficient
- Dissolution
- Effect of temperature

5. Assay developments
6. Stability analysis
   - Solution stability
   - Solid state stability
     - Hydrolysis
     - Oxidation
     - Pyrolysis/elevated temperature studies
     - Photolysis
   - Stability under high humidity condition
7. Active drug compatibility with excipients or excipient compatibility

Organoleptic Properties

Organoleptic properties provide useful information mainly color, odor and taste of the drug substance. A few examples of these three organoleptic properties are tabulated in Table 1.1.

Table 1.1: Organoleptic properties of drug substance

Color	Odor	Taste
White	Pungent	Acidic
Off white	Sulfurous	Bitter
Cream yellow	Fruity	Intense
Tan	Aromatic	Sweet
Shiny	Odorless	Bland (smooth)

Organoleptic properties help in identification of the drug substance as well as it help to identify any degradation. For example, when oxidation reaction produces a colored degraded product, it will often be detected by human eye. Smell can be an effect method by which chemical and microbiological instabilities can be detected.

Purity

The preformulation scientists must have knowledge of the purity of a drug substance. Thin layer chromatography (TLC) and high

pressure liquid chromatography (HPLC) are of very wide ranging applicability and are excellent tools for characterizing the chemical homogeneity of many types of materials. Paper chromatography and gas chromatography can also be used in the determination of chemical homogeneity. All of these techniques can be designed to give a quantitative estimate of purity.

Bulk Characterization

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 during preformulation with the smallest particle size as is practical to facilitate preparation of homogeneous samples and maximize the drug's surface area for interactions.

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 a relationship between particle size and apparent solubility of a drug.

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

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, size influence the flow and the mixing efficiency of powders and granules. Size can also be a factor in stability; fine materials are relatively more open to attack from atmospheric oxygen, the humidity, and interacting excipients than are coarse materials.

Particle Size Determination (Table 1.2)

1. Simple method—microscopy with help of light microscope and sieving (but sieving is a less useful technique at preformulation stage due to lack of bulk material)
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—Andreasen pipette method

Table 1.2: Common techniques for measurement

Technique	Particle size (um)
Microscopic	1–100
Sieve	>50
Sedimentation	>1
Permeability	>1
Centrifugal	<50
Light scattering	0.5–50

Surface Area

Reason for controlling the particle size is that changes will alter the available surface area and consequently affect dissolution and potentially bioavailability.

Methods of Determination

BET nitrogen absorption: A precise measurement of surface area is based on Brunauer–Emmette–Teller (BET) theory of adsorption. Most substances adsorb a monomolecular layer of gas under certain condition of partial pressure of gas and temperature. Here nitrogen absorption in which a layer of nitrogen molecule is absorbed to the sample surface at –196°C. Once surface absorption reached equilibrium the sample is heated to room temperature, the nitrogen

gas is desorbed and its volume is measured and converted to the number of absorbed molecule via the ideal gas low.

SEM (scanning electron microscope): Surface morphology can be observed by a SEM which serves to confirm qualitatively a physical observation related to surface area.

Density

Bulk density of a compound varies substantially with the method of crystallization, milling or formulation. It is a great importance when one considers the size of high capsule product or the homogeneity of a low dose formulation in which there are larger difference in drug and excipient densities.

Bulk density = Mass of powder/Bulk volume

Density problem is easily correlated by milling, slugging, formulation and method of measurement.

Wettability

Wetting is an adsorption process in which an intimate contact of the solid phase is achieved. The process is important in the following ways:

1. Intimate contact of solids or liquids with liquid is an initial step towards the preparation of suspension and emulsion.
2. In granulation prior to tabletting, the powders are mixed with a liquid binding agents. The success of this process in part depends on the wetting and spreading of the liquid over the solid.
3. Film coating requires the wetting and spreading of liquids (containing coating material) over the tablet surface.
4. Dissolution of a tablet or a capsule necessitates the penetration of liquid into pores of the dosage form.

Surfactant are used to aid wetting of powders, because of their following properties:

a. Lowering the interfacial tension
b. Lowering of contact angle between the solids and liquids
c. Displacing air and permit the intimate contact

Contact angle (Fig. 1.1) is used as an indicator to evaluate the efficiency of a wetting agent. Contact angle can be defined as angle between the liquid droplets and surface over which it spreads.

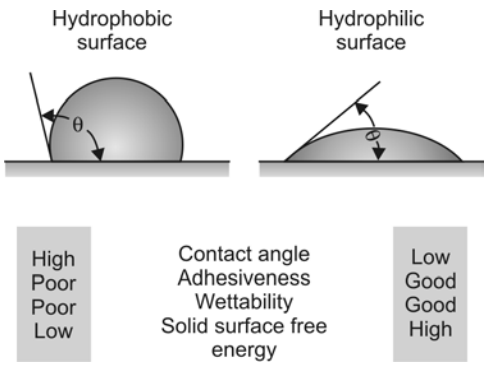


Fig. 1.1: Contact angle, an indicator of adhesiveness, wettability and surface free energy

At equilibrium, Young's equation (Fig. 1.2) define relationship between contact angle, solid/liquid interfacial free energy, solid free energy and liquid free energy.

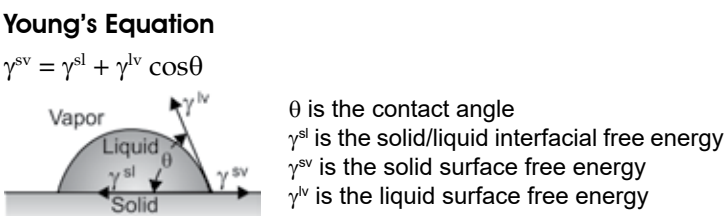


Fig. 1.2: Young's equation

Examples, with contact angle values:
θ = 0° then a drop of water on a glass surface
θ > 90° a drop of fatty acid on a clean glass surface or a drop of water on a paraffin coated surface
θ = 109° a drop of water on Teflon

Hygroscopicity

Many drug substances, particularly water-soluble salt forms, have a tendency to adsorb atmospheric moisture. Adsorption and equilibrium moisture can depend upon the atmospheric humidity, temperature, surface area, exposure and mechanism for moisture uptake.

Deliquescent materials adsorb sufficient water to dissolve completely such as sodium chloride on a humid day. Other hygroscopic substances adsorb water because of hydrate formation or specific site adsorption.

With most hygroscopic materials, changes in moisture levels can greatly influence many important parameters, such as chemical stability, flowability and compatibility.

Analytical methods for monitoring the moisture level include:

a. Gravimetry
b. TGA (thermogravimetric analysis)
c. Karl Fischer titration
d. Gas chromatography

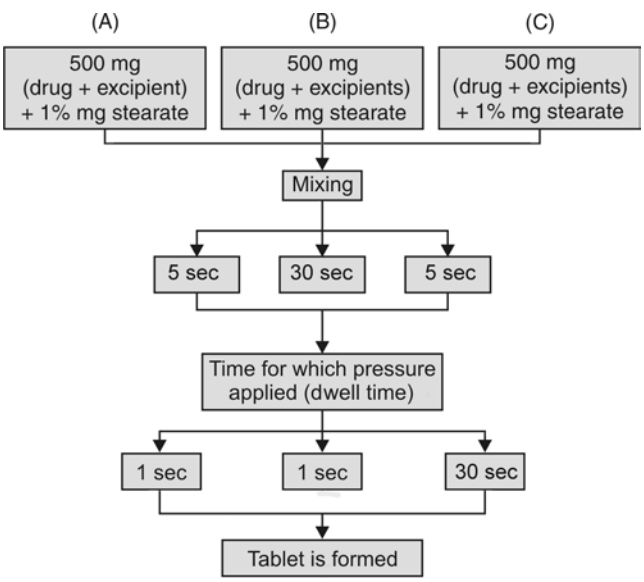
Compression characteristics

Benefit to be derived from compression testing is an indication of whether the drug is elastic, plastic or brittle. In order to make a good tablet, there is a need for brittle fracture and plastic flow, elasticity is also often present, but it is not a desirable property. For example, if the drug is plastic material the diluent should compact by brittle fracture (e.g. lactose). If the drug is brittle material it is best to mix it with a plastic excipient such as microcrystalline cellulose.

There are three methods by which we can formulate the drug substance into tablets:

1. Direct compression
2. Wet granulation
3. Slugging/dry granulation

Method of compression testing



Plastic strength is more in 'C' tablets as pressure applied for longer time (dwell time more).

Crystallinity and Polymorphism

Crystallinity

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

Habit is the description of the outer appearance of a crystal internal structure is the molecular arrangement within the solid.

A single internal structure for a compound can have several different habits depending upon the environment for growing crystals. Changes with internal structure usually alter the crystal habit while such chemical changes as conversion of a sodium salt to its free acid form produce both a change in internal structure and crystal habit.

Characterization of a solid volume

1. Verifying that the solid is the expected chemical compound.
2. Characterizing the internal structure.
3. Describing the habit of the crystal.

The internal structure of a compound can be classified as given in Table 1.3.

Table 1.3: Various crystal system (Fig. 1.3)

S. 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≠c	Nickel sulfide
3.	Orthorhombic	$\alpha=\beta=\gamma= 90°$	a≠b≠c	$KMnO_4$
4.	Monoclinic	$\alpha=\gamma=90°$ and $\beta \neq 90°$	a≠b≠c	Sucrose
5.	Triclinic (asymmetric)	$\alpha\neq\beta\neq\gamma\neq90°$	a≠b≠c	$CuSO_4$
6.	Trigonal (rhombohedral)	$\alpha=\beta=\gamma\neq90°$	a=b=c	Sodium nitrate
7.	Hexagonal	$\alpha=\beta=90°$ and $\gamma=120°$	a=b≠c	$AgNO_3$

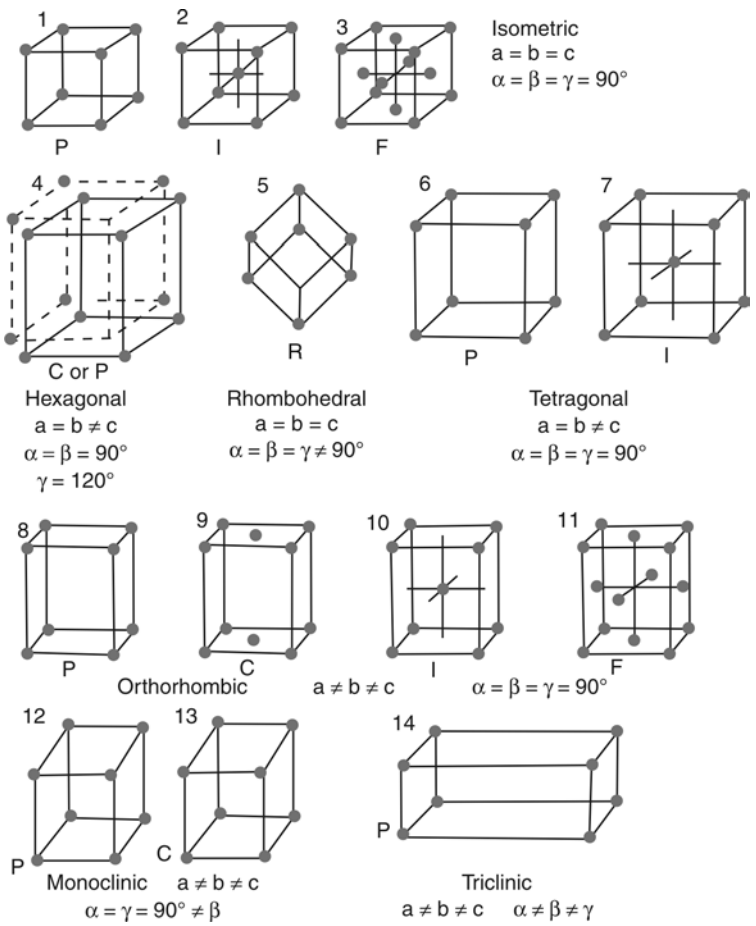


Fig. 1.3: Different crystal systems

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 was more available after oral administration as compared to less soluble pure A form and their mixture.

Amorphous forms are typically prepared by rapid precipitation, lypophilization 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. sulfur.
2. **Monotropic:** It is the one in which transition from one polymorph to another will be irreversible.

Depending upon their relative stability, one of the several polymorphic form will be physically more stable than others. Stable polymorph represent lowest energy state, has highest melting point and least aqueous solubility. The remaining polymorph are called as 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-stoichiometer adducts such as inclusion or clathrates, 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 as pseudopolymorph and phenomenon called pseudopolymorphism.

When the incorporated solvent is water, the complex is called a hydrate and the term hemihydrate, monohydrate and dehydrate 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. Solvent including polymorphic changes are: water, methanol, ethanol, acetone, chloroform, n-propanol, isopropyl alcohol, n-butan, n-pentanol, benzene and toluene. The presence of traces of solvent (either water or organic) is usual in early batches of new drug candidates, as residue from the precipitation process in the final crystallization. These can become molecular addition to the crystal and change in its habit.

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 behavior of the compound dispersed in silicon oil using hot stage microscopy. Pseudopolymorphs will evolve a gas (steam or solvent vapor) causing bubbling of the oil. True polymorphs merely melt, forming a second globular phase. The temperature at which the solvent volatilizes will be close to the boiling point of the solvent and can be used for identification.

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.

a. 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 1.4 and 1.5).
Carr 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.

Table 1.4: Grading of the powder for their flow properties, according to Carr index

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

*Adding the glidant, e.g. 0.2% aerosil should improve the flow.

Table 1.5: Compressibility and flowability of pharmaceutical excipients

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

b. 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. 1.4 and Tables 1.6 and 1.7).

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

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

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

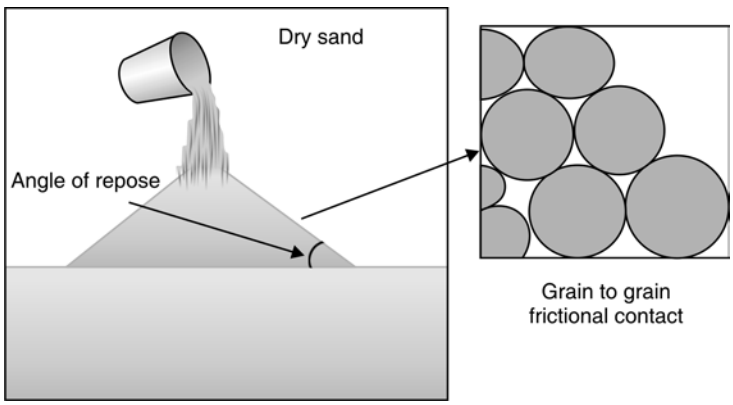


Fig. 1.4: Showing angle of repose of dry sand. Spherical the particle lesser the angle of repose and better will be the flow

The lower the angle of repose, the better the flow property. Certain observations are made:

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

Table 1.6: 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

*Adding glidant, e.g. 0.2% aerosol, may improve flow.

Table 1.7: Angle of repose of some pharmaceutical excipients

Substance	Angle of repose (θ°)
Calcium state NF	10
Dextrose	25
Lactose USP	15
Lactose (spray dried) USP	20

Contd.

Table 1.7: Angle of repose of some pharmaceutical excipients (Contd.)

Substance	Angle of repose (θ°)
Magnesium oxide	20
Microcrystalline cellulose USP	15
Starch NF	15
Stearic acid NF	15
Talc USP	15
Sodium bicarbonate USP	20

Physiochemical Parameters

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 absorption (Fig. 1.5).

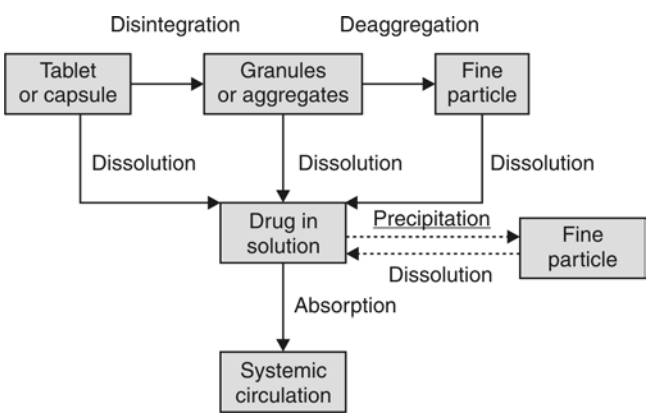


Fig. 1.5: Solubility or dissolution as critical step during absorption

Kaplan (1972) suggested that unless a compound has an aqueous solubility in excess of 1% (10 mg/ml) over the pH range 1–7 at 37 °C then potential bioabsorption problem may occur.

Analytical methods that are particularly useful for solubility measurements include HPLC, UV spectroscopy, fluorescence spectroscopy and gas chromatography. For most drug, 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 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 is an acidic solution compared with its aqueous solubility suggested a weak base and an increase in alkali, a weak acid. In both cases, a dissociation constant (pKa) will be measurable and salt should form. An increase in both acidic and alkaline solubility suggests either amphoteric or zwitter-ion behavior; in this case, there will be two pKa, one acidic and one basic. No change in solubility suggests a non-ionizable neutral molecule with no measurable pKa. Here solubility manipulations will require either solvents or complexation.

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 by two temperatures:

a. 4 or 5°C to ensure good physical stability and to extend short-term storage and chemical stability until more definitive data is available.
b. 37°C to support biopharmaceutical evaluation.

pH Solubility Profile

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, 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} or 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

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

$$= [BH^+]_S \left(1 + \frac{Ka}{H^+}\right)$$

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

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

$$= [B]_S \left(1 + \frac{H^+}{Ka}\right)$$

Corresponding equation for acidic compound:

$$S_t\ pH < pH_{max} = [AH]_S \left(1 + \frac{Ka}{[H^+]}\right)$$

$$S_t\ pH > pH_{max} = [A^-]_S \left(1 + \frac{H^+}{Ka}\right)$$

Since ionizable compounds may be available in free or salt form, one could use either in solubility experiments, e.g. Serajuddin and Karwoski studied the solubility behavior of phenazopyridine free base and its hydrochloride salt over pH range 1 to 10. Phenazopyridine, a free with pKa of 5.2, exhibits maximum solubility at pH 3.45 (pH_{max}). It should be noted here that, depending upon the starting material, in the region of pH_{max} experimentally determined. Solubilities are higher than the equilibrium solubilities. This phenomenon described as supersaturation. Supersaturated solutions are metastable and will precipitated excess solute in due course on standing.

Solubility Product/Common Ion Effect

In a saturated solution of a salt with some undissolved solid, there exists an equilibrium between the excess solid and the ions resulting from the dissociation of the salt in solution. For a hydrochloride salt represented as BH^+Cl^-, the equilibrium is

$$BH^+Cl^-\ (s) \rightleftharpoons BH^+ + Cl^-$$

where, B is the base compound, BH^+ and Cl^- represents hydrated ion in solution. The corresponding equilibrium constant K is given by

$$K = \frac{[BH^+][Cl^-]}{[BH^+Cl^-]_{solid}} \quad \ldots (1)$$

As a solid the activity of $[BH^+Cl^-]_{solid}$ is constant, then equation becomes

$$K_{sp} = [BH^+]\ [Cl^-] \quad \ldots (2)$$

For an ionizable drug as mentioned earlier total solubility S_T is sum total of $[BH^+]_s$ and [B] since $[BH^+]_s >> [B]$, equation (2) becomes,

$$K_{sp} = S_T [Cl]^- \quad \ldots (3)$$

Equation (3) indicates that total solubility of a hydrochloride salt would decrease with an increase in the chloride ion concentration. This phenomenon is known as common ion effect.

Since the gastric contents are high in Cl^- ion concentration, the common ion effect phenomenon suggest that one should use salts other than the hydrochloride to benefit fully from the enhanced solubility due to a salt form. Despite this, many drugs are used as hydrochloride salts. This is because solubilities of most hydrochloride salts is so high that the suppression of solubility due to common ion effect under *in vivo* conditions is not of sufficient magnitude to affect dissolution or bioavailablity of these compounds.

Solubilization

When the drug substance under consideration is not an acidic or basic compound, or when the acidic or basic character of the compound is not amenable to the formation of a stable salt, other means of enhancing the solubility may be explored. Like use of **Co-solvent**. A general means of increasing solubility is the addition of a co-solvent to the aqueous system. The solubility of poorly soluble non-electrolytes can often be improved by orders of magnitude with suitable co-solvent such as ethanol, propylene glycol and glycerin. These co-solvents solubilize drug molecule by disrupting the hydrophobic interactions of water at the non-polar solute/water interface. The extent of solubilization due to the addition of co-solvent depends on the chemical structure of the drug, i.e. the more non-polar the solute, the greater is the solubilization achieved by co-solvent addition. Take example of hydrocortisone and hydrocortisone-21-heptonate. The lipophilic ester (i.e. hydrocortisone-21-heptonate) is solubilized to more extent by addition of propylene glycol than by the more polar parent compound (hydrocortisone).

pKa Determination/Dissociation Constant

The amount of drug that exists in unionized form is a function of dissociation constant (pKa) of drug and pH of the fluid at the absorption site.

It is customary to express the dissociation constants of both acidic and basic drugs by pKa values. The lower the pKa of an acidic drug, stronger the acid, i.e. greater the proportion of ionized form at a particular pH. The higher the pKa of basic drug the stronger the

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 and Hasselbalch equation.

$$\text{For weak acid: } pH = pKa + \log \frac{\text{Ionized drug concentration}}{\text{Unionized drug concentration}}$$

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

$$\text{For weak base: } pH = pKa + \log \frac{\text{Unionized drug concentration}}{\text{Ionized drug concentration}}$$

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

The above equations used:

- To determine the pKa by following changes in solubility.
- To allow the prediction of solubility at any pH provided that the intrinsic solubility (C_0) and pKa are known.
- To facilitate the selection of suitable salt forming compounds and predicts the solubility and pH properties of the salts.

A pKa value can be determined by a variety of analytic methods:

- Buffer, temperature, ionic strength and co-solvent affect the pKa value and should be controlled for these determinations.
- The preferred method is the detection of spectral shifts by UV or visible spectroscopy, since dilute solution can be analyzed directly.
- A second method, potentiometric titration, offers maximum sensitivity for compounds with pKa values in the range of 3 to 10 but is often hindered by precipitation of the unionized form during the titration since a high drug concentration is usually require to obtain a significant titration curve.
- To prevent precipitation, a co-solvent such as methanol or dimethyl sulfoxide can be incorporated.
- **General information:** 75% of the all drugs are weak base (20% are weak acid and the remaining 5% are non-ionic, amphoteric or alcohols). It is, therefore, appropriate to consider this equation.

Salt formation

Most drugs are either weak acid or weak bases. One of the easiest approach to enhance the solubility and dissolution rate of such drugs to convert them into their salt forms. Generally, with weakly acid drugs a strong base salt is prepared as the sodium or potassium salts of barbiturates and sulfonamide. In case of weakly basic drug, a strong acid salt is prepared like the HCl or sulfate salts of several alkaloidal drugs, e.g. consequences of changing chlordiazepoxide to various salts form (Table 1.8).

Table 1.8: Solubility of chlordiazepoxide and its various salts

Salts	pKa	Salt pH	Solubility
Chlordiazepoxide base	4.80	8.30	2.0
Hydrochloride	–6.10	2.53	2165 (1)
Sulfate	–3.00	2.53	Freely soluble
Besylate	0.70	2.53	Freely soluble
Malate	1.92	3.36	57.1
Tartrate	3.00	3.90	17.9
Benzoate	4.20	4.50	6.0
Acetate (2)	4.76	4.78	4.1

(1) Maximum solubility of chlordiazepoxide hydrochloride achieve at pH 2.89 is governed by crystal lattice energy, a common ion.
(2) Chlordiazepoxide acetate may not form. pKa too high and close to drug.

The dissolution rate of a particular salt is usually much greater than the parent drug. Sodium and potassium salts of weak acids dissolve much rapidly than the parent acid. Some comparative data are shown in Table 1.9.

Table 1.9: Solubility of sodium salts of weak acid

Drug	pKa	pH at (C_s)	Dissolution rate (mg cm^{-2}/min) × 10^2	
			Dissolution media	
			0.1 M HCl (pH 1.5)	Phosphate buffer (pH 6.8)
Salicylic acid	3.0	2.40	1.7	27
Sodium salicylate		8.78	1870	2500
Benzoic acid	4.2	2.88	2.1	14
Sodium benzoate		9.35	980	1770
Sulfathiazole	7.3	4.97	<0.1	0.5
Sodium sulfathiazole		10.75	550	810

Solvents

The first choice for a solvent is obviously water. However, although the drug may be freely soluble, some are unstable in an aqueous solution. Accordingly, water-miscible solvents must be used.

- As co-solvents in formulation to improve solubility or stability.
- In analysis to facilitate extraction and separation (chromatography).

Oils are used in emulsions, topical (creams and ointments), intramuscular injection and liquid-fill oral preparation (soft and hard gelatin capsules) when aqueous pH and co-solvent solubility and stability are unpalatable.

Aqueous methanol is widely used in HPLC and is the standard solvent in samples extraction during analysis and stability testing (Table 1.10).

Table 1.10: Recommended solvents for preformulation screening

Solvent	Dielectric constant (ε)	Solubility parameters (δ)	Applications
Water	80	24.4	Formulation
Methanol	32	14.7	Extraction and separation
0.1 M HCl (pH 1.07)	–	–	Dissolution (gastric), basic extraction
0.1 M NaOH (pH 13.1)	–	–	Acidic extraction
Buffer pH (7)	–	–	Dissolution (intestinal)
Ethanol	24	12.7	Formulation, extraction
Propylene glycol	32	12.6	Formulation
Glycoyl	43	16.5	Formulation
PEG 300 or 400	35	–	Formulation

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 unionized drug distributed between the organic and aqueous phases at equilibrium.

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

For series of compounds, the partition coefficient can provide an empiric handle in screening for some biologic properties. 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.

- Solubility in both aqueous and mixed solvents.
- Drug absorption *in vivo*: applied to a homologous drug series for structure activity relationship.
- Partition chromatography: Choice of column (HPLC) or TLC and choice of miscible phase (eluent).
- Extraction of crude drugs.
- 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. It has been suggested that the solubility of drug exceeded 10 mg/ml at pH 7 then no bioavailability problem were to be expected.

An equation which describes the process of 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. 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:

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

Intrinsic dissolution

When dissolution is solely controlled by the diffusion, the rate of dissolution is directly proportional to the dissolution rate of a solid in its own solution is adequately described by Noyes–Nernst equation.

$$\frac{dC}{dt} = \frac{SD(C_s - C)}{hV}$$

where dC/dt = dissolution rate, S = surface area of dissolved solid, D = diffusion coefficient, C = solvent concentration in the bulk medium, h = diffusion layer thickness, V = volume of dissolved medium, C_s = solute concentration in the diffusion layer.

During early phase of dissolution, $C_s >> C$ and is essential equal to saturation solubility C_s, surface area A and volume V can be held constant under this condition and at a constant temperature and agitation above equation becomes:

$$\frac{dC}{dt} = KC_s, \text{ where, } K = SD/hV = \text{constant}$$

Intrinsic dissolution rate is generally expressed as

$$\text{mg dissolved} \times (\text{min}^{-1}\text{cm}^{-2})$$

This constant rate differs from the dissolution from conventional dosage form which is known as total dissolution (mg/min) where the exposed surface area (S) is uncontrolled as disintegration, deaggregation and dissolution process. According to the intrinsic dissolution rate, it is independent of formulation effects and measure the intrinsic properties of the drug and salt as a function of dissolution media effects, e.g. pH, ionic strength and counter ions.

Influence of some parameters on dissolution rate of drug:

- Diffusion coefficient of drug.
- Surface area of solid drug.
- Water/oil partition coefficient of drug.
- Concentration gradient.
- Thickness of stagnant layer.

Effect of Temperature

The heat of solution, ΔH_s, represents the heat released or absorbed when a mole of solute is dissolved in a large quantity of solvent. Most commonly, the solution process is endothermic, or ΔH_s is positive, and thus increasing the solution temperature increase the drug solubility. For such solutes as lithium chloride and other hydrochloride salts that are ionized when dissolved, the process is exothermic (negative ΔH_s) such that higher temperature suppress the solubility.

Heat of the solution is determined from solubility values for saturated solutions equilibrated at controlled temperatures over the range of interest. Typically, the temperature range should include 5°C, 25°C, 37°C and 50°C. The working equation for determining ΔH_s is

$$\ln S = \frac{-\Delta H_s}{R}\left(\frac{1}{T}\right) + C$$

S = molar solubility, R = Carr's constant, T = temperature (°K)

Over elevated temperature ranges, a semi-logarithmic plot of solubility against reciprocal temperature is linear and ΔH_s is obtained from the slope.

Assay Development

The majority of preformulation method development is Chromatographic with differing detection mechanism.

- HPLC (High-performance liquid chromatography): It is of two types—(i) normal phase HPLC: Chromatographic method that uses polar stationary phase and (ii) reversed phase HPLC: Chromatographic method that uses non-polar stationary phase
- GC (Gas chromatography)
- TLC (Thin layer chromatography)

Specialized method development can also be performed using capillary electrophoresis and supercritical fluid chromatography.

The analyted program consists of:

- Method development
- Forced degradation studies
- Validation

Stability Studies

The stability studies are done to determine shelf-life, and co-related specifications, and it must be taken into account of the

chemistry of the active ingredient and its likely vulnerability to degrade by oxidation, hydrolysis, isomerization, polymerization, decarboxylation, moisture, heat and light. Properly conducted stability study 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 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 physicochemical parameters, such as the presence of additives as well as the 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 (12 months) and accelerated stability studies (least 6 months) have to be carried out (Table 1.11).

Table 1.11: 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	12 months
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 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.

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:

- UV spectroscopy
- Thin layer chromatography (TLC)
- High-performance liquid chromatography (HPLC)
- Differential scanning calorimetry (DSC)

Chemical stability analysis includes:

1. Solution stability
2. Solid state stability
   - Hydrolysis
   - Oxidation
   - Pyrolysis/elevated temperature studies
   - Photolysis
3. Stability under high humidity condition

Solution Stability

The primary objective of this phase of preformulation research is identification of conditions necessary to form a stable solution. These studies should include the effect of pH, ionic strength, co-solvent, light, temperature and oxygen.

Solution stability investigations usually commence with probing experiments to confirm decay at the extramax of pH and temperature (e.g. 0.1 N HCl, water and 0.1 N NaOH all at 90°C).

These intentionally degraded samples may be used to confirm assay specificity as well as to provide estimate for maximum rates of degradation.

Since most solution in pharmaceutics are intended for parental root of administration, this initial pH-rate study should be conducted at a constant ionic strength that is compatible with physiological media.

Ionic strength can be calculated as:

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

m_i is the molar concentration of the ion, Z_i is the valency.

All ionic species (even the drug molecule) in the buffer solution must be considered in computing ionic strength. The apparent pH of a buffer solution also varies, when there is co-solvent is present. For example, pH rate profile of ampicillin.

Solid State Stability

The primary objectives of this investigations are identification of stable storage conditions for drug in the solid state and identification of compatible excipient for a formulation.

Chemical unstability normally results from either of the following reaction:

- Hydrolysis
- Oxidation
- Photolysis
- Pyrolysis

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 types, i.e. ionic and molecular forms 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.

A number of conditions catalyzes the breakdown.

- Presence of OH^- ion
- Presence of H_3O^+ ion
- Presence of divalent metal ion is quicker than molecular ion
- Ionic hydrolysis (protolysis)
- Heat
- Light
- Solution polarity and ionic strength
- High drug concentration

Oxidation: Oxidation is a loss of electrons and an oxidizing agent must be able to take electrons. In organic chemistry, oxidation is synonymous with dehydrogenation (the loss of hydrogen). There are two types of oxidation:

1. Oxidation processes that produced slowly under the influence of atmospheric oxygen.
2. Oxidation processes that involve the reversible loss of electrons without the addition of oxygen, e.g. oxidation of morphine, adrenaline, fixed oils, fats, volatile oils and phenol compounds.

Oxidation can be avoided by the use of polyhydroxy phenol anti-oxidant, e.g. hydroquinone. Most anti-oxidant function by providing electrons or labile H^+, which will be accepted by any free radical to terminate the chain reaction.

Pyrolysis/elevated temperature studies: The elevated temperatures most commonly used are 40°, 50° and 60°C in conjunction with ambient humidity. Occasionally, higher temperatures are used. The samples stored at the highest temperature should be examined for physical and chemical changes at weekly intervals, and any change, when compared to an appropriate control (usually a sample stored at 5°C), should be noted. If a substantial change is seen, samples stored at lower temperatures are examined. If no change is seen after 30 days at 60°C, the stability prognosis is excellent. Corroborative evidence must be obtained by monitoring the samples stored at lower temperatures for longer durations. Samples stored at room temperature and at 5°C may be followed for as long as 6 months. The data obtained at elevated temperatures may be extrapolated using the Arrhenius treatment to determine the degradation rate at a lower temperature.

Not all solid-state reactions are amenable to Arrhenius treatment. Their heterogeneous nature makes elucidation of the kinetic order

and prediction difficult. Long-term lower temperature studies are, therefore, an essential part of a good stability program.

Arrhenius equation

$$K = A\ e^{-E_a/RT}$$

E_a: activation energy; R: gas constant

$$log K = log A - (E_a/2.303RT)$$

Plotting the rate of reaction (K) against 1/T allows the calculation of rate at any temperature and, therefore, a prediction of shelf-life (t_{90}, time to 90% potency). This forms the basis of many accelerated stability tests.

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 1.12).

Table 1.12: 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:

- Cause decomposition
- Be retained or transferred
- Converted to heat
- Result in emission of light at a new wavelength (fluorescence, phosphorescence)

Natural sunlight lies in the wavelength range 290–780 nm of which only the higher energy (UV) (290–320 nm) cause photodegradation of drugs. These photolysis can be prevented by:

- Suitable packing: Using amber colored glass bottles, card board outers and aluminum foil overwraps and blisters.
- Clean flint glass absorbs around 80% in the 290–320 nm range whereas amber glass absorbs more than 95% plastic containers, by comparison absorbs only half of this amount of radiation.

Photostability testing (Fig. 1.6): Exposure of the drug substance to 400 and 900 footcandles of illumination for 4- and 2-week periods, respectively, is adequate to provide some idea of photosensitivity. Over these periods, the samples should be examined frequently for change in appearance and for chemical loss, and they should be compared against samples stored under the same conditions but protected from light.

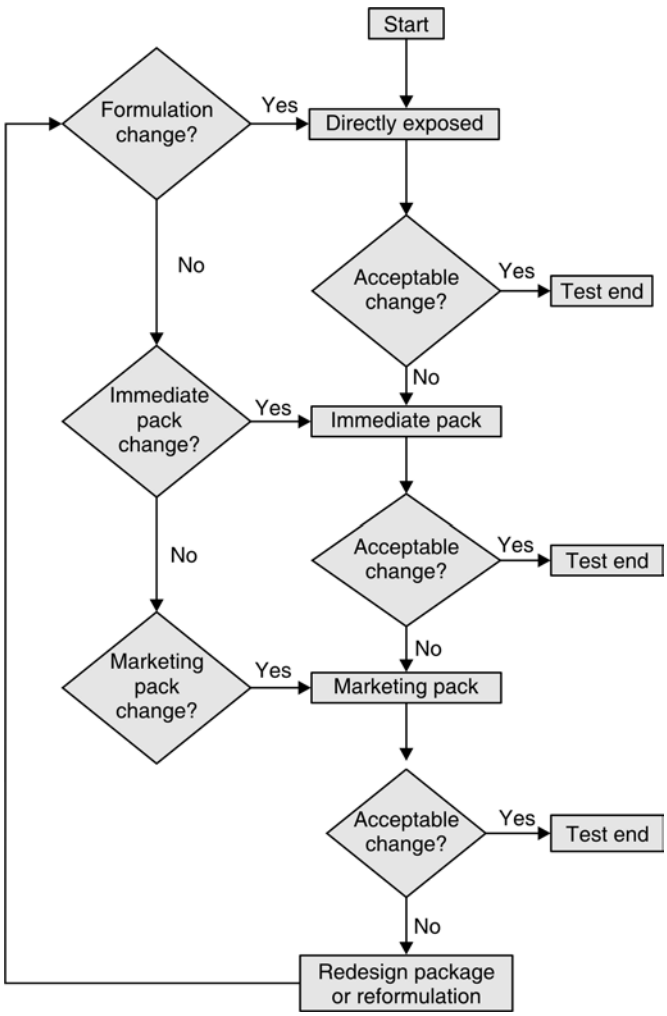


Fig. 1.6: Chart for photostability testing of drug products

Stability under High Humidity Conditions

In the presence of moisture, many drug substances hydrolyze, react with other excipients, or oxidize. These reactions can be accelerated by exposing the solid drug to different relative humidity conditions. Controlled humidity environments can be readily obtained using laboratory desiccators containing saturated solution of various salts. The closed desiccators in turn are placed in an oven to provide a constant temperature, e.g. decarboxylation of p-aminosalicylic acid show a dependence on the ambient moisture. So preformulation data of this nature are useful in determining if the material should be protected and stored in controlled low-humidity environment or if the use of an aqueous-based granulation system should be avoided. They may also caution against the use of excipient that adsorb moisture.

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. Incompatibility between excipient and drug substance can be detected by:

1. Differential scanning chromatography.
2. By evaluating organoleptic characteristics 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 physicochemical 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.

Table 1.13: 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/Sodium lauryl sulfate	–
OXC/Mannitol	208.63
OXC/Colloidal silica	206.11
OXC/Lactose	199.40
OXC/Magnesium stearate	206.64

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 1.13). The absence of the melting endotherm of OXC from the mixture with SLS may be due to the dissolution of the drug in the melting of the SLS.

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 cephalexin, observation tabulated in Table 1.14.

Table 1.14: Physical compatibility studies

Composition	Description		
	Initial	2 weeks	4 weeks
Cephalexin	White to off white powder	No color change	No color change
Cephalexin + Micro-crystalline 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

Contd.

Table 1.14: Physical compatibility studies (Contd.)

Composition	Description		
	Initial	2 weeks	4 weeks
Cephalexin + HPMC 15 pcs	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 + 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

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

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 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 physicochemical 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

- *Introduction:* Preformulation studies begin when a new chemical entity shows sufficient pharmacological promise and it may be a viable candidate for studies in man. Preformulation studies primarily include the study of the relevant physicochemical parameters for that dosage form and extensive stability studies so that the appropriate dosage form can be designed.
- *Aim:* To establish the physicochemical properties of a new drug (API); to establish the data on drug excipient compatibility; and to establish its (API) kinetic rate profile.
- *Stability studies:* Some commonly evaluated parameters: Organoleptic properties; purity; particle size, shape and surface area; solubility; dissolution; partition coefficient, ionization constant and Kp; crystal properties and polymorphism; density, hygroscopicity, flowability, wettability, etc.
- *Organoleptic properties:* Color—Stability problems, improve appearance by including dye in body or coating. Taste—Palatability problems, flavors and excipients may be added. Odor—Degradation products, e.g. aspirin, stable form of drug to be used, flavors and excipients may be used.
- *Purity studies are essential for further studies to be carried out safely:* Impurities may make a compound toxic or render it unstable. TLC, HPLC, GC and paper chromatography used. HPLC—Impurity index (II) and homogeneity index (HI), DTA, gravimetric analysis and melting point by hot stage microscopy are other techniques.
- *Techniques used for characterizing purity:* Thin layer chromatography (TLC), high pressure liquid chromatography and gas chromatography (GC). Impurity index (II) defined as the ratio of all responses (peak areas) due to components other than the main one to the total area response. Homogeneity index (HI) defined as the ratio of the response (peak area) due to the main component to the total response.
- *Particle size, shape and surface area:* Various chemical and physical properties of drug substances are affected by their particle size distribution and shapes. Size and shape also influence the dissolution of poorly soluble drugs which in turn influence bioavailability.

- Particle size determination microscopy is the simplest technique of estimating size ranges and shapes, e.g. light microscope, electron microscope. Andreasen pipette is based on the rate difference of sedimentation of different particles, but techniques like this are seldom used due to their tedious nature. Sieving 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 available.
- *Common techniques for measuring fine particles of various sizes:* Technique particle size (mm) microscopic 1–100, sieve >50, sedimentation >1, elutriation 1–50, centrifugal <50, permeability >1, light scattering 0.5–50.
- *Solubility determinations:* The solubility of drug is an important physicochemical property because it effects the rate of drug release into dissolution medium and consequently, the bioavailability of the drug, and therapeutic efficiency of the pharmaceutical product.
- *Common solvents used for solubility determination:* Benzyl alcohol, isopropyl alcohol, tweens, polysorbates, castor oil, peanut oil, sesame oil, buffer at various pHs, water, polyethylene glycols, propylene glycol, glycerin, sorbitol, ethyl alcohol, methanol.
- *Intrinsic solubility:* The solubility should ideally be measured at two temperatures. The minimum density of water occurs at 4°C. This leads to a minimum aqueous solubility. 37°C to support biopharmaceutical evaluation.
- *pKa determination:* It is the negative logarithm of dissociation constant. It describes about the chemical nature of API/NCE. It is mainly determined by using the Henderson–Hasselbalch equation. For acidic compounds, pH = pKa + log (unionized drug)/(ionized drug). For basic compounds, pH = pKb + log (ionized drug)/(unionized drug).
- *Partition coefficient:* 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. The partition coefficient is commonly determined using an oil phase of octanol or chloroform and water. 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 substances 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. Different polymorphs also lead to different morphology, tensile strength and density of power bed which all contribute to compression characteristics of materials.

- *Powder flow properties:* When limited amounts of drugs are available, 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. An increase in crystal size or a more uniform shape will lead to a small angle of repose and a smaller Carr's index.
- *Bulk density:* 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 affects their flow properties. Carr's compressibility index can be used to predict the flow properties based on density measurement. Carr's index (%) = (Tapped density – Poured density) × 100/Tapped density.
- *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 prospect is excellent.
- *Stability under high humidity conditions:* The preformulation data of this nature are useful in determining, if the material should be protected and stored in controlled low humidity environment or if non-aqueous solvent be used during formulation.
- *Photolytic stability:* Many drugs fade on exposure light. Though the extent of degradations small and limited to the exposed surface area, it presents an aesthetic problem. Exposure of drug 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 allows for rapid evaluation. A shallow layer of drug exposed to a sufficient headspace volume ensures that the system is not oxygen limited. Samples are kept in desiccators equipped with three-way stop cocks, which are alternatively evacuated and flooded with desired atmosphere. The process is repeated 3 or 4 times to ensure 100% desired atmosphere. Results may be useful in predicting, if an antioxidant is required in the formulation or if the 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. Mixtures should be examined under nitrogen to estimate oxidation and paralytic effect

at a standard heating rate on DSC, over a temperature range, which will encompass any thermal changes due to both the drug and appearance or disappearance one or more peaks in thermograms of drug excipient mixtures are considered of indication of interaction.

Long Answer Type Questions

Q1. What do you understand by the term preformulation? Discuss in brief about the objectives of preformulation studies.

Q2. Enumerate the various factors involved in preformulation studies. Discuss in detail about organoleptic properties.

Q3. Write short notes on following in context to preformulation studies.

a. Bulk characterization

b. Gibbs–Kelvin relation

c. Crystallinity and polymorphism

Q4. Discuss in detail about physicochemical parameters related to preformulation.

Q5. How solubility and common ion effect play role in preformulation studies?

Q6. Discuss in brief the significance of pka determination and dissociation constant in preformulation studies.

Q7. Make a chart for drug X, which is to be formulated in the form of tablet and carry out its preformulation studies at ambient condition for two months?

Q8. What are the various methods of drug excipient compatibility studies? How can incompatibilities between excipients and drugs be detected?

Q9. Write in brief about assay development and stability analysis used for preformulation studies.

Q10. Write short notes on:

a. Contact angle b. Surface area

c. Young's equation d. Method of compression testing

Objective Type Questions

1. Gibbs–Kelvin is relationship between …….. and apparent solubility of a drug.
2. When a substance exists in more than one crystalline form than the phenomenon is called as ……..
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 physicochemical 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. UV spectroscopy
   b. IR spectroscopy
   c. High-performance liquid chromatography (HPLC)
   d. Differential scanning calorimetry (DSC)
6. Match the following based on particle size determination:

S. No.	Method		Instrument
1.	Simple method	A	Based on rate difference of sedimentation of different particle
2.	Andreasen pipette	B	Microscopy
3.	ROYCO	C	Based on light scattering
4.	HIAC	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.	Angle 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. b
6. 1 (b), 2(a), (3)c, (4) d
7. 1 (c), 2(d), 3(a), (4) b
8. 1 (b), 2 (c), 3 (d), 4 (a)