CHAPTER

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Preformulation

Muhammad Qamar -Uz- Zaman
Assistant Professor
Department Of Pharmacy
The Islamia University Bahawalpur.

INTRODUCTION

Preformulation studies are described as the process of optimizing the delivery of drug through determination of physicochemical properties of the new compound that could affect drug performance and development efficacious, stable and safe dosage form. Preformulation activities range from supporting discovery's identification of new active agents to characterizing physical properties necessary for the design of dosage forms. To develop an adequate formulation, it is necessary to fully understand the physical and chemical properties of the drug substance. The goals of preformulation are to:

- To establish the necessary physicochemical parameters of a new drug substance
- To determine its kinetic rate profile
- To establish its physical characteristics
- To establish its compatibility with common excipients. The study of drug-excipient interactions therefore represents the intersection of solid-state organic chemistry and solid-state pharmaceutical physics.

A preformulation program of a new drug candidate is divided into:

1. **Preliminary Preformulation:** At this stage, one may merely know the identity of the drug candidate. It is necessary to acquire the information necessary to interpret the solid-state data to be acquired in later stages. Pre-preformulation data is intrinsic to the drug candidate, and independent of the perturbing influence of physical phases.
   - Empirical and structural formula
   - Molecular weight
   - Whether drug is acidic/basic/non-ionic
   - pH dependence of partition coefficient
   - Solubility tendencies.

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The sophistication of computational programs is reaching the point that one may calculate many of these quantities to an acceptable degree of accuracy.

Through the proper completion of preliminary-preformulation work, it is possible to develop an optimal design for the actual preformulation program of study. In addition, the preliminary preformulation results allow one to have a feel for the validity of the experimental results.

2. **Preformulation Program:** It includes characterization of the bulk drug substance, establishment of solubility characteristics, and evaluation of stability in various media and studies of drug-excipient incompatibilities.
   - Acid or base ionization constants
   - Solubility in aqueous and non-aqueous solvents
   - pH dependence of solubility
   - Partition coefficient profile
   - Polymorphism
   - Hygroscopicity
   - Particle size analysis
   - Drug stability
   - Powder flow characteristics
   - Compatibility studies.

PHYSICOCHEMICAL PARAMETERS

Physico-chemical studies of a new drug substance include the studies of the following parameters:

- pKa, if drug is an acid or base
- Solubility
- Partition coefficient
- Dissolution
- Crystallinity
- Polymorphism
- Melting point
- Particle size analysis.

pK_a Determination

The pK_a (or ionization constant, dissociation constant) is defined as the negative logarithm of the equilibrium coefficient of the neutral and

charged forms of a compound. This allows the proportion of neutral and charged species at any pH to be calculated, as well as the basic or acidic properties of the compound to be defined. Determination of pK_a for a drug capable of ionization within a pH range of 1 to 10 is important since solubility and consequently absorption, can be altered by orders of magnitude with changing pH. 75% of all drugs are weak bases, 20% are weak acids and only 5% are non-ionic, amphoteric or alcohols. It is therefore appropriate to consider the Henderson-Hasselbalch equation for weak bases and acids.

The concept of pK_a is derived from the Henderson-Hasselbalch equation:

For an acid:

$$pH = pKa + \log \frac{\text{ionized conc. (salt)}}{\text{unionized conc. (acid)}}$$

For a base:

$$pH = pKa + \log \frac{\text{unionized conc. (base)}}{\text{ionized conc. (salt)}}$$

These equations can be used to determine pK_a following changes in solubility; to predict solubility at any pH and to facilitate the selection of suitable salt-forming compounds.

Since, the pH of body fluids varies, the absorption of a drug from various body fluids will differ and may dictate to some extent the type of dosage form and route of administration preferred for a given drug. Different ionic species of a molecule differ in physical chemical and biological properties and so it is important to be able to predict which ionic form of the molecule is present at the site of action. The partition coefficient is also a very useful parameter, which may be used in combination with the pK_a to predict the distribution of a drug compound in a biological system. pK_a values can be determined by potentiometry, conductivity and spectroscopy methods.

Solubility Analysis

Drugs having limited solubility (less than 1%) in fluids of gastrointestinal tract often exhibit poor or erratic absorption unless dosage forms are specifically tailored for drug. Therefore a solubility of less than 1 mg/mL indicates the need for a salt, particularly if the drug will be formulated as a tablet or capsule. The solubility of acids and bases is of course a function of pH **(Figure 3.1)**.

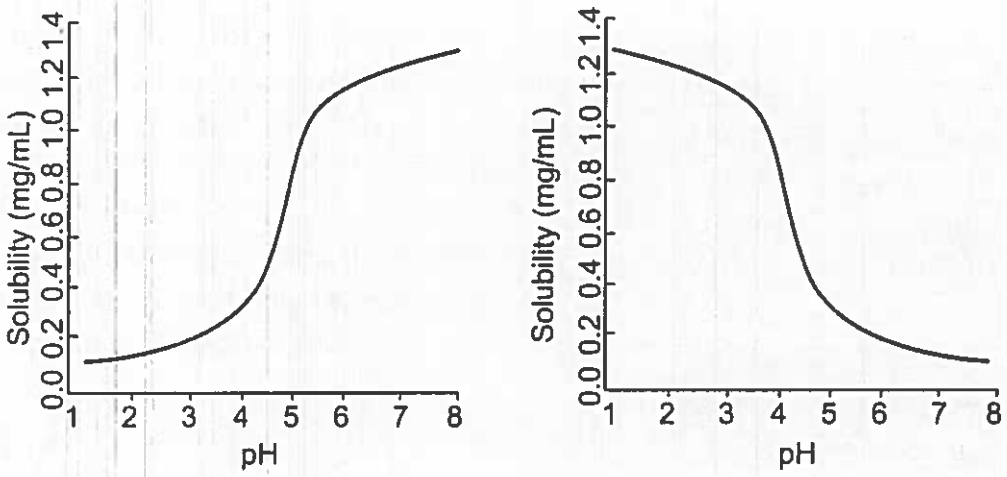


Figure 3.1: Effect of pH on solubility of weak acid (A) and weak base (B)

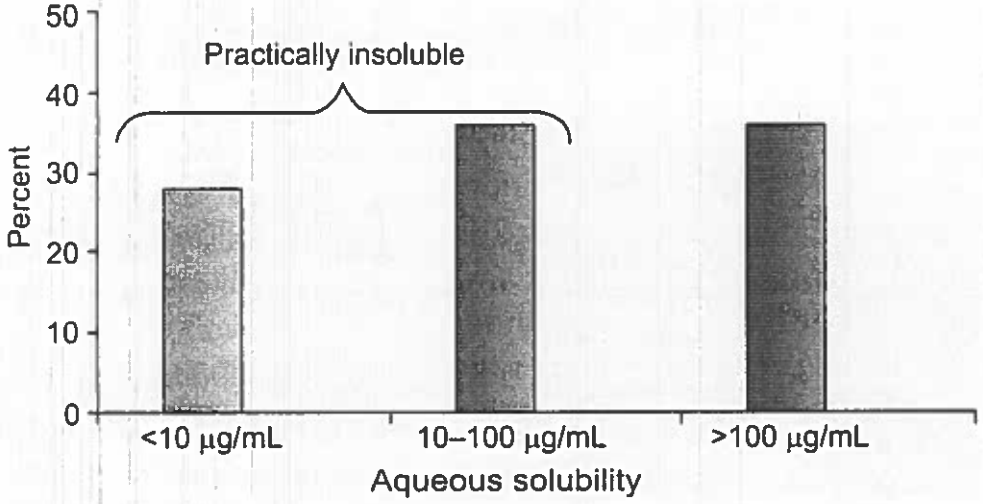


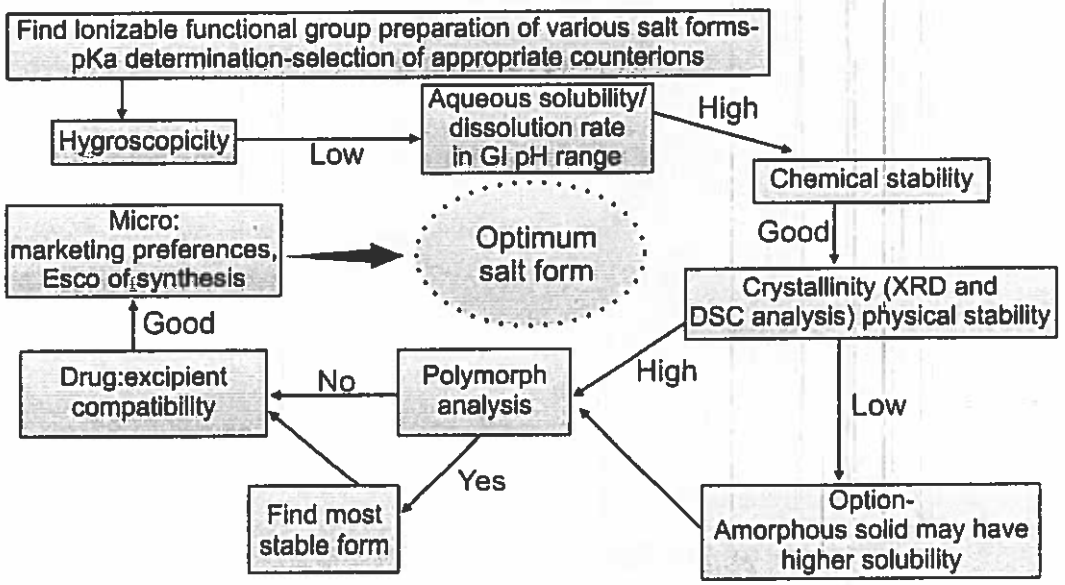
Figure 3.2: Recent trends in aqueous solubility of discovery compounds

Preformulation solubility studies focus on drug-solvent system that could occur during the delivery of a drug candidate. Solubility determinations are necessary both for stability reasons and for formulation reasons **(Figure 3.2)**. For drug candidates with either poor solubility or insufficient solubility for projected solution dosage forms, preformulation studies should also include experiments to identify possible mechanisms for solubilization. Solubility is determined by exposing an excess of drug to the liquid in question and assaying after equilibrium has been established. Analytical methods that are useful for solubility measurements include HPLC, UV spectroscopy and gas chromatography. Solubility values that are useful in a candidate's early development are those in distilled water, 0.9% sodium chloride, 0.01N hydrochloric acid, 0.1N sodium hydroxide, all at room temperature and at pH 7.4 buffer at 37°C.

A major improvement in solubility can be achieved by forming a salt. Salts prepared from strong acids or bases are freely soluble but very hygroscopic. This lead to instability in tablet or capsule formulation. It is better to use a weaker acid or base to form the salt, provided solubility requirements are met. The un-ionized (free) form of weak acids and bases may not be ideal molecular form for development. Salts are formed by reacting with an appropriate counter-acid or counter-base. Salts have different physical properties than their free forms. The dissolution rate of a particular salt is usually much greater than that of the parent drug. Changes in dissolution rate and solubility affect the rate and extent of absorption and changes on hygroscopicity and stability influence formulation. Improving oral absorption by increasing the dissolution rate is often a goal of the salt expansion step. Therefore each new drug candidate has to be examined to choose the most suitable salt, because each potential salt will behave differently and require separate preformulation screening **(Flow chart 3.1)**.

Converting the free acid/base form to a salt form is an important option to explore when trying to improve solubility and oral bioavailability. Of the 21 new molecular entities approved by US FDA in 2003, ten were salt forms. Selection of the right counter ion with optimum physiochemical characteristics is crucial to drug development. Consideration of the new form's physical chemical properties, processability under various manufacturing conditions and bioavailability must be made.

Flow chart 3.1: Salt screening decision tree



When a drug substance is poorly water soluble it often falls upon the preformulator to design solution systems, which achieve an adequate concentration for a solution dosage form. There are three approaches to:

- Solubilization by co-solvents
- Complexation
- Micellar solubilization.

Changing the aqueous phase drastically is an approach in solubilization of drugs. The solubility of poorly soluble non-electrolytes can often be improved by orders of magnitude with suitable co-solvents. Co-solvents commonly used are ethanol, glycerin, propylene glycols and polyoxyethylene glycols. These co-solvents solubilize drug molecules by disrupting the hydrophobic interactions of water at the nonpolar solute/water interfaces. If more than one co-solvent is used, then ternary diagrams **(Figure 3.3)** may be employed to visualize where maximum solubility occurs.

For hydrophobic nonionizable substances solubility can be improved by addition of nonpolar solvents. The relative polarities of solvents can be scaled using dielectric constant (ε), solubility parameter (δ), interfacial (γ) and hydrophilic-lipophilic balance (HLB). The solubility is usually tied to the dielectric constant, as shown in **Figure 3.4**, the solubility is often log-linear when plotted as a function of inverse

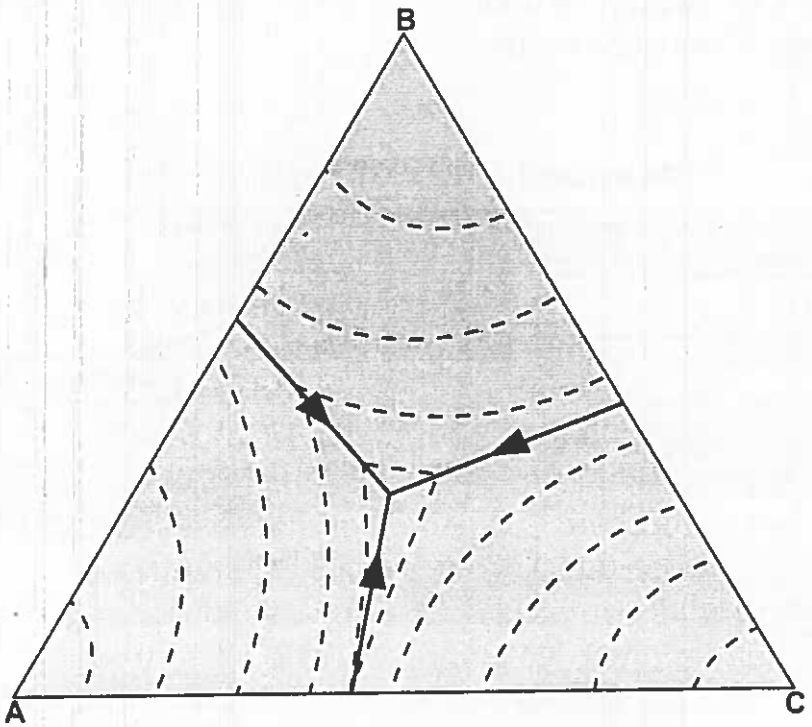


Figure 3.3: Ternary phase diagram

dielectric constant, ε. The optimum water/solvent ratio for another solvent can be calculated from known dielectric constant relationship.

$$\ln S = \frac{-e_1}{e + e_2}$$

where *e* terms are constant.

Water-miscible solvents are commonly used in formulations to improve solubility, stability and also in analysis to facilitate extraction and separation by chromatography. Oils are used in emulsions, creams, ointments, intramuscular injections and soft gelatin capsules when aqueous and stability are unattainable. **Table 3.1** shows a range of solvents used for preformulation studies.

Surfactants such as sorbates will solubilize drug compounds when surfactant is present in excess of its critical micelle concentration (CMC). Surfactants also aid in wetting down of a solvent and are often used in dissolution tests of poorly soluble drugs.

Drug substances may complex with complexing agents. The most important ligands are cyclodextrins, caffeine, urea and polyoxyethylene glycols. Complexed species have higher biological absorption rates than the native compound.

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:

$$P_{o/w} = \left(\frac{C_{oil}}{C_{water}}\right)\text{equilibrium}$$

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

Table 3.1: Solvents for preformulation screening

Solvent	*Dielectric Constant (ε)*	*Solubility Parameter (δ)*
Distilled water	80	24.4
Ethanol	24	12.7
Propylene glycol	32	12.6
Glycerol	43	16.5
PEG-400	35	9.9

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. Octanol-water partition coefficient log P is used in QSAR studies and rational drug design as a measure of molecular hydrophobicity. Hydrophobicity affects drug absorption, bioavailability, hydrophobic drug-receptor interactions, metabolism of molecules, as well as their toxicity. Factors such as absorption, excretion and penetration of the CNS may be related to the log P value of a drug and in certain cases predictions made. Drugs should be designed with the lowest possible log P to reduce toxicity, non-specific binding, increase ease of formulation and bioavailability.

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. The shake flask method is the method for measuring log P values. The UV absorbance of an aqueous solution is measured before and after being shaken with a known volume of octanol. HPLC may be used to estimate log P values. Compounds with known log P's are injected onto a C_{18} reverse phase HPLC column and their capacity factors used to create a calibration curve. Unknown compounds are then injected and their capacity factors used to predict log P. Strictly this technique is only valid for neutral molecules. Charged molecules have a far more complex retention behavior than simple partition.

Although it appears that the partition coefficient may be the best predictor of absorption rate, the effect of dissolution rate, pK_a, and solubility on absorption must not be neglected. Log D is the log distribution coefficient at a particular pH. This is not constant and will vary according to the protogenic nature of the molecule. Log D at pH 7.4 is often quoted to give an indication of the lipophilicity of a drug at the pH of blood plasma.

$$\text{Distribution Coefficient, } D = \frac{[\text{Unionized}]_{(o)}}{[\text{Unionized}]_{(aq)} + [\text{Ionized}]_{(aq)}}$$

Log D is related to log P and the pK_a by the following equations:

For Acids:

$$\log D_{(pH)} = \log P - \log [1 + 10^{(pH.pKa)}]$$

For Bases:

$$\log D_{(pH)} = \log P - \log [1 + 10^{(pKa.pH)}]$$

Relationships between log P and activity are often found in series where structural modifications have not significantly affected the pK_a values. Hansch in 1964 showed that these relationships were often parabolic **(Figure 3.4)**; hence the relationship often leads to an optimum value for the log P for a desired activity or selective distribution. Relationships of the type are:

Activity = m log P + k' (linear)

Activity = m log P – c (log P) 2 – k (parabolic)

Activity = m log P – c (b log P +1) – k (rectilinear)

where m, k and c are constants.

For both CNS penetration and gastric absorption many studies show a parabolic relationship with an optimum log P value of around 2. Evidence for this comes from a wide variety of experiments in the literature from brain concentration of radiolabelled compounds to CNS behavioral studies. The literature review reveals some general guidelines about the optimum log P values for certain classes of drugs. When designing drug molecules some thought should be given to the following, some studies have found:

- Optimum CNS penetration around log P = 2 ± 0.7 (Hansch)
- Optimum oral absorption around log P = 1.8
- Optimum intestinal absorption log P =1.35
- Optimum colonic absorption log P = 1.32

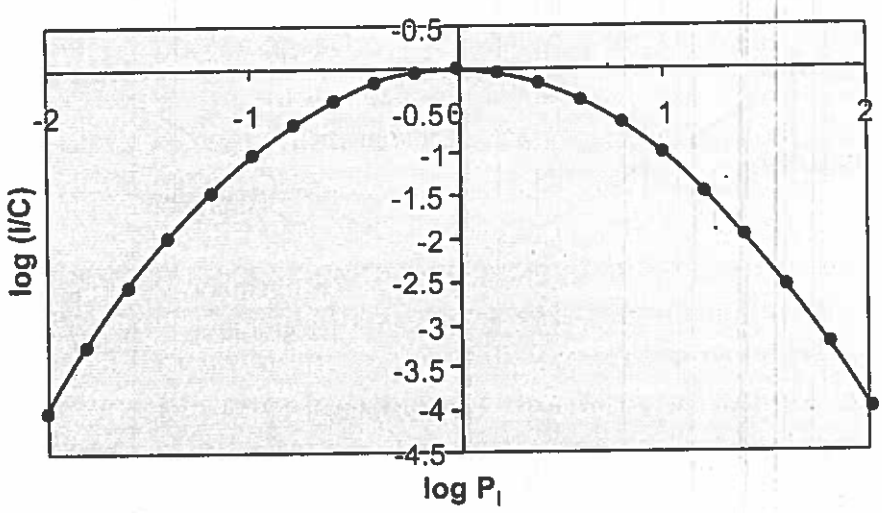


Figure 3.4: Log P versus biological activity

- Optimum sub-lingual absorption log P = 5.5
- Optimum percutaneous absorption log P = 2.6 (and low molecular weight)

Formulation and dosage forms:

- Low log P (below 0): Injectable
- Medium log P (0–3): Oral
- High log P (3–4): Transdermal
- Very high log P (4–7): Toxic build up in fatty tissues.

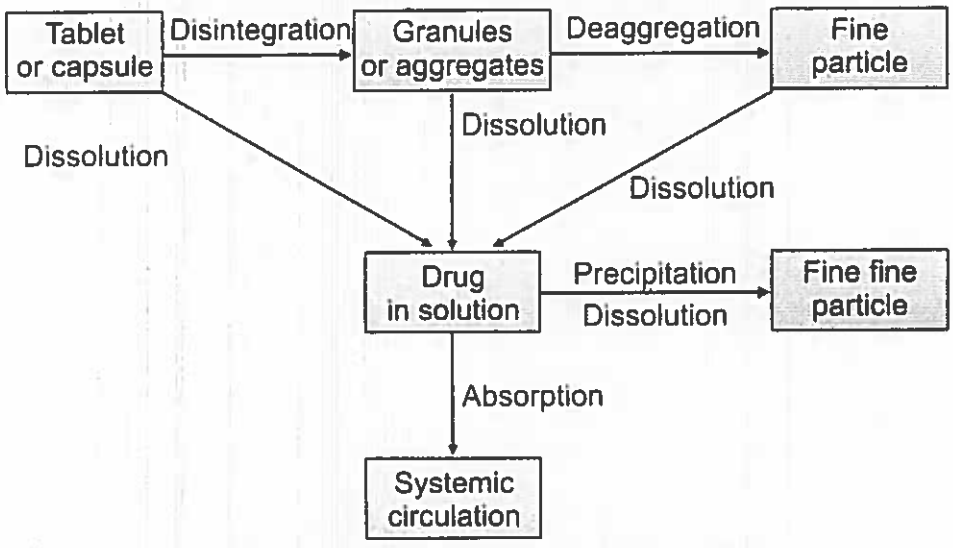
Dissolution

Dissolution of a drug particle is controlled by several physicochemical properties including chemical form, crystal habit, particle size, solubility, surface area and wetting properties **(Flow chart 3.2)**. The dissolution rate of a drug is the only important where it is the rate-limiting step in the absorption process. It has been suggested that when solubility of drug exceed 10 mg/mL at pH 7, then no bioavailability problems are expected. Below 1 mg/mL such problems were quite possible and salt formation could improve absorption and solubility by controlling the pH of the microenvironment independently of the drug and dosage form position within GIT.

The dissolution rate of a substance in which surface area is constant during dissolution is described by modified Noyes-Whitney equation:

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

Flow chart 3.2: Drug has to be in solution to get absorbed



where D is the diffusion coefficient, h is the thickness of diffusion layer at solid-liquid interface, A is the surface area of drug exposed to dissolution media, V is the volume of media, C_s is the concenteration of a saturated solution of the solute in the dissolution medium at the experimental temperature, and C is the concenteration of drug in solution at time t. The dissolution rate is given by dc/dt. If the surface area of drug is held constant and $C_s >> C$, the equation can be rearranged and integrated to give the following equation:

$$\frac{W}{A} = kt$$

where the constant k is defined as:

$$k = \frac{D}{h} C_s$$

and W is the weight (mg) of drug dissolved in time t.

A plot of W versus t gives a straight line with slope equal to intrinsic dissolution rate constant k, usually expressed in units of $mg/cm^2/min$.

The measurement of intrinsic dissolution is a tool in the functionality and characterization of bulk drug. The intrinsic dissolution rate (IDR) is defined as the dissolution rate of pure drug under conditions of constant surface area. The intrinsic dissolution rate and bioavailability are influenced by its solid state properties-crystallinity, amorphism, polymorphism, particle size and surface area, hydration, solvation, etc. Intrinsic dissolution is a method which utilizes a compressed disc of known area (a constant surface), effectively eliminating surface area and surface electrical charges as dissolution variables. The dissolution rate obtained by this method is IDR and is characteristic of each solid compound in a given solvent under the fixed experimental conditions. Each new drug candidate should be measured in 0.05 M HCl, phosphate buffer pH 7.0 and distilled water. To calculate intrinsic dissolution rate plot the cumulative amount of test specimen dissolved per unit area of compacted pellet against time. Linear regression is then performed and intrinsic dissolution rate is determined from the slope of regression line. The value is generally expressed as mg dissolved per minute per centimeters squared and is useful in predicting probable absorption problems due to dissolution rate. Intrinsic rates of greater than 1.0 $mg/min/cm^2$ have negligible problems with dissolution rate limitations, but rates less than 0.1 $mg/min/cm^2$ suggest problems with dissolution rate limitations. The importance of improvements in IDR due to microenvironmental pH control lies in the improvement of in vivo of a salt over the parent drug.

Crystal Morphology

Solids are either amorphous or crystalline. Crystalline substances are characterized by existing in crystal lattices. Crystallization is the process of formation of solid crystals from a uniform solution. Crystallization is also a chemical solid-liquid separation technique. Crystal formation is a special characteristic of a solid in which molecules self-organize into regular, repeating molecular patterns. Crystal system is a category of space groups, which characterize symmetry of structures in three dimensions with translational symmetry in three directions, having a discrete symmetry group.

The shape or habit of a crystal of a given substance may vary but the angles between the faces are always constant. A crystal is made up of atoms, ions, or molecules in a regular geometric arrangement or lattice constantly repeated in three dimensions. This repeating pattern is known as the unit cell. There are 7 crystal systems:

1. Triclinic, all cases not satisfying the requirements of any other system; thus there is no other symmetry than translational symmetry, or the only extra kind is inversion
2. Monoclinic, requires either 1 twofold axis of rotation or 1 mirror plane
3. Orthorombic, requires either 3 twofold axes of rotation or 1 twofold axis of rotation and two mirror planes
4. Tetragonal, requires 1 fourfold axis of rotation
5. Rhombohedral, also called trigonal, requires 1 threefold axis of rotation
6. Hexagonal, requires 1 sixfold axis of rotation
7. Isometric or cubic, requires 4 threefold axes of rotation.

There are 2, 13, 59, 68, 25, 27, and 36 space groups per crystal system, respectively, together 230. **Table 3.2** gives a breakdown of the various different things per crystal system.

Crystals are characterized by repetitious spacing of constituent atoms or molecules in a three-dimensional array, whereas amorphous forms have atoms or molecules randomly placed as in a liquid **(Figure 3.5)**. Crystal habit and the internal structure of a drug can affect bulk and physicochemical properties, which range from flowability to chemical stability. Crystal habit can be modified by:

- Excessive supersaturation
- Change in cooling rate and agitation
- Change of crystallizing solvent
- Addition of co-solvents or other solutes and ions.

Table 3.2: Crystal systems

Crystal System	*No. of Point Groups*	*No. of Bravais Lattices*	*No. of Space Groups*
Triclinic	2	1	2
Monoclinic	3	2	13
Orthorhombic	3	4	59
Tetragonal	7	2	68
Rhombohedral (Trigonal)	5	1	25
Hexagonal	7	1	27
Cubic	5	3	36
Total	32	14	230

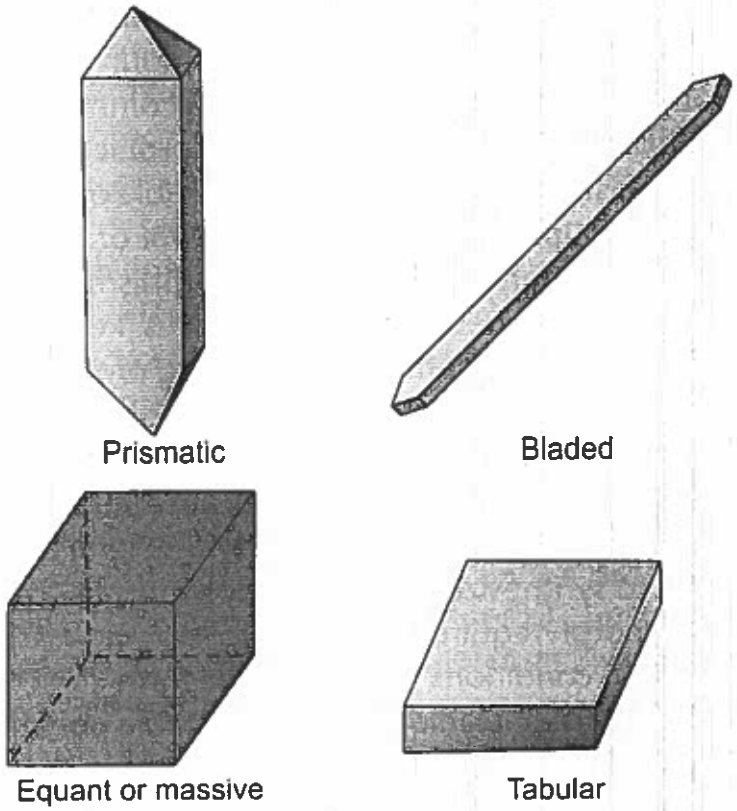


Figure 3.5: Few examples of crystal habits

Microscopy is used to study basic crystallography and to determine crystal morphology (structure and habit). Most pharmaceutical powders have crystals in the range of 0.5–300 µm. However the distributions are often smaller, 0.5–50 µm, to ensure good blend homogeneity and rapid dissolution. For most preformulation work a 10 × eyepiece and a 10 × objective are mostly used for regular powders, while 10 × 20 is used for micronized powder.

Amorphous forms have atoms or molecules randomly placed as in liquid and are prepared by rapid precipitation, lyophilization or rapid cooling of liquid melts. Amorphous forms are of higher thermodynamic energy than corresponding crystalline forms therefore solubility as well as dissolution rates are generally greater than crystalline forms. Upon storage, amorphous solids tend to revert to more stable forms. This thermodynamic instability, which can occur during bulk processing or within dosage forms, is a major disadvantage.

Polymorphism

Crystalline forms have different arrangements and/or conformations of the molecules in the crystal lattice. Amorphous forms consist of disordered arrangements of molecules that do not possess a distinguishable crystal lattice. Solvates are crystal forms containing either stoichiometric or nonstoichiometric amounts of a solvent. If the incorporated solvent is water, the solvate is commonly known as a hydrate. When a drug substance exists in polymorphic forms, it is said to exhibit polymorphism. Polymorphism is remarkable particularly within certain structural groups: 63% of barbiturates, 67% of steroids and 40% sulfonamides exhibit polymorphism. Polymorphism is an important aspect of physical properties of drug substances. Polymorphism can affect the quality, safety, and efficacy of the drug product.

One of the characteristics of a metastable polymorph is that it is more soluble than its stable counterpart. Knowledge of polymorphic forms is of importance in preformulation because suspension systems should never be made with a metastable form. Several techniques are used to attempt to synthesize metastable polymorphs. Preparation of metastable polymorph requires:

i. Supersaturating conditions for metastable form
ii. Crystallization of metastable state before stable polymorph forms
iii. Stable conditions for the metastable polymorph so that conversion to stable form is prevented.

Since polymorphism is so important, it should be routine in preformulation of solids; DSC traces, X-ray powder diffraction and solubility determinations should be carried out. In preformulation the following should be considered:

- How many polymorphs exist?
- How stable are metastable polymorphs?
- Is there any amorphous glass?
- Can the metastable form be stabilized?

- What is the solubility of each form?
- Will a more soluble form survive processing and storage?

There are a number of methods that can be used to characterize polymorphs of a drug substance. Demonstration of a nonequivalent structure by single crystal X-ray diffraction is currently regarded as the definitive evidence of polymorphism. X-ray powder diffraction can also be used to support the existence of polymorphs. Other methods, including microscopy, thermal analysis (e.g. differential scanning calorimetry, thermal gravimetric analysis, and hot-stage microscopy), and spectroscopy (e.g. infrared [IR], Raman, solid-state nuclear magnetic resonance [ssNMR]) are helpful to further characterize polymorphic forms.

Polymorphic forms of a drug substance can have different chemical and physical properties, including melting point, chemical reactivity, apparent solubility, dissolution rate, optical and mechanical properties, vapor pressure, and density. These properties can have a direct effect on the ability to process and/or manufacture the drug substance and the drug product, as well as on drug product stability, dissolution, and bioavailability as described below:

a. **Influences on solubility, dissolution, and bioavailability (BA) and bioequivalence (BE):** The solid-state properties of a drug substance can have a significant influence on the solubility of the drug substance. Since polymorphic forms differ in their internal solid-state structure, a drug substance that exists in various polymorphic forms can have different aqueous solubilities and dissolution rates. When there are differences in the solubilities of the various polymorphic forms, we recommend that you focus on the potential effect such differences can have on drug product bioavailability (BA) and bioequivalence. Whether drug product BA/BE can be affected by the differences in solubilities of the various polymorphic forms depends on the various physiological factors that govern the rate and extent of drug absorption including gastrointestinal motility, drug dissolution, and intestinal permeability.

   For a drug whose absorption is only limited by its dissolution, large differences in the solubilities of the various polymorphic forms are likely to affect BA/BE. On the other hand, for a drug whose absorption is only limited by its intestinal permeability, differences in the solubilities of the various polymorphic forms are less likely to affect BA/BE. Furthermore, when the solubilities of the polymorphic forms are sufficiently high and drug dissolution is rapid in relation to

gastric emptying, differences in the solubilities of the polymorphic forms are unlikely to affect BA/BE.

b. **Influences on manufacturing of the drug product:** Drug substance polymorphic forms can also exhibit different physical and mechanical properties, including hygroscopicity, particle shape, density, flowability, and compactibility, which in turn may affect processing of the drug substance and/or manufacturing of the drug product. The effect of polymorphism on pharmaceutical processing also depends on the formulation and the manufacturing process. For a drug product manufactured by direct compression, the solid-state properties of the active ingredient will likely be critical to the manufacture of the drug product, particularly when it constitutes the bulk of the tablet mass. On the other hand, for a drug product manufactured by wet granulation, the solid-state properties of the active ingredient are often masked by the resultant granulation; therefore such properties of the active ingredient are less likely to affect the manufacture of the drug product. In the context of the effect of polymorphism on pharmaceutical processing, what is most relevant is the ability to consistently manufacture a drug product that conforms to applicable in-process controls and release specifications.

   Polymorphic forms of the drug substance can undergo phase conversion when exposed to a range of manufacturing processes, such as drying, milling, micronization, wet granulation, spray drying, and compaction. Exposure to environmental conditions such as humidity and temperature can also induce polymorph conversion. The extent of conversion generally depends on the relative stability of the polymorphs, kinetic barriers for phase conversion, and applied stress. Nonetheless, phase conversion generally is not of serious concern, provided that the conversion occurs consistently, as a part of a validated manufacturing process where critical manufacturing process variables are well understood and controlled and where drug product BA/BE has been demonstrated.

c. **Influence on stability:** Polymorphs can have different physical and chemical (reactivity) properties. The most stable polymorphic form of a drug substance is often chosen during development based on the minimal potential for conversion to another polymorphic form and on its greater chemical stability. However, a metastable form can be chosen for various reasons, including bioavailability enhancement. Nonetheless, because drug product stability is

affected by a multitude of other factors, including formulation, manufacturing process, and packaging, it is the stability of the drug product, and not stability of the drug substance polymorphic form that should be the most relevant measure of drug quality.

Melting Point Determination

The melting point of a substance is the temperature at which the material changes from a solid to a liquid state. Pure crystalline substances have a clear, sharply defined melting point. During the melting process, all of the energy added to a substance is consumed as heat of fusion, and the temperature remains constant. Purity may be assessed by determining melting point of compound. Melting point and solubility are related via the latent heat of fusion, which is the amount of heat generated during melting or fusion. A crystal with weak bonds has a low melting point and low heat of fusion. A strong lattice leads to higher melting point and a high heat of fusion. Polymorphs differ in melting point and solubility. Melting point can be determined by capillary method, differential scanning calorimeter (DSC) and differential thermal analysis (DTA).

Differential scanning calorimetry (DSC) is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature **(Figure 3.6)**. Both the sample and reference are maintained at very nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned. The basic principle underlying this technique is that, when the sample undergoes a physical transformation such as phase transitions, more (or less) heat will need to flow to it than the reference to maintain both at the same temperature. Whether more or less heat must flow to the sample depends on whether the process is exothermic or endothermic. For example, as a solid sample melts to a liquid it will require more heat flowing to the sample to increase its temperature at the same rate as the reference. This is due to the absorption of heat by the sample as it undergoes the endothermic phase transition from solid to liquid. Likewise, as the sample undergoes exothermic processes (such as crystallization) less heat is required to raise the sample temperature. By observing the difference in heat flow between the sample and

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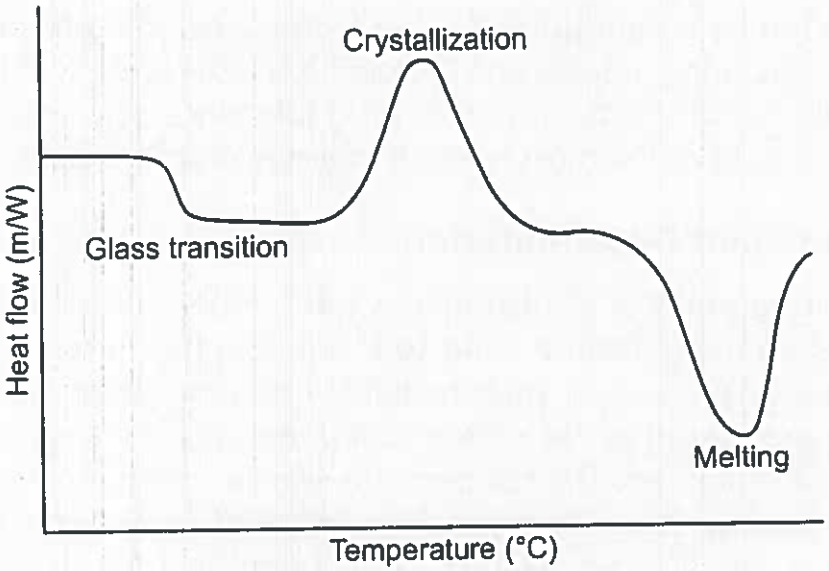

Figure 3.6: A schematic DSC curve

reference, differential scanning calorimeters are able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle phase changes, such as glass transitions. DSC is widely used in industrial settings as a quality control instrument due to its applicability in evaluating sample purity and for studying polymer curing. The result of a DSC experiment is a heating or cooling curve. This curve can be used to calculate enthalpies of transitions. This is done by integrating the peak corresponding to a given transition. It can be shown that the enthalpy of transition can be expressed using the following equation:

$$\Delta H = KA$$

where ΔH is the enthalpy of transition, K is the calorimetric constant, and A is the area under the curve. The calometric constant will vary instrument to instrument, and can be determined by analyzing a well-characterized sample with known enthalpies of transition.

An alternative technique, which shares much in common with DSC, is differential thermal analysis (DTA). In this technique it is the heat flow to the sample and reference that remains the same rather than the temperature. When the sample and reference are heated identically phase changes and other thermal processes cause a difference in temperature between the sample and reference. Both DSC and DTA provide similar information; DSC is the more widely used of the two techniques.

Introduction DTA involves heating or cooling a test sample and an inert reference under identical conditions, while recording any temperature deference between the sample and reference. This

deferential temperature is then plotted against time, or against temperature. Changes in the sample, which lead to the absorption or evolution of heat, can be detected relative to the inert reference. Deferential temperatures can also arise between two inert samples when their response to the applied heat treatment is not identical. DTA can therefore be used to study thermal properties and phase changes which do not lead to a change in enthalpy. The baseline of the DTA curve should then exhibit discontinuities at the transition temperatures and the slope of the curve at any point will depend on the microstructural constitution at that temperature.

In the pharmaceutical industry it is necessary to have well-characterized drug compounds in order to define processing parameters. For instance, if it is necessary to deliver a drug in the amorphous form, it is desirable to process the drug at temperatures below those at which crystallization can occur. A DTA curve can be used as a fingerprint for identification purposes, to evaluate drug and polymer purities.

Particle Size Analysis

Flow properties, dissolution, chemical reactivity and formulation homogeneity are directly affected by size, shape and surface morphology of drug particles. Small particles are important in low-dose high-potency drug candidates and for any drug whose aqueous solubility is poor. Particle size reduction to extremely small size (<10μm) may be inadvisable as it act as dissolution rate-limiting steps. During preformulation smallest particle size for each new drug candidate should be determined. There are numerous methods of particle size analysis such as sieving, microscopy, coulter counter and laser light scattering. Microscopy can be used for qualitative particle shape and size analysis, image analysis is often required to automate the complete task assigning shape values to large number of particles. Light scattering can also provide morphology data for some shapes of powder. Computer interfacing of image analysis techniques offer several advantages. Surface morphology may be observed by scanning electron microscopy (SEM).

DRUG AND PRODUCT STABILITY

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration

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dating periods. Pharmaceutical products should ideally have shelf-life of at least 3 years and the potency should not fall below 95% under recommended storage conditions. By investigating the intrinsic stability of drug it is possible to select formulation approaches and indicate types of excipients, specific additives and packaging. Drug degradation may occur by: Hydrolysis, oxidation, photolysis, trace metal catalysis.

Temperature

Temperature has a high degree of influence on all varieties of chemical reactions and usually they are accelerated by a raise in temperature. It is said that typically a 10°C increase in temperature produces a 2 to 5 fold increase in decomposition. The effect of temperature on the rate constant k is indicated by the Arrhenius equation:

$$K = Ae^{-(Ea/RT)}$$

$$\log k = \log A - (E_a/2.303\ RT)$$

For drug compounds whose decomposition is log K is temperature, if log K is plotted against 1/T, a straight line is obtained. This is kown as Arrhenius plot **(Figure 3.7)**. The constants E_a and A may be determined

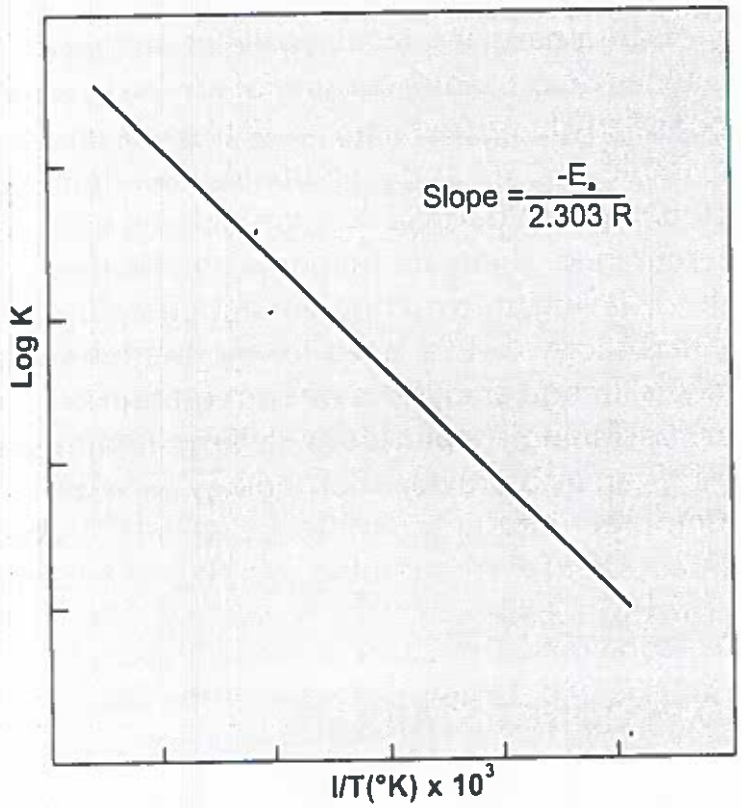


Figure 3.7: An Arrhenius plot

from the slope and intercept of this line, which are equal to $-E_a/2.303R$ and log A respectively.

Influence of pH

Acidic and alkaline pH influence the rate of decomposition of most drugs. Many drugs are stable between pH 4 and 8. Weekly acidic and basic drugs show good solubility when they are ionized and they also decompose faster when they are ionized. So if the pH of a drug solution has to be adjusted to improve solubility and the resultant pH leads to instability then a way out of this tricky problem is to introduce a water-miscible solvent into the product. It will increase stability by (A) Suppressing ionization, (B) Reducing the extreme pH required to achieve solubility, (C) Enhancing solubility and (D) Reducing the water activity by reducing the polarity of the solvent.

Reactions catalyzed by pH are monitored by measuring degradation rates against pH, keeping temperature, ionic strength and solvent concentration constant. Some buffers such as acetate, citrate, lactate, phosphate and ascorbate buffers are utilized to prevent drastic change in pH.

In early stages of preformulation it is desirable to determine what is optimum buffer and its concentration, whether there is kinetic salt effect and what is the best pH for solutions of the compound at 25°C **(Figure 3.8)**.

Hydrolysis

The term "hydrolyze" is used to include almost any reaction with water. Hydrolytic reactions involve nucleophilic attack of labile bonds by water on the drug in solution and are first order. When this attack is by a solvent other than water it is known as solvolysis. Hydrolysis is considered to be the major cause of deterioration of drugs, especially for those in aqueous solution. The molecules having ester or amide functional groups are most susceptible to hydrolysis. Anesthetics, antibiotics, vitamins and barbiturates are examples for drugs that decompose due to hydrolysis. Although hydrolysis will occur principally with drugs in aqueous solution, suspensions and solid dosage forms are also susceptible to hydrolytic attack.

- Hydrolysis reactions involve nucleophilic attack of labile bonds by water on the drug in solution
- The reactions involving lactam groups are fastest and are followed by those involving esters, amides and imides in that order

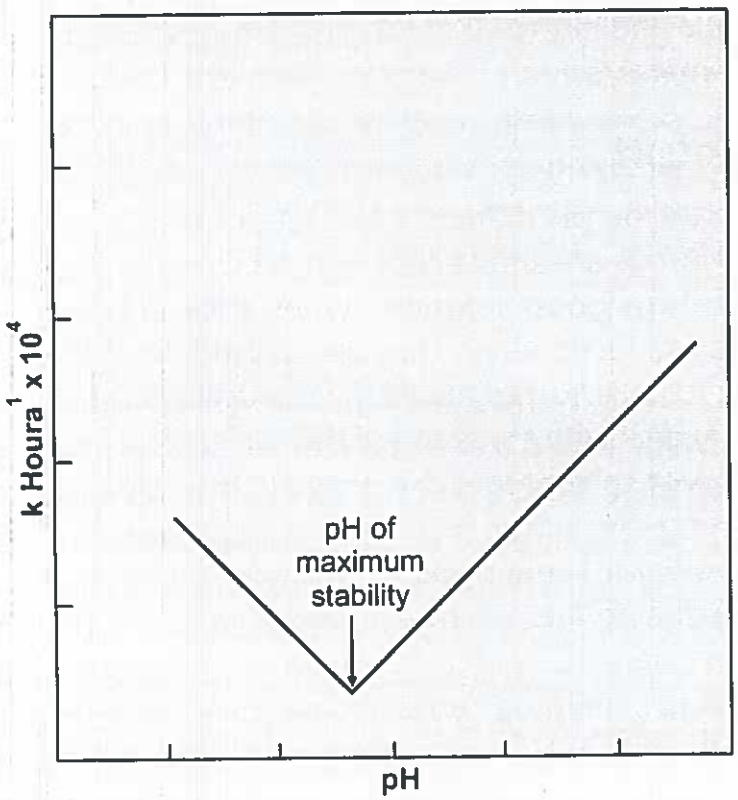


Figure 3.8: pH inflection plot of maximum stability

- These reactions usually follow first order
- If this type of reaction happens due to any other solvent it is called solvolysis
- These reactions are catalyzed by: (A) H_3O^+ (B) Presence of divalent metal ions (C) Ionic hydrolysis (Protolysis) (D) Heat (E) Light (F) Solution (G) High drug concentrations.

Replacement of water with nonaqueous solvents (e.g. ethanol, glycerin, mannitol) decrease the velocity of hydrolysis. There have been several reports on the influence of surfactants in reducing the rate of hydrolysis. The hydrolytic rates may be decreased by complex formation due to steric effects. Modification of chemical structure causes a decrease in hydrolytic rate of many drugs; this may be due to steric and/or polar effect of substituent group. Another technique sometimes employed to increase the stability of drugs undergoing hydrolysis is to reduce their solution solubility by forming less soluble salts or esters of the drugs.

Oxidation

Oxidation involves addition of oxygen or the removal of hydrogen. Oxidation reactions are chemical reactions that involve the transfer of

Table 3.3: Examples of antioxidants

For Aqueous Systems	*For Oily Systems*
Acetylcysteine	Ascorbyl palmitate
Ascorbic acid	Butylated hydroxyanisole
Sodium metabisulfite	Butylated hydroxytoluene
Sodium sulfite	Hydroquinone
Sodium thiosulfate	Lecithin
Sulfur dioxide	Propyl gallate
Thioglycerol	Tocopherol

electrons from one substance to an oxidizing agent. The most common form of oxidative degradation is autoxidation, which involves a free radical chain process (see *Chapter 7*). Oxidation is controlled by the environment, i.e. light, trace metals, oxygen and oxidizing agents. The oxidative decomposition of drugs is responsible for the instability of considerable number of pharmaceutical preparations.

- Highly unsaturated compounds are more susceptible to autoxidation
- Presence of free carboxylic acid group enhances reactivity of organic molecules
- Dilution of an oxidizable compound with an inert solvent decreases the rate of oxidation
- The rate of oxidation of an organic compound is increased with increase in temperature of storage
- Pro-oxidants accelerate the rate of autoxidation.

Antioxidants are molecules that slow or prevent the oxidation of other chemicals. Antioxidants slow these reactions either by reacting with intermediates and halting the oxidation reaction directly, or reacting with the oxidizing agent themselves and preventing the oxidation reaction from occurring. Water soluble antioxidants act by undergoing oxidation in place of drug whereas oil soluble antioxidants act by inhibiting free radical chain process **(Table 3.3)**.

Photolysis

When molecules absorb energy and are energized or activated they go to a high energy state and then release that energy in a chemical reaction and come back to their original position. When this energy of activation is supplied by light and is absorbed by the compound the decomposition reaction is called photolytic. The activated species then returns to

Table 3.4: Relationship between wavelength and associated energy of various forms of light

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

ground state by either: (A) Emitting light of a different frequency (this is fluorescene or phosphorescene) or (B) Decomposition (photolysis). Oxidation to a great extent and hydrolysis to some extent are catalyzed by light and photo-induced reactions are common in steroids.

The energy associated with the radiation increases as its wavelength decreases, so that the energy of UV is greater than that of visible which is greater than that of IR **(Table 3.4)**. The higher energy range of visible light and UV light cause photolysis.

Photolysis is prevented by suitable packing in low actinic amber colored bottles, cardboard outers and in aluminum foil over wraps and blisters. Clear flint glass absorbs around 80% in 290–320 nm, whereas amber glass absorbs more than 95%. Plastic containers absorb only 50%.

Hygroscopicity

Deliquescent materials adsorb sufficient water from the atmosphere to dissolve completely. A substance that loses water to form a lower hydrate or become anhydrous is termed efflorescent. Hygroscopic substances adsorb water because of hydrate formation or specific site adsorption. With most hygroscopic substances, changes in moisture level can influence chemical stability, flowability and compactibility. Therefore pharmaceutical air-conditioning is usually set below 50% RH and hygroscopic substances are stored below 40% RH. To test hygroscopicity samples of drug are stored in open containers and then exposed to an atmosphere of controlled relative humidity by storing it over saturated salt solutions in desiccators. Moisture uptake is determined at different time points and storage period (0 to 12 weeks). Moisture level in samples can be determined by using various analytical techniques such as gravimetry, Karl Fischer titration and gas chromatography.

Solid State Stability

The objective of this investigation is identification of stable storage conditions for drugs in the solid state and identification of compatible

excipients for a formulation. Many solid state reactions occur in solution. Major source of the solvent are—residual moisture or solvent from wet granulation; moisture sorbed (in) onto excipients, such as starch and lactose; moisture in the capsule shell may migrate through direct contact or vapor phase; a melt of the drug itself or an ingredient in the formulation that has a low melting point and a solvate or hydrate that loses its lattice solvent with time and temperature fluctuation. Examples of few known incompatibilities are given in **Table 3.5**.

To determine solid state stability profile of a new compound, weighed samples are placed in open screw cap vials and are exposed directly to varying conditions of temperatures, humidity, and light intensities up to 12 weeks **(Table 3.6)**. Samples are then analyzed for drug content by HPLC; polymorph evaluation by DSC and IR.

Table 3.5: Known incompatibilities

Functional Group	*Incompatibilities*	*Type of Reaction*
Primary amine	Mono and disaccharides	Amine-aldehyde and amine-acetal
Ester, cyclic, lactose	Basic components	Ring opening, ester-base hydrolysis
Carbonyl, hydroxyl	Silanol	Hydrogen bonding
Aldehyde	Amine, carbohydrates	Aldehyde-amine, Schiff base or glycosylamine formation
Carboxyl	Bases	Salt formation
Alcohol	Oxygen	Oxidation to aldehydes and ketones
Sulfhydryl	Oxygen	Dimerization
Phenol	Metals	Complexation
Gelatin capsule shell	Cationic surfactants	Denaturation

Table 3.6: Storage conditions for determining bulk stability for a new drug candidate

Parameter	*Storage Conditions**
Heat (°C)	4, 20, 30, 40/75%Rh, 50 and 75
Moisture uptake	30, 45, 60, 75 and 90% Rh at room temperature
pH	1,3, 5, 7, 9 and 11 at room temperature and 37°C
Light	UV (254 and 366 nm) and visible at room temperature

*Room temperature can vary between 15 and 25°C.

A useful relationship for analyzing solid state stability data assumes that a compound must partially liquefy prior to decomposition. Given that the mole fraction of the solid that has liquefied (F_m) is directly proportional to its decay rate:

$$\ln K_{app} \alpha \ln F_m = \frac{\Delta H}{R}\left[\frac{1}{T} - \frac{1}{T_m}\right]$$

where ΔH is the molar heat of fusion, T_m is absolute melting point (in °Kelvin), T is absolute temperature of stability study and R is the gas constant.

Once bulk drug stability is determined compatibility with excipients commonly used to formulate solid dosage forms must be established. The number of excipients may be reduced by considering the results of solid state and solution stability profiles.

POWDER FLOW PROPERTIES

The specific bulk characteristics and properties of a powder that affect flow, which can in principle be measured are known as flow properties. Powders may be classified as free-flowing or cohesive (non-flowing). Flow properties are affected by changes in particle size, shape, density, electrostatic charges and adsorbed moisture. A preformulaton study assesses the derived parameters for drug powder flow properties- bulk density, Carr's index, Hausner ratio and angle of repose.

The bulk and tapped density of drug powder are often measured for processability. The tapped density is measured for two primary purposes: (i) The tapped value is more reproducibly measured than the bulk value, and (ii) The "flowability" of a powder is inferred from the ratio of these two measured densities. The true density (ρ_t) of a powder sample is the weight per unit volume of the material with no air spaces between particles, and is determined for computation of void volume or porosity (E) of packed powder beds. Apparent bulk density (ρ_b) is determined by pouring perceived bulk drug into a graduated cylinder and measuring the volume and weight "as is". Packed or tapped density (ρ_p) is determined by placing a graduated cylinder containing known mass of drug on a mechanical tapper, which is operated for a fixed number of taps until the powder bed volume has reached the minimum. Using the weight of drug in cylinder and minimum volume, the tapped density can be computed.

$$E(\%) = \left[1 - \frac{\rho_b}{\rho_t}\right] \times 100$$

One method of assessing flow properties is the angle of repose. Angle of repose is the maximum angle that can be obtained between the freestanding surface of powder heap and the horizontal plane as shown in **Figure 3.11**. Such measurement provides qualitative assessment of the internal cohesive and frictional effects. Angle of repose and flow rate depend on particle size, shape and surface roughness. Flow properties are frequently enhanced by the use of glidants. Powder is allowed to flow freely through a funnel onto the center of an upturned petri dish of known radius **(Figure 3.9)**. When the powder reaches the side of the petri dish the height of the cylindrical cone is determined. From the petri dish radius (r) and cone height (h), the angle of repose (θ) can be calculated.

$$\text{Tan}\,\theta = \frac{h}{r}$$

Carr's index (I_C) is a measure of interparticulate forces. If the interparticulate forces are high, powders will have a low bulk density because bridging will occur between particles **(Table 3.7)**. This results in a large Carr's index and a large change in volume caused

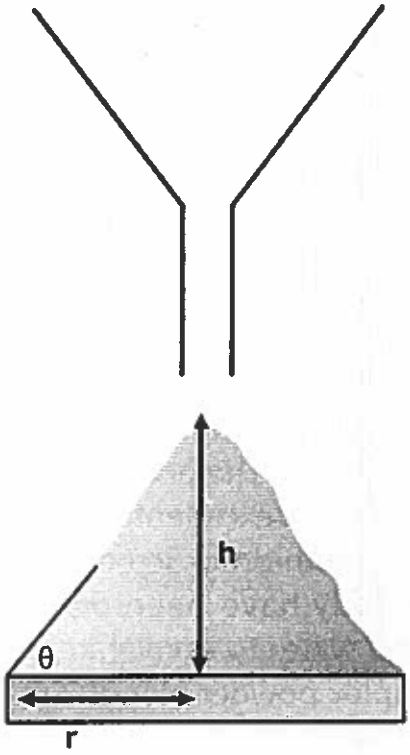


Figure 3.9: Measurement of angle of repose

Table 3.7: Carr's index, Hausner ratio and angle of repose as an indication of flow properties

Carr's Index (%)	Hausner Ratio	Angle of Repose (degrees)	Type of Flow
5–15	-	< 20	Excellent
12–16	< 1.25	20–30	Good
18–21	-	30–34	Fair to passable*
23–35	> 1.25	-	Poor
33–38	-	> 40	Very poor
> 40	-	-	Extremely poor

* May be improved by addition of glidant such as 0.2% Aerosil.

by tapping. If the interparticulate forces are low, particles will have little affinity for one another, and will compact spontaneously. Under these circumstances, Carr's index is small and little change in apparent density is induced by tapping. A similar index is Hausner ratio (H_R); values less than 1.25 indicate good flow whereas greater than 1.25 indicates poor flow.

$$I_C = \frac{\rho_p - \rho_b}{\rho_p} \times 100$$

$$H_R = \frac{\rho_p}{\rho_p}$$

COMPATIBILITY TESTS

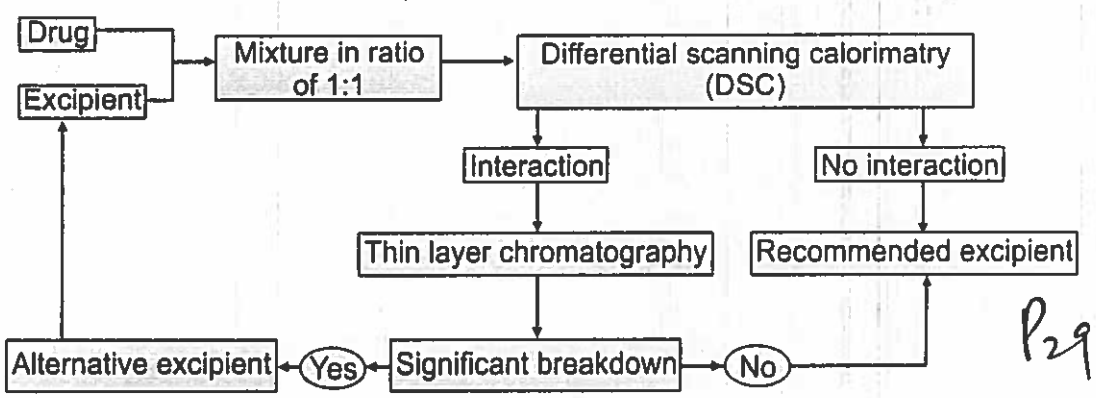
Excipients serve many roles and are the backbone of a formulation. The successful formulation of a stable and effective dosage form depend upon the careful selection of excipients. Excipients constitute the mass or greater volume in the usual enteral or parenteral formulations. Prior to attemting the first formulation with a new drug, most research groups carry out compatibility testing. The principle is to make up reasonably rationed mixtures of drug and excipient, to ascertain which excipients may be reasonably used with drug. Screens to detect drug-excipient incompatibilities recently have been developed using elevated temperature and added water to accelerate potential interactions in ternary and more complex powder blends. Such methods have been shown to be capable of rapidly detecting chemical incompatibilities and giving good correlations with results using powder blends of drug

and excipients at elevated temperatures and humidity. Interactions occur more frequently between excipient and active principle than between excipient and excipient. However, when evaluating potential pharmaceutical excipient interactions, it must however be considered that the kinetics of chemical reactions involving solutions are very high, whereas in the case of solid formulations they are low, if not negligible.

The analytical techniques commonly used for drug-excipient compatibility studies include high performance liquid chromatography (HPLC), thin layer chromatography (TLC), ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform-infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) and microcalorimetric methods. The DSC is used to measure specific heat capacity (100 to 1200°C) and heats of transition as well as to detect the temperature of phase changes and melting points in the range of 20 to 1500°C. An interaction on DSC will show changes in melting point, peak shape and area. Chemical interactions are indicated by the appearance of new peaks or there is alteration in exo- or endothermic peaks. When an interaction is suspected but thermal changes are small, the incompatibility should be confirmed by TLC. Heat conduction microcalorimetry has been used as a method to evaluate drug stability and drug-excipient stability. Microcalorimetry method gives no direct information about the chemical nature of reaction occurring between drug and excipient. **Flow chart 3.3** shows a general scheme to identify chemically compatible excipients using DSC.

Several examples of compatibility test for solid dosage form exist. Generally HPLC, DSC and TLC are used to evaluate the compatibilities. Aside from magnesium stearate, dicalcium phosphate and lactose are the excipients that are most often found incompatible with a drug. The primary excipients recommended for initial screening for solid dosage forms are lactose monohydrate, dicalcium phosphate dehydrate,

Flow chart 3.3: Drug-excipient compatibility study using DSC



dicalcium phosphate anhydrous, microcrystalline cellulose, maize starch, modified starch, ploy vinylpyrrolidone, sodium starch glycollate, sodium croscarmellose, magnesium stearate, stearic acid, colloidal silica.

Frequently a broad screen of stability is performed on the initial small sample used for initial preformulation; this is referred to as "forced decomposed studies". In this study a new drug candidate is exposed to acid degradation, base degradation, aqueous degradation, drug powder degradation and light degradation. The pH profile is most important part of liquid compatibilities. This will allow formulation of solutions of injections and for oral products as well, at a pH and using buffers that will give the best stability.

Aqueous compatibility studies are generally carried out by placing the drug in a solution of the additive. For example these can be a heavy metal (with or without chelating agent) or an antioxidant (in either oxygen or nitrogen atmosphere). Usually both flint and amber vials are used. This will indicate the drug's susceptibility to oxidation, hydrolysis, photolysis and heavy metals. Compatibility studies with ethanol, glycerin, sucrose, corn syrup, preservatives, antioxidants and buffers are usually carried out. With non-aqueous liquids it is advisable to test for compatibilities with ointment excipients and polymers.

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