Unit-I 07 Hours

Pre-formulation Studies: Introduction to pre-formulation, goals and objectives, study of physicochemical characteristics of drug substances.

Physical properties: Physical form (crystal & amorphous), particle size, shape, flow properties, solubility profile (pKa, pH, partition coefficient), polymorphism.

Chemical Properties: Hydrolysis, oxidation, reduction, racemization, polymerization. BCS classification of drugs & its significance.

Application of pre-formulation considerations in the development of solid, liquid oral and parenteral dosage forms and its impact on stability of dosage forms.

PREFORMULATION STUDIES

INTRODUCTION

Preformulation studies were evolved in 1950 & early 1960.Preformulation testing is the first step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass produced.

Preformulation investigations are designed to deliver all necessary data especially physicochemical, physico- mechanical and bio pharmaceutical properties of drug substances, excipients and packaging materials.

Preformulation during Drug Discovery

Apart from helping formulation development, preformulation studies also help in lead identification during drug discovery phase. A new chemical entity should possess optimal biopharmaceutical properties to become a drug molecule. Mere possession of potency and selectivity does not ensure ‘drug ability’.

Preformulation studies help in assessing the ‘drug ability’ of a molecule. Preformulation can thus be considered as critical decision-making tool during both – drug discovery and development phase. A comprehensive understanding of physicochemical properties and its effect on biological performance, allows selection of potential lead molecules and in identification of drug delivery challenges.

Objectives

 To develop the elegant dosage forms (stable, effective & safe}

 It is important to have an understanding of the physical description of a drug substance before dosage form development.

 It is 1st step in rational development of a dosage form of a drug subt before dosage form development.

Goals

 To establish the physico-chemical parameters of new drug substance.

 To establish the physical characteristics

 To establish the kinetic rate profile.

 To establish the compatibility with the common excipient.

 To choose the correct form of a drug substance.

3. PREFORMULATIONPARAMETERS:

A. PHYSICAL CHARACTERISTICS.

1) Organoleptic properties

2) Bulk characteristics

a) Solid state characteristics

b) Flow properties

c) densities

d) compressibility

e) crystalline

f) polymorphism

g) hygroscopicity

3) Solubility analysis

a) Ionization constant(Pka)

b) Partition co-efficient

c) Solubilization

d) Thermal effect

e) Common ion effect(Ksp)

f) Dissolution

4) Stability analysis

a) Solution-state stability

b) Solid-state stability

c) Drug-excipients compatibility

B. CHEMICAL CHARACTERISTICS

1) Hydrolysis

2) Oxidation

3) Photolysis

4) Recemization

5) Polymerization

6) Isomerization

1. ORGANOLEPTIC PROPERTIES

A typical preformulation program should begin with the description of the drug substance. The color, odour and taste of the new drug must be recorded using descriptive terminology. The color, odour and taste of the new drug must be recorded using descriptive terminology. It is important to establish a standard terminology to describe these properties in order to avoid confusion among scientists using different terms to describe the same property. A list of some descriptive terms to describe the most commonly encountered colors, tastes and odours of pharmaceutical powders is provided in table. The color of all the early batches of the new drug must be recorded using the descriptive terminology. A record of color of the early batches is very useful in establishing appropriate specifications for later production. When the color attributes are undesirable or variable, incorporation of a dye in the body or coating of the final product could be recommended.

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Table 1: Terminology to describe organoleptic properties of pharmaceutical powders.

Colour	Odour	Taste
Off-white	Pungent	Acidic
Cream yellow	Sulfurous	Bitter
Tan	Fruity	Bland
Shiny	Aromatic	Intense
	Odouerless	Sweet
		Tasteless

2. BULK CHARACTERISTICS:

a) Solid state characteristics:

Powders are masses of solid particles or granules surrounded by air (or other fluid)and it is the solid plus fluid combination that significantly affects the bulk properties of the powder. It is perhaps the most complicating characteristic because the amount of fluid can be highly variable. Powders are probably the least predictable of all materials in relation to flow ability because of the large number of factors that can change their rheological properties. Physical characteristics of the particles, such as size, shape, angularity, size variability and hardness will all affect flow properties. External factors such as humidity, conveying environment, vibration and perhaps most importantly aeration will compound the problem.

Particle size and size distribution:

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 behaviour.For example, the bioavailability of griseofulvin and phenacetin is directly related to the particle size distributions of these drug. It is now 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 sub divided state than as a coarse material.Size also plays a role in the homogeneity of the final tablet. When large differences in size exist between the active components and excipients, mutual sieving (de- mixing) effects can occur making thorough mixing difficult or if attained difficult to maintain during the subsequent processing steps.

Table .2: Common Techniques for Measuring

Technique	Particle size (micro meters)
Microscopic	1-100
Sieve	>5
Sedimentation	>1
Elutriation	1-50
Centrifugal	<50
Permeability	>1
light scattering	0.5-50

b) POWDER FLOW PROPERTIES:

The flow properties of powders are critical for an efficient tableting operation. A good flow of the powder or granulation to be compressed is necessary to assure efficient mixing and acceptable weight uniformity for the compressed tablets. If a drug is identified at the preformulation stage to be "poorly flowable," the problem can be solved by selecting appropriate excipients. In some cases, drug powders may have to be precompressed or granulated to improve their flow properties. Some of these methods are angle of repose, flow through an orifice, compressibility index, shear cell, etc. Changes in particle size and shape are generally very apparent; an increase in crystal size or a more uniform shape will lead to a smaller angle of repose and smaller carr's index.

Angle of Repose:

The maximum angle which is formed between the surface of pile of powder and horizontal surface is called the angle of repose. For most pharmaceutical powders, the angle-of repose values range from 25 to 45°, with lower values indicating better flow characteristics.

$\tan \theta = h / r$

h = height of heap of pile, r = radius of base of pile

c) Densities:

The ratio of mass to volume is known as density

Types of density:

(a) Bulk density: It is obtained by measuring the volume of known mass of powder that passed through the screen.

(b) Tapped density: It is obtained by mechanically tapping the measuring cylinder containing powder.

(c) True density: It actual density of the solid material.

(d) Granule density: may affect compressibility, tablet porosity, disintegration, dissolution

d) Compressibility:

"Compressibility" of a powder can be defined as the ability to decrease in volume under pressure and "compactability as the ability of the powdered material to be compressed into a tablet of specified tensile strength. It can be used to predict the flow properties based on density measurement.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{pored density} * 100}{\text{Tapped density}}$$

e) Crystallinity:

Generally most of drugs exist in solid state. Very few are in liquid state like valproic acid and even less in gaseous form like some general anesthetics. A crystal structure is a unique arrangement of atoms in a crystal. Physical properties affected by the solid-state properties can influence both the choice of the delivery system and the activity of the drug, as determined by the rate of delivery. Chemical stability, as affected by the physical properties, can be significant. A crystalline particle is characterized by definite external and internal structures. Crystal habit describes the external shape of a crystal, whereas polymorphic state refers to the definite arrangement of molecules inside the crystal lattice. Crystallization is invariably employed as the final step for the purification of a solid. The use of different solvents and processing conditions may alter the habit of recrystallized particles, besides modifying the polymorphic state of the solid.

f)Polymorphism:

Many drug substances can exist in more than one crystalline form with different space lattice arrangements. This property is known as polymorphism. The different crystal forms are called polymorphs. When polymorphism occurs, the molecules arrange themselves in two or more different ways in the crystal; either they may be packed differently in the crystal lattice or there may be differences in the orientation or conformation of the molecules at the lattice sites.

Methods to identify polymorphism

- Optical crystallography
- Hot stage microscopy
- X- Ray Diffraction method
- NMR technique
- FTIR technique.
- Microcalorimetry
- Thermal methods

 Melting point determination

g) Hygroscopisity:

Many compounds and salts are sensitive to the presence of water vapour or moisture. When compounds interact with moisture, they retain the water by bulk or surface adsorption, capillary condensation, chemical reaction and, in extreme cases, a solution (deliquescence). Deliquescence is where a solid dissolves and saturates a thin film of water on its surface. It has been shown that when moisture is absorbed to the extent that deliquescence takes place at a certain critical relative humidity, the liquid film surrounding the solid is saturated. This process is dictated by vapour diffusion and heat transport rates.

Moisture is also an important factor that can affect the stability of candidate drugs and their formulations. Sorption of water molecules onto a candidate drug (or excipient) can often induce hydrolysis. In this situation, by sorbing onto the drug-excipient mixture, the water molecules may ionize either or both of them and induce a reaction. For example, we have found that a primary amine, when mixed with lactose was apparently stable even when stored at 90°C for 12 weeks. However, when the experiment was carried out in the presence of moisture, extensive degradation by way of the well-known Mailliard reaction took place. Other properties such as crystal structure, powder flow, compaction, lubricity, dissolution rate and polymer film permeability may also be affected by moisture adsorption.

Table 2: Different classes of hygroscopic substances

Hygroscopicity Classification		
Class 1	Non-Hygroscopic	Essentially no moisture increases occur at relative humidities below 90%.
Class 2	Slightly hygroscopic	Essentially no moisture in occur at relative humidity below 80%
Class 3	Moderately hygroscopic	Moisture Content does not increase more than 5% after storage for 1 week at relative humidity below 60%
Class 4	Very hygroscopic	Moisture content increase may occur at relative humidity as low as 40 to 50%

3. Solubility Analysis:

An important Physical-chemical property of a drug substance is solubility, especially aqueous solubility. A drug must possess some aqueous solubility for therapeutic efficacy in the physiological P H range of 1 to 8. For a drug to enter into systemic circulation, to exert therapeutic effect, it must be first in solution form. If solubility of drug substance is less than desirable, than consideration must be given to increase its solubility. Poor solubility **(< 10mg/ml)** may exist incomplete or erratic absorption over PH rang 1-7 at 37°C. However, knowledge of two fundamental properties is mandatory for a new compound

i) Intrinsic solubility(Co)

ii) Dissociation constant (Pka).

i) Intrinsic Solubility (Co)

The intrinsic solubility should be measured at two temp: **4 to 5°C** to ensure good physical stability and to extend short term storage and chemical stabilityuntil more definite data is available. **37° C** to support biopharmaceutical evaluation. The solubility of weakly acidic and weakly basic drug as function of PH can be predicted with help of equation,

S = So	{1 + (K1/ [H+])}	For weak acid.
S = So	{1+ ([H+]/ K2)}	For weak base.

Where, S = solubility at given PH.

So = intrinsic solubility of neutral form.

K1 = dissociation constant for the weak acid.

K2 = dissociation constant for weak base.

a) Ionization Constant(PKA):

Many drugs are either weakly acidic or basic compounds and, in solution, depending on the Ph value, exist as ionized or un-ionized species. The un- ionized species are more lipid-soluble and hence more readily absorbed. The gastrointestinal absorption of weakly acidic or basic drugs is thus related to the fraction of the drug in solution that is un- ionized. The conditions that suppress ionization favor absorption. The factors that are important in the absorption of weakly acidic and basic compounds are the pH at the site of absorption, the ionization constant, and the lipid solubility of the un- ionized species. These factors together constitute the widely accepted pH partition theory.

The relative concentrations of un-ionized and ionized forms of a weakly acidic or basic drug in a solution at a given pH can be readily calculated using the Henderson-Hasselbalch equations:

$$pH = pKa + \log \frac{[\text{Un- ionized form}]}{[\text{ionized form}]} \quad \text{for bases}$$

$$pH = pKa + \log \frac{[\text{Ionized form}]}{[\text{un ionized form}]} \quad \text{for acids}$$

Weakly acidic compounds (pKa< 4.3) were absorbed relatively rapidly;Those with pKa values ranging between 2.0 and 4.3 were absorbed more slowly; and strong acids (pKa> 2.4) were hardly absorbed.For bases, those with pKa values smaller than 8.5 were absorbed relatively rapidly; those with a pK a between 9 and 12 were absorbed more slowly; and completely ionized quaternary ammonium compounds were not absorbed. In pharmacokinetic area, the extent of ionization is imp. affect of its extent and absorption,

distribution, elimination. The extent of Pka , in many cases, highly dependent on PH of the medium containing the drug.

Determination of Pka:

- Potentiometric Titration
- Spectrophotometric Determination
- Dissolution rate method
- Liquid-Liquid Partition method

b). Partition Coefficient:

The lipophilicity of an organic compound is usually described in terms of a partition coefficient; log *P*, which can be defined as the ratio of the concentration of the unionized compound, at equilibrium, between organic and aqueous phases:

$$Po/w = (C\ oil/water)equilibrium$$

Or

$$\log P = \frac{(un\ ionized\ compound)org}{(un\ ionized\ compound)aq}$$

This ratio is known as the partition coefficient or distribution coefficient and is essentially independent of concentration of dilute solutions of a given solute species. log*P* = 0 means that the compound is equally soluble in water and in the partitioning solvent. If the compound has a log *P* = 5, then the compound is 100,000 times more soluble in the partitioning solvent. A log *P* = –2 means that the compound is 100 times more soluble in water, i.e., it is quite hydrophilic. 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. Lipids occurring in living membranes are complex and difficult to obtain in pure form. An indication of the relative lipid solubility, however, can be obtained by determining how a drug substance distributes itself between water and an immiscible organic solvent. When a solute is added to two immiscible liquids that are in contact with each other, it will distribute itself between the two phases in a fixed ratio. This ratio is known as the partition coefficient, or distribution coefficient, and is essentially independent of concentration of dilute solutions of a given solute species. Various organic solvents such as chloroform, ether, amyl acetate, isopropylmyristate, carbon tetrachloride, and n - Octanol can be used in the determination of the partition coefficient, with the latter gaining increasing acceptance.

Methods of finding Partition coefficient:

1) Shake-flask method

2) Chromatographic method.

3) Counter current and filter probe method.

4) Tomlinson's filter probe method.

5) Microelectrometrictitratation method

6) Automated instrument is now available.

Applications of Partition coefficient:

- Measure of Lipophilic character of molecules.
- Recovery of antibiotics from fermentation broth.
- Extraction of drug from biological fluid for therapeutic monitoring.
- Absorption of drug from dosage forms. (Ointments, Suppositories, Transdermal patches).
- Study of distribution of flavouring oil between oil & water in emulsion.

c). Solubilization:

For drug candidates, with either poor water solubility or insufficient solubility for projected solution dosage form, preformulation study should include limited experiments to identify possible mechanism for solubilization.

Methods for Increasing Solubility:

- Change in pH
- Co-Solvency
- Dielectric Constant
- Solubilization by Surfactant
- Complexation
- Hydrotropy
- Chemical Modification of drug

d) Thermal Effect:

We determine the effect of temp. on the solubility of drug candidate. This can be determined by measuring heat of solution i.e. **HS**

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

Where, S = molar solubility at temp. T (° K)

R = gas constant.

Heat of solution represents the heat released or absorbed when mole of solute is dissolved in large quantity of solvent. It is determined from solubility value for saturated solution equilibrated at controlled temperature over the range of interested. Typically the temperature range should include 5 °C, 25°C, 37°C and 50°C.If heat of solution is positive (endothermic process) thus, increasing solution temp. Increased the drug solubility. For **non-electrolyte and un-ionized** form of weak acid and weak bases dissolved in water, heat of solution range from 4 to 8 Kcal/mol.

e) Common Ion Effect:

A common interaction with solvent, which often overlooked, is the common ion effect. The addition of common ion often reduces the solubility of slightly soluble electrolyte. This salting out results from the removal of the water molecule as the solvent due to competing hydration of other ions. So, weakly basic drug which are given as HCL salts have decreased solubility in acidic (HCL) solution.

Eg.Chlortetracycline, methacyclin, papaverine, cyproheptadine, bromhexine,Triamterene

To identify a common ion interaction, the intrinsic dissolution rate of hydrochloride salt should be compared between, Water and water containing 1.2%W/V NACL 0.05M HCL and 0.9%W/V NACL in 0.05M After this, if solubility is not decreased than we can give drug in chloride salt, otherwise it should be eliminated.

f) Dissolution:

In many instances, dissolution rate in the fluids at the absorption site, is the rate limiting steps in the absorption process. This is true for the drug administered orally in the solid dosage forms such as tablet, capsule, and suspension as well as drug administered I.M. in form of pellets or suspension. Dissolution is of 2 types.

a) Intrinsic dissolution

b) Particulate dissolution

a)Intrinsic Dissolution

The dissolution rate of a solid in its own solution is adequately describedby the Noyes-Nernst equation:

$$dC / dt = \frac{AD\,(Cs - C)}{hv}$$

Where,

dC / dt = dissolution rate

A = surface area of the dissolving solid

D = diffusion coefficientge 1405

C = solute concentration in the bulk medium

h = diffusion layer thickness

V = volume of the dissolution medium

Cs = solute concentration in the diffusion layer

During the early phase of dissolution, Cs » C and is essentially equal to saturation solubility S. Surface area A and volume V can be held constant. Under these conditions and at constant temperature and agitation, Equation reduces to

dC / dt = KS

Where K =AD/hV = constant.

Dissolution rate as expressed in Equation is termed the intrinsic *dissolution rate* and is characteristic of each solid compound in a given solvent under fixed hydrodynamic conditions. The intrinsic dissolution rate in a fixed volume of solvent is generally expressed as mg dissolved x(min-1 cm- Z).

Knowledge of this value helps the preformulation scientist in predicting if absorption would be dissolution rate-limited.

Particulate dissolution:

It will determine dissolution of drug at different surface area. It is used to study the influence on dissolution of particle size, surface area and mixing with excipient. So, if particle size has no influence on dissolution than other method like addition of surfactant will be considered.

4. STABILITY STUDIES:

Incompatibility- general aspects

When we mix two or more API and / or excipient with each other & if they are antagonistic & affect adversely the safety, therapeutic efficacy, appearance or elegance then they are said to be incompatible.

(A). Solid State Stability Studies:

Solid state reactions are much **slower** and more **difficult to interpret** than solution state reactions, due to a reduced no. of molecular contacts between drug and excipient molecules and to the occurrence of multiple phase reactions.

Sample A	Sample B	Sample C
• Prepare a small mixture of drug and excipient. • Place above mix in vial. • Palce a rubber closure on vial and dip the stopper in molten carnuba wax to render it hermetically sealed.	Sample preparation method is same as sample A but 5% moisture is added in mixture.	Drug itself without any excipient is taken as a sample for solid state stability study.

 All the samples of drug-Excipient blends are kept for 1-3 weeks at specified storage conditions.

 Then sample is physically observed for (1) caking (2) liquefaction (3) Discoloration (4)

odor (5) gel formation.

 It is then assayed by TLC or HPLC or DSC.

 Whenever feasible, the degradation products are identified by MASS SPECTROSCOPY,

NMR or other relevant analytical techniques.

(B) Solution State Stability Studies:

It is easier to detect liquid state reactions as compared to solid state reactions. For detection of unknown liquid incompatibilities, the program set up is same as solid dosage forms. Now according to ―Stability guidelines‖ by FDA states that:

Following conditions be evaluated in studies on solutions or suspensions of bulk drug substances:

1) Acidic or alkaline pH.

2) Presence of added substances- chelating agents, stabilizers etc.

3) High Oxygen and Nitrogen atmospheres.

4) Effect of stress testing conditions……

Methodology:-

 Place the drug in the solution of additives.

 Both flint and amber vials are used.

 Autoclave conditions are employed in many cases. This will provide information about

Susceptibility to oxidation.

Susceptibility to light exposure.

Susceptibility to heavy metals.

 In case of oral liquids, compatibility with ethanol, glycerine, sucrose, preservatives and

buffers are usually carried out.

(C) Drug-Excipient Compatibility Studies:

In the tablet dosage form the drug is in intimate contact with one or more excipients; the latter could affect the stability of the drug. Knowledge of drug-excipient interactions is therefore very useful to the formulator in selecting appropriate excipients. This information may already be in existence for known drugs. For new drugs or new excipents, the preformulation scientist must generate the needed information. A typical tablet contains binders, disintegrants, lubricants, and fillers. Compatibility screening for a new drug must consider two or more excipients from each class. The ratio of drug to excipient used in these tests is very much subject to the discretion of the preformulation scientist.

Importance of Drug Excipient Compatibility Study:-

 Stability of the dosage form can be maximized. Any physical or chemical interaction between drug and excipient can affect bioavailability and stability of drug.

 It helps to avoid the surprise problems. By performing DECS we can know the possible reaction before formulating final dosage form.

 It bridges the drug discovery and drug development. Drug discovery can emerge only new chemical entity. It becomes drug prod uct after formulation and processing with excipients.

 By using DECS data we can select the suitable type of the excipient with the chemical entities emerging in drug discovery programs. DECS data is essential for IND (investigational new drug) submission. Now, USFDA has made it compulsory to submit DECS data for any new coming formulation before its approval.

Analytical techniques used to detect Drug-

Excipient Compatibility:

1) Thermal methods of analysis

I. DSC- Differential Scanning Calorimetry

II. DTA- Differential Thermal Analysis

2) Accelerated Stability Study

3) FT-IR Spectroscopy

4) DRS-Diffuse Reflectance Spectroscopy

5) Chromatography

I. SIC-Self Interactive Chromatography

II. TLC-Thin Layer Chromatography

III. HPLC-High Pressure Liquid Chromatography

6) Miscellaneous

I. Radiolabelled Techniques

II.Vapour Pressure Osmometry

III. Fluorescence Spectroscopy

B. CHEMICAL CHARACTERISTICS

a) Hydrolysis-It involves nucleophilicattack of labile groups eg: lactam ester amide imide. When the attack is by the solvent other than water, then it is known as solvolysis. It generally follows 2^{nd} order kinetics as there are two reacting species, water and API. In aqueous solution, water is in excess so the reaction is 1st order. Conditions that catalyze the breakdown are Presence of hydroxyl ion, hydride ion, divalent ion and

heat, light, ionic hydrolysis, solution polarity and ionic strength, high drug concentration. Hydrolysis can be prevented by Adjusting the PH.As most of the potent drugs are weakly acidic or weakly basic in nature. Formulate the drug solution close to it's PH of optimum stability or by Addition of water miscible solvent in formulation or by Using Optimum buffer concentration to suppress

ionization or by Addition of surfactant such as nonionic, cationic and anionic surfactant stabilizes the drug against base catalysis or the solubility of pharmaceuticals undergoing ester hydrolysis can be reduced by forming less soluble salts or ester of drug. eg: phosphate ester of Clindamycin or Store with desiccants, using complexing agents.

b) **Oxidation:**

It is a very commonpathway for drug degradation in liquid and solid formulations. Oxidation occurs in two ways

1. Auto- oxidation

2. Free radical chain process.

Reaction of any material with molecular oxygen producing free radicals by hemolytic bond fission of a covalent bond. These radicals are highly unsaturated and readily accept electron from other substance causing oxidation is called Autooxidation. Free radical chain process involves Initiation, Propagation, Hydro peroxide decomposition and Termination. Factors affecting oxidation process are Oxygen concentration, light, heavy metals particularly those having two or more valence state (copper, iron, nickel, cobalt), hydrogen and hydroxyl ion, temperature. Oxidation can be Prevented by Reducing oxygen content oxidative degradation of drug takes place in an aqueous solution, so the oxygen content can be decreased by boiling water or by storing the formulation in in a dark and cool condition or by addition of an antioxidant/reducing agent /chain inhibitors of radical induced decomposition. Antioxidants are of two types based on Solubility. Oil soluble and Water soluble. Oil Soluble Antioxidants are Free radical acceptors and inhibit free radical chain process eg: hydroquinone, propylgallate, lecithin whereas Water soluble Antioxidants Oxidizes itself and prevents oxidation of drug Eg: sodium metabisulphate, sodium bisulfate, thioglycolic acid, thioglycerol.

c) Reduction: is a relatively more common pathway of drug metabolic process. Hepatic microsomescatalyze diverse reductive chemical reaction* and require NADPH for this purpose. Azo and nitro reduction is catalyzed by cytochrome P- 450.Chloral hydrate is reduced to it's active metabolite trichloroethanol by alcohol dehydrogenase. Reduction of prednisolone and cortisone results in the formation of their active metabolites hydrocortisone. Azo dyes used as coloring agents in pharmaceutical products or foods are reduced to form amines in the liver and by the intestinal flora.

d) Photolysis: Mechanism of photodecomposition: Electronic configuration of drug overlaps with the spectrum of sunlight or any artificial light where energy is absorbed by the electron resulting in excitation. As they are unstable, they release the acquired energy and return to the ground state by decomposing the drug. The phenomenon where molecules or excipients which absorb energy but do not participate themselves directly in the reaction but transfer the energy to others which cause cellular damage by inducing radical formation is known as photosensitization. Photosentizer Convert oxygen from its ground state to singlet excited state and Generate superoxide molecule which is an anion radical and acts as a powerful oxidizing agent.

Photo Decomposition Pathway

1. N-dealkylation: eg:Dipenhydramine, Chloroquine, Methotrexate

2. Dehalogenation: eg:-Chlorpropamide, Furesemide

3. Dehydrogenation of ca++ channel blockers

4. Decarboxylation in anti-inflammatory drugs:

Naproxen, Flurbiprofen, Benzoxaprofen

5. Oxidation:- Chlorpromazine and other

phenothiazines give n- oxides in the presence of sunlight.

6. Isomerization and cyclization:-

Noradrenaline, Doxapine

7. Rearrangement: Metronidazole and oxidiazine yellow color Photodecomposition canbe Prevented by-suitable packing, antioxidant, protection of drug from light, avoiding sunbath, photostabilizer, coating.

e) Polymerization:

 It is a continuous reaction between molecules.

 More than one monomer reacts to form a polymer.

 Eg. Darkening of glucose solution is attributed to polymerization of breakdown

 product [5- (hydroxyl methyl) furfural].

 Eg. Polymerization of HCHO to para-HCHO which crystallizes out from the solution.

f) Racemization:

 The interconversion from one isomer to another can lead to different P'cokinetic

 properties (ADME) as well as different P'cological& toxicological effect.

 Eg. L-epinephrine is 15 to 20 times more active than D-form, while activity of racemic mixture is just one half of the L-form.

 It follows first order kinetics.

 It depends on temperature, solvent, catalyst & presence or absence of light.

CONCLUSION

Preformulation studies have a significant part to play in anticipating formulation problems and identifying logical paths in both liquid and solid dosage form Technology. By comparing the physicochemical properties of each drug candidate within a therapeutic group, the Preformulation scientist can assist the synthetic chemist to identify the optimum molecule, provide the biologist with suitable vehicles to elicit

pharmacological response. Stability studies in solution will indicate the feasibility of parental or other liquid dosage form and can identify methods of stabilization. In parallel solid-state stability by DSC, TLC and HPLC in the presence of tablet and capsule excipient will indicate the most acceptable vehicles for solid dosage form. This review article gives details of above studies with respect to any sustained release dosage forms can be developed without preformulation studies.

Guideline

Stability Testing of New Drug Substances and Products

1.1 Objective of the Guideline

• defines stability data package or drug substance and drug product for registration

application,

• within three regions of ICH, EC, Japan USA

• does not cover testing for registration in or export to other areas of the world

• Alternative approaches if scientifically reasons

1.2 Scope of the guideline

• Registration application for New Molecular Entity (NME) and associated Drug product.

• Not covered:

- clinical trial applications

1. Introduction

1.3 General Principles

• Purpose of stability testing is to provide evidence how quality varies with time under influence as

- temperature

- humidity

- light,

• establish re-test period for drug substances

• establish shelf life for drug products

• recommend storage conditions

• Test conditions based on analysis of effects of climatic conditions in the three

regions of the EC, Japan, USA

• mean kinetic temperature can be derived from climatic data

• world can thereby divided into four climatic zone I-IV

• This guideline addresses climatic zones I and II

• Stability information generated in one of the three regions is mutually acceptable to

the other two provided:

- information is consistent with this guideline,

- labelling is in accord with national/ regional requirements.

The four Climatic Zones

Climatic Zone Definition Storage conditions

Climatic Zone	Definition	Storage conditions
I	Temperate climate	21°C/ 45% r.h.
II	Subtropical and Mediterranean climate	25°C/60%r.h.
III	Hot, dry climate	30°C/35%r.h
IV	Hot, humid climate	30°C/70%r.h.

Criteria used to classify a site according to climatic zone

Criteria	Guide values for individual climatic zone			
	I	II	III	IV
Mean annual temperature measured in the open air	up to 15°C	> 15 – 22°C	> 22°C	> 22°C
Calculated mean annual Temperature (< 19°C)	up to 20.5°C	> 20.5 – 24°C	> 24	> 24
Mean annual Water vapour partial pressure	up to 11 mbar	> 11 – 18 mbar	up to 15 mbar	> 15 mbar

1. Guidelines Drug Substance Drug Product

1.1 Drug Substance		2.2 Drug Product	
2.1.1	General	2.2.1	General
2.1.2	Stress Testing	2.2.2	Photostability Testing
2.1.3	Selection of Batches	2.2.3	Selection of Batches
2.1.4	Container Closure System	2.2.4	Container Closure System
2.1.5	Specification	2.2.5	Specification
2.1.6	Testing frequency	2.2.6	Testing Frequency
2.1.7	Storage Conditions	2.2.7	Storage Conditions
2.1.8	Stability Commitment	2.2.8	Stability Commitment
2.1.9	Evaluation	2.2.9	Evaluation
2.1.10	Statements/Labelling	2.2.10	Statements/Labelling

2.1.2 Stress Testing

Stress Testing

 help identify likely degradation products but only those which are formed under accelerated and long term storage conditions

 establish degradation pathway

 establish intrinsic stability of molecule

 validate indicating power of analytical procedure

 depends on individual drug substance and type of drug product

 carried out on a single batch

 should include effect of

• temperature e.g. 50°C, 60°C, 70°C etc.

• humidity e.g. 75% or greater

• oxidation

• hydrolysis across a wide range of pH

• photostability as described in ICH Q1B

Results from these studies form an integral part of information provided to regulatory

authorities

2.1.3 Selection of Batches

Data from formal stability studies should be provided

• a least three primary batches

• manufactured to a minimum of pilot scale

• same synthetic route

• method of manufacture and procedure should simulate final process

• quality representative of quality to be made on production scale

Other supporting data can be provided

2.1.4 Container Closure System

 Container closure system same or simulates packaging proposed for storage and distribution

2.1.5 Specification

 Specification:

• list of tests,

• reference to analytical procedure,

• proposed acceptance criteria

 Test Attributes

• attributes that are susceptible to change during storage,

• influence quality, safety and/or efficacy

• Should cover physical, chemical, biological, microbiological attributes

 Analytical procedures

• validated stability indicating

• replication depending on results from validation studies

The following requirements for replication can be fixed:

RSD ≤ 1% single analysis

RSD > 1% 3fold analysis

The initial assay at time point 0 should be always analysed 3fold

2.1.6 Testing Frequency

 General: every 3 months first year, every 6 months

second year, than annually through proposed re-test period: e.g. 0, 3, 6, 9, 12, 18, 24, 36, 48, 60 months

 Accelerated storage condition: 0, 3, 6 months. Where expectation to approach significant change, increasing testing necessary: adding samples at final time point or forth time point in study design: 0, 3, 2 x 6 or 0, 1, 3, 6 months

2.1.7 Storage Conditions

Long term testing should cover a minimum of 12 months duration on at least three primary batches at time of submission and should be continued sufficient to cover the proposed retest period.

 General case

Study	Storage condition	Study
Long term*	25°C ± 2°C/60% ± 5% or 30°C ± 2°C/65% ± 5%	12 months
Intermediate**	30°C ± 2°C/65% ± 5%	6 months
Accelerated	40°C ± 2°C/75% ± 5%	6 months

2.1.7 Storage Conditions

 Drug substance intended for storage in a refrigerator

Study	Storage condition	Minimum time period at submission
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% ± 5%	6 months

If significant change between 3 and 6 months at accelerated testing proposed re-test data based on real time data.

If significant change within 3 months discussion should address excursions outside label storage. Single batch shorter than 3 months with more frequent testing.

 Drug substance intended for storage in a freezer

Study	Storage condition	Minimum time period at submission
Long term	- 20 °C ± 5°C	12 months

Re-test period based on real time data at long term storage condition.

In absence of accelerated storage condition testing on a single batch at an elevated temperature e.g. 5°C ± 3°C to address short term excursions

2.1.8 Stability Commitment

Re-test period not covered

When long term stability data do not cover proposed re-test period granted at time of approval commitment should be made to continue post approval to establish re-test period

Commitment not necessary

Submission includes data on three production batches covering proposed re-test period

Commitment required

• Submission includes data from 3 production batches, commitment to continue through proposed re-test period.

• Fewer than three production batches commitment continue with these studies through proposed re-test period and place additional production batches to a total of three on long term stability through proposed re-test period

• No Production batches commitment to place first three production batches on long term stability studies through proposed re-test period.

2.1.9 Evaluation

Re-test period

Purpose of stability studies is to establish a re-test period applicable to all further batches of the drug substance manufactured under similar circumstances.

It is based on results of physical, chemical, biological and microbiological tests from

three batches.

No formal statistical analyses

The data may show so little degradation and so little variability that it is apparent from looking at the data that the requested re-test period will be granted. Under these circumstances normally unnecessary to go through the formal statistical analyses; providing a justification for the omission should be sufficient.

Statistical evaluation

Data on a quantitative attribute that changes with time: Determination of the time at which the 95% one sided confidence limit for the mean curve intersects the acceptance criterion etc,

2.1.9 Evaluation

Extrapolation

Limited extrapolation of the real time data beyond the observed range to extend the re-test period can be undertaken at approval time, if justified.

Justification should be based on

• Knowledge on mechanism of degradation

• results of accelerated testing,

• goodness of fit of mathematical model

• existence of supporting data and batch size

2.1.11 Statements/Labelling

 Storage Statement

Storage statement established for labelling should be in accordance with national/regional requirements.

Statement based on stability evaluation

 Re-test date

Re-test date derived from stability information.

The re-test date should be displaced on the container label

2.2 Drug Product

 2.2.1 General

Design of the formal stability studies should be based on

• knowledge and properties of drug substance,

• experience gained from clinical formulation studies.

 2.2.2 Photostability Testing

One primary batch, standard conditions according to ICH Q1B

2.2.3 Selection of Batches

 Required are at least three primary batches.

• Same formulation and in same container closure system as proposed for marketing.

• Manufacturing process should simulate that applied to production batches.

• Same quality and meeting specifications as that intended for marketing.

 Two of the three batches at least pilot scale third can be smaller

• for solid oral dosage forms pilot scale is generally on tenth that of full production scale or

• 100000 tablets or capsules, whichever is larger.

 Drug products should be manufactured by using different batches of the drug substance.

 Stability studies should be performed on each individual strength and container size of the drug product unless bracketing or matrixing is applied.

 Other supporting data can be provided.

2.2.4 Container Closure System

 Container closure system proposed for marketing (if appropriate any secondary packaging and container label)

 Supporting information:

• results of open storage of stress testing

• studies in other packaging materials

2.2.5 Specification

 Specification is a list of

• tests, test attributes

• reference to analytical procedures

• proposed acceptance criteria release and shelf life

 Test attributes

• attributes susceptible to change during storage

• may influence quality, safety and/or efficacy

• should cover physical, chemical, biological, microbiological attributes.

 Analytical procedures

• fully validated and stability indicating

• Replication will depend on results of validation studies:

Following requirements were fixed:

RSD ≤ 1.5% single analysis

RSD > 1.5% 3fold analysis

Initial analysis generally 3fold

 Acceptance Criteria

• based on all available stability information

• differences between release and shelf life acceptance criteria justified

• difference for antimicrobial preservative content supported by validated correlation of chemical content and preservative effectiveness

• Single primary batch should be tested for antimicrobial preservative effectiveness

at proposed shelf life

2.2.6 Testing Frequency

 Long term studies

• first year every three months. 0, 3, 6, 9, 12

• second year every six months: 12, 18, 24

• third year and longer annually: 24, 36, 48, 60

 Accelerated studies

• general minimum three time points: 0,3,6 months

• expectation of significant change increases testing adding samples at final time point or forth time point: 0, 3, 2x6 or 0, 1, 3, 6 months

 Intermediate storage condition studies

Minimum four time points, including initial and final e.g.: 0,6,9,12 months, at time of submission 0,6 months

 Reduced design

Matrixing or bracketing for reduction of testing frequency if justified

2.2.7 Storage conditions

Storage conditions and lengths of studies sufficient to cover storage shipment and subsequent use.

Stability testing of products after constitution or dilution should be conducted to provide information for labelling of *storage condition* and *in-use period.*

Primary batches initial and final time point, at least at 12 months.

 Long term testing should cover al least 12 months

 Should be continued to cover proposed shelf life

 Accelerated testing to evaluate short term excursions outside label storage condition

 2.2.7.1 General case

Study	Storage condition	Minimum time period at submission
Long term*	25°C ± 2°C/60% ± 5% or 30°C ± 2°C/65% ± 5%	12 months
Intermediate**	30°C ± 2°C/65% ± 5%	6 months
Accelerated	40°C ± 2°C/75% ± 5%	6 months

*It is up to the applicant, to decide whether long term stability is performed at 25°C ± 2°C/60% ± 5% or 30°C ± 2°C/65% ± 5%.

** If 30°C ± 2°C/65% ± 5% is the long-term condition, there is no intermediate condition.

Testing at intermediate storage condition if significant change at accelerated testing

2.2.7 Storage Conditions

Significant change

• A 5% change in assay from initial value,

• Any degradation product's exceeding its acceptance criterion,

• Failure to meet acceptance criteria for appearance, physical attributes, and functionality test.

- Some changes as softening of suppositories, melting of creams are acceptable.

• Failure to meet acceptance criteria for dissolution for 12 units.

2.2.7.2 Drug products in impermeable container

Studies can be conducted under any controlled or ambient humidity condition

2.2.7.3 Drug products in semi-permeable container

• Evaluation for potential water loss for aqueous-based products in semi-permeable containers

• Evaluation under condition of low relative humidity

Study	Storage condition	Minimum time period at submission
Long term	25°C ± 2°C/40% ± 5% or 30°C ± 2°C/35%± 5%	12 months
Intermediate	30°C ± 2°C/35%± 5%	6 months
Accelerated	40°C ± 2°C/not more than 25%	6 months
Accelerated	40°C ± 2°C/75% ± 5%	6 months

*It is up to the applicant, to decide whether long term stability is performed at 25°C ± 2°C/60% ± 5% or 30°C ± 2°C/65% ± 5%.

** If 30°C ± 2°C/65% ± 5% is the long-term condition, there is no intermediate condition

2.2.7 Storage Conditions

 A significant change in water loss alone during 6 months accelerated testing does not necessitate storage at inter-mediate condition, but no significant water loss at 25°C/40%.

 A significant change is a 5% water loss after 3 months accelerated testing

 For small containers (1 ml or less) more than 5% loss after 3 months may be appropriate

 Alternative Testing

• Storage under general storage conditions and calculate water loss by determining permeation coefficient or using calculated ratio of water loss.

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Table with calculated ratio of water loss

Alternative relative Humidity	Reference relative humidity	Ratio of water loss rates at a given temperature
60% r.h.	25% r.h	2.4
60% r.h.	40% r.h.	1.5
65% r.h	35% r.h.	1.9
75% r.h.	25% r.h.	3.0

2.2.7 Storage Conditions

 2.2.7.4 Drug products intended for storage in a refrigerator

Study	Storage condition	Minimum time period at submission
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% ± 5%	6 months

• If significant change between 3 and 6 months at 25°C/60% shelf life based on real time data

• If significant change within 3 months, discussion about short term excursions outside label storage.

• As possible support one batch shorter than 3 months and more frequent testing

 2.2.7.5 Drug products intended storage in freezer

Study	Storage condition	Minimum time period at submission
Long term	- 20 °C ± 5°C	12 months

• Shelf life based on real time data

• testing on a single batch at 5°C for appropriate time period

2.2.8 Stability Commitment

Proposed shelf life not covered

When long term stability data do not cover proposed shelf life granted at time of approval commitment should be made to continue post approval to establish the shelf life

Commitment not necessary

Submission includes data on three production batches covering proposed shelf life

Commitment required

• Submission includes data from 3 production batches, commitment to continue through proposed shelf life

• Fewer than three production batches commitment continue with these studies through proposed shelf life and place additional production batches to a total of three on long term and accelerated stability testing through proposed shelf life.

• No Production batches commitment to place first three production batches on long term and accelerated stability testing through proposed shelf life.

2.2.9 Evaluation

Stability information

• Systematic approach in presentation and evaluation of stability information.

• Should include results from physical, chemical, biological and microbiological tests.

Purpose of stability studies

Establish shelf life and storage instructions applicable for all further batches manufactured and packed under similar circumstances.

No formal statistical analyses

Where data show so little degradation and so little variability that it is apparent from looking at the data, the requested shelf life will be grated without formal statistical analyses. But justification for omission

Formal statistical analyses

For quantitative attributes which change with time determination of time at which the 95% one sided confidence limit for the mean curve intersects the acceptance criteria.

Data of batches can be combined if

• batch to batch variability is small

- slope of regression line

- zero time intercepts

 Extrapolation

Limited extrapolation can be undertaken at approval time with justification

2.2.10 Statements/Labelling

 Storage Statement

• Storage statement for labelling in accordance with national/regional requirements

• Based on the stability evaluation

• Direct link between label storage statement and demonstrated stability.

• Expiration date should be displayed on container label.