1 INTRODUCTION:

1.1 Introduction of Biopharmaceutics Classification System (BCS)

The Biopharmaceutics Classification System is a system to differentiate the drugs on the basis of their solubility and permeability. It is a guide for predicting the intestinal drug absorption provided by the U.S. Food and Drug Administration. The introduction of the Biopharmaceutics Classification System (BCS) in 1995 was the result of continuous efforts on mathematical analysis for the elucidation of the kinetics and dynamics of the drug process in the gastrointestinal tract (GI). The fundamental basis for the BCS was established by Amidon.

This system restricts the drug absorption prediction using the parameters drug solubility and intestinal permeability. The solubility classification is based on a United States Pharmacopoeia (USP). The intestinal permeability classification is based on a comparison to the intravenous injection. All those factors are highly important because 85% of the most sold drugs are orally administered [1].

According to the Biopharmaceutics Classification System, drug substances are classified as follows: [2,3]

- **Class I - high permeability, high solubility**
  - Example: Metoprolol
  - Those compounds are well absorbed and their absorption rate is usually higher than excretion.

- **Class II - high permeability, low solubility**
  - Example: Glibenclamide, Bicalutamide, Ezetimibe
  - The bioavailability of those products is limited by their solvation rate. A correlation between the in vivo bioavailability and the in vitro solvation can be found.

- **Class III - low permeability, high solubility**
  - Example: Cimetidine
  - The absorption is limited by the permeation rate but the drug is solvated very fast. If the formulation does not change the permeability or gastro-intestinal duration time, then class I criteria can be applied.
• Class IV - low permeability, low solubility
  o Example: Hydrochlorothiazide
  o Those compounds have a poor bioavailability. Usually they are not well absorbed over the intestinal mucosa and a high variability is expected.

The drugs are classified in BCS on the basis of following parameters:
1. Solubility
2. Permeability
3. Dissolution

The class boundaries for these parameters are [4,5]:

1. Solubility class boundaries- It is based on the highest dose strength of an immediate release product. A drug is considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1 to 7.5. The volume estimate of 250 mL is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with a glass of water.

2. Permeability class boundaries- It is based indirectly on the extent of absorption of a drug substance in humans and directly on the measurement of rates of mass transfer across human intestinal membrane. Alternatively non-human systems capable of predicting drug absorption in humans can be used (such as in-vitro culture methods). A drug substance is considered highly permeable when the extent of absorption in humans is determined to be 90% or more of the administered dose based on a mass-balance determination or in comparison to an intravenous dose.

3. Dissolution class boundaries- An immediate release product is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 15 minutes using USP Dissolution apparatus 1 at 100 RPM or Apparatus 2 at 50 RPM in a volume of 900 mL or less in the following media: 0.1 N Hydrochloric acid simulated gastric fluid or pH 4.5 buffer and pH 6.8 buffer or simulated intestinal fluid.
Chapter 1: Introduction

1.2 Objectives and concept of BCS:

- To improve the efficiency of the drug development and review process by recommending a strategy for identifying expendable clinical bioequivalence test.
- To recommend a class of immediate-release (IR) solid oral dosage forms for which bioequivalence may be assessed based on in vitro dissolution tests.
- To recommend methods for classification according to dosage form dissolution along with the solubility–permeability characteristics of the drug product [6].

Challenges of formulation development related to BCS II drugs:

Class II drugs which are highly permeable but exhibit low solubility/insoluble in aqueous medium are challenging to formulate. To formulate it in to liquid formulation solubility is the prerequisite. Once we make the drug soluble; the stability of the formulation system becomes a major challenge for formulator. Therefore a proper balance of solubility and stability needs to build in for final liquid formulation with the sound knowledge of science and technology. Class II drugs are very difficult to predict because of the large variability in the absorption or dissolution kinetics and the lack of an adequate in vitro system for evaluating the dissolution behavior. For example, to predict the in vivo absorption kinetics of griseofulvin (categorized as BCS Class II), it is orally administrated as a powder to rats, based on the Gastrointestinal–Transit–Absorption model (GITA model), which consists of the absorption, dissolution, and GI-transit processes. Using the dissolution rate constants (Kdis) of griseofulvin obtained with FaSSIF (fasted-state simulated intestinal fluid), FeSSIF (fed-state simulated intestinal fluid), and other simulated media, simulation lines did not describe the observed mean plasma profile at all.

A very important factor affecting the bioavailability of the drug substance after oral intake is the dissolution. i.e. the rate of dissolving of the drug substance, particularly in gastric fluid.
Oral administration of nebivolol hydrochloride is impeded by the poor dissolution when in a normal crystalline form. In the course of the investigations towards improving the bioavailability of nebivolol hydrochloride, the product was micronized. Unfortunately, as the dissolution of micronized nebivolol hydrochloride is even worse than nebivolol hydrochloride in normal crystalline form. (US patent 5,759,580) However, it was found that when nebivolol hydrochloride in micronized form was formulated in a composition with surfactant, polysorbate, it had an appropriate dissolution profile. Thus, the problem of bioavailability of the drug substance was resolved.[7]

- It is estimated that 40% or more of active substances being identified through combinatorial screening programs are poorly soluble in water. When these molecules are formulated using conventional methods, the performance of the drug in preclinical screens is often times erratic and highly variable.

- In the clinic, conventional formulations of poorly-water-soluble drugs are frequently plagued with problems such as poor and highly variable bioavailability. The drug absorption and dosage form is often affected by the fed–fasted state of the patient and its onset of action is slower than anticipated. All of these issues lead to sub-optimal dose and poor performance. Generally, it is more expeditious and cost effective to chemically redesign the molecule, than to move a blemished molecule through the development process.

1.3 Introduction to liquid formulation [8, 9]:

- Liquid formulation are mainly divided in to two classes Monophasic and Biphasic. monophasic consist of for oral, external, special use and parenterals, while biphasic further diverge in to liquid in liquid and solid in liquid.
LIQUIDS

Monophasic
1. for Oral use
2. for External use
3. for Special use
4. Parenteral
   1. Oral use (Emulsion)
   2. External use

Biphasic
Liquid in liquid
Solid in liquid

Figure 1.2 Classification of liquid formulation

1.4 Liquid formulation development [9-15]

Currently, there are fewer formulation approaches available for compounds that are poorly soluble in water. The most direct approach for enhancing solubility is to generate a salt. If, however, the compound is non-ionizable, solubility concerns are generally addressed by micronization and/or the development of oil-based solutions for injectable formulation. In addition, co-solvents, surfactants or complexing agents such as cyclodextrins have been employed. Reasonable success has also been met in formulating water-insoluble drugs using emulsion, microemulsion and solid dispersion technology. Although some of these approaches have been successfully utilized, especially for highly potent compounds with low dose requirements, there is a growing need for more effective and versatile ways to handle formulation issues associated with poorly-water-soluble molecules. This class of molecule could have tremendous impact on discovery effectiveness and improve the performance of products suffering from formulation-related issues.
Liquid formulation: factor affecting formulation,

- Route of administration
- Volume of injection
- Vehicle in which medicament is to be dissolved or suspended
- Osmolality of solution
- Need for a preservative
- pH of the solution
- Stabilizers
- Specific gravity for spinal injection
- Particle size and elegance for suspension

Liquid formulation pharmacokinetics:

In majority of the cases injectable products (true solution) are exempted from the bio-study as compare to solid orals, with logical justification. However for oral liquid which requires bioavailability study.

Merits of intravenous route of formulation over other routes of administration:

- Easy to handle
- Exact dose can be administered
- Purity & sterility of the medicament
- Stability of drug incompatibility overcome
- First pass gastric metabolism avoided
- Can easily administered to patients having problem in swallowing, patients having traumatic condition
Table 1.1: List of poorly water soluble drugs

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Drug Name</th>
<th>Drug Name</th>
<th>Drug Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difluprednate</td>
<td>Zaltoprofen</td>
<td>Meoxicam</td>
<td>Diclofenac</td>
</tr>
<tr>
<td>Aceclofenac</td>
<td>Amphotericin</td>
<td>Griosfulvin</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>Carprofen</td>
<td>Cyclophosphamid</td>
<td>Paracetamol</td>
<td>Indomethacin</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Betulinic acid</td>
<td>Piroxicam</td>
<td>Ketoprofen</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Docetaxel</td>
<td>Difluprednate</td>
<td>Lornocxicam</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Lornocxicam</td>
<td>Temazepam</td>
<td>Ethodolac</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>Bromazepam</td>
<td>Camazepam</td>
<td>Atorvastatin</td>
</tr>
<tr>
<td>Clobazepam</td>
<td>Clonazepam</td>
<td>Clotiazepam</td>
<td>Fluvastatin</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>Clorazepat</td>
<td>Diazepam</td>
<td>Pravastatin</td>
</tr>
<tr>
<td>Estazolam</td>
<td>Fluorazepam</td>
<td>Flunitrazepam</td>
<td>Lefunamid</td>
</tr>
<tr>
<td>Fluortemazepam</td>
<td>Ketazolam</td>
<td>Lorazepam</td>
<td>Rifunamid</td>
</tr>
<tr>
<td>Loprazolam</td>
<td>Lormetazepam</td>
<td>Medazepam</td>
<td>Glimeperide</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Nitrazepam</td>
<td>Nimetazepam</td>
<td>Oxazepam</td>
</tr>
<tr>
<td>Prazepam</td>
<td>Quazepam</td>
<td>Triazolam</td>
<td>Prazepam</td>
</tr>
<tr>
<td>Nebivolol</td>
<td>Drotaverine</td>
<td>Mycophenolate</td>
<td>Amiodarone</td>
</tr>
<tr>
<td>hydrochloride</td>
<td>Hydrochloride</td>
<td>Mofetil</td>
<td>hydrochloride</td>
</tr>
<tr>
<td>Trimcinolone</td>
<td>Methyl prednisol</td>
<td>Dronedarone</td>
<td>Milrinone</td>
</tr>
<tr>
<td>aceamide</td>
<td>acetate</td>
<td>hydrochloride</td>
<td></td>
</tr>
<tr>
<td>Nepafenc</td>
<td>Cyclosporine</td>
<td>Citalopram</td>
<td>Propofol</td>
</tr>
</tbody>
</table>

1.5 Challenges [16]:

Challenges for development of a liquid injectable formulation of a poorly water soluble drugs.

- Search for a suitable solvent which will give desire solubility and at the same which is non toxic and safe to patients.
- Stabilize the liquid formulation in to suitable solvent system up to its shelf life.
- To formulate lyophilized injection product into ready to use injection, which will reduce the overall manufacturing time & cost. Also give an appropriate dose amount to patients.
- Ease to medical practitioner to handle ready to use injection rather than lyophilized powder formulation.
However to develop the injectable formulation of poorly water soluble drug which is difficult and require many more precaution as compare to solid dosage form.

1.6 Importance of increasing solubility of BCS II type[17-18]:

**INDs and NDAs**

BCS-based biowaivers are applicable to the to-be marketed formulation when changes in components, composition, or method of manufacture occur to the clinical trial formulation, as long as the dosage forms have rapid and similar in vitro dissolution profiles. This approach is useful only when the drug substance is highly soluble and highly permeable (BCS Class I) and the pre- and post-change formulations are pharmaceutical equivalents. These are intended only for BE studies and are not applicable to food-effect BA studies or other pharmacokinetic studies.

**ANDAs**

Biowaivers can be requested for rapidly dissolving immediate-release (IR) test products containing highly soluble and highly permeable drug substances if the reference listed drug (RLD) is also rapidly dissolving and the test products exhibit dissolution profiles similar to the RLD. This approach is useful when the test and reference dosage forms are pharmaceutical equivalents.

**Post approval Changes**

Biowaivers can be requested for significant post approval changes (e.g., Level 3 changes in components and compositions) to a rapidly dissolving, immediate-release (IR) product containing a highly soluble, highly permeable drug substance, provided that dissolution remains rapid for the post-change product and both pre- and post change products exhibit similar dissolution profiles. The BCS enables pharma manufacturers to reduce the cost of scale-up and post approval changes to certain oral drug products (rapidly dissolving drug products of Class I drug). The BCS-based biowaivers apply during both pre- (IND/NDA and ANDA) and post approval phases. Considering the uncertainties associated with in vitro dissolution tests, the proposed biowaivers are as follows.

**Data Supporting High Solubility**

Data supporting high solubility of the test drug substance should include:

- A description of test methods including information on analytical methods and composition of the buffer solutions.
- Chemical structure, molecular weight, nature of the drug substance (acid, base, amphoteric, or neutral), and dissociation constants.
• Test results (mean, standard deviation, and coefficient of variation) summarized in a table under solution pH, drug solubility (e.g., mg/mL), and volume of media required to dissolve the highest dose strength.
• A graphic representation of mean pH–solubility profile.

**Data Supporting High Permeability**

Data supporting high permeability of the test drug substance should include:

• For human pharmacokinetic studies, information on study design and methods used along with the pharmacokinetic data.

The most powerful approach to improvement of class IV drugs is to return to the lead optimization phase of discovery and select a drug candidate with more appropriate physiochemical properties. (Fig.1.1)

• For direct permeability methods, information supporting the suitability of a selected method that encompasses a description of the study method; criteria for selection of human subjects, animals, or epithelial cell line; drug concentrations in the donor fluid; description of the analytical method and method used to calculate extent of absorption or permeability; and where appropriate, information on efflux potential (e.g., bidirectional transport data).

• A list of selected model drugs along with data on extent of absorption in humans (mean, standard deviation, coefficient of variation) used to establish suitability of a method, permeability values for each model drug (mean, standard deviation, coefficient of variation), permeability class of each model drug, and a plot of the extent of absorption as a function of permeability (mean ± standard deviation or 95% confidence interval) with identification of the low/high permeability class boundary and selected internal standard. Information to support high permeability of a test drug substance should include permeability data on the test drug substance, the internal standards (mean, standard deviation, and coefficient of variation), stability information, data supporting passive transport mechanism where appropriate, and methods used to establish high permeability of the test drug substance.

Table 1.2 provides a brief indication of the main formulation options and advantages and disadvantages of each approach.
### 1.2: Options for formulation of poorly water-soluble drugs

<table>
<thead>
<tr>
<th>Technology</th>
<th>Type of formulation</th>
<th>Potential advantage</th>
<th>Potential disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-dispersing ‘solid solutions’ with Surfactants</td>
<td>Solid oral &amp; Liquid</td>
<td>Steric hindrance to aggregation built into product, amenable to extrusion</td>
<td>Physical stability of product questionable—drug or polymer may crystallize</td>
</tr>
<tr>
<td>‘Solid solutions’ — drug immobilized in Polymer</td>
<td>Novel drug delivery system</td>
<td>Freedom to operate, new extrusion technology offers solvent-free continuous Process</td>
<td>Physical stability of product questionable—drug or polymer may crystallize</td>
</tr>
<tr>
<td>Nanocrystals obtained by Dense gas Technology</td>
<td>Nano particulate injectable drug delivery system</td>
<td>Alternative nanocrystal processing method, still room to develop new IP</td>
<td>Unproven technology, secondary process required to avoid aggregation of nanocrystals</td>
</tr>
<tr>
<td>Nanocrystals obtained by Ball-milling</td>
<td>Solid oral</td>
<td>Established products on the market, experienced technology provider (Elan), solid dosage form possible</td>
<td>Available only under license, secondary process required to avoid aggregation of Nanocrystals</td>
</tr>
<tr>
<td>Micronization</td>
<td>Tablet &amp; Capsule</td>
<td>Known technology, freedom to operate, solid dosage form</td>
<td>Insufficient improvement in dissolution rate</td>
</tr>
<tr>
<td>Lipid solutions (LFCS Type I lipid systems)</td>
<td>Novel drug delivery system</td>
<td>Freedom to operate, safe and effective for lipophilic actives, drug is presented in solution avoiding the dissolution step</td>
<td>Limited to highly lipophilic or very potent drugs, requires encapsulation Self-emulsifying drug delivery systems</td>
</tr>
<tr>
<td>Surfactant-co solvent systems (LFCS Type IV ‘lipid’ systems)</td>
<td>Injectable</td>
<td>Relatively high solvent capacity for typical APIs</td>
<td>Surfactant may be poorly tolerated in chronic use, significant threat of drug precipitation on dilution</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>------------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Solid or semi-solid SEDDS</td>
<td>Novel drug delivery system</td>
<td>Could be prepared as a free flowing powder or compressed into tablet form</td>
<td>Surfactant may be poorly tolerated in chronic use, reduced problem of capsule leakage, physical stability of product questionable drug or polymer may crystallize</td>
</tr>
<tr>
<td>Self-emulsifying drug delivery systems (SEDDS) and SMEDDS (LCFS Type II or Type III lipid systems)</td>
<td>Novel drug delivery system</td>
<td>Prior art available, dispersion leads to rapid absorption and reduced variability, absorption not dependent on digestion</td>
<td>Surfactant may be poorly tolerated in chronic use, soft gel or hard gel capsule can be used in principle but seal must be effective</td>
</tr>
</tbody>
</table>

However, to identify an appropriate vehicle with satisfactory solubility as well as chemical and physical stability can take significant time and resources. For compounds that do not dissolve in a small set of commonly used excipients, there are a large number of possible embodiments that can be tested (e.g., combinations of excipients, ratios, and processing conditions).

An urgent need has always existed for safe and effective delivery of poorly soluble drugs. Insolubility is caused by either a limited ability to hydrogen bond with water (hydrophobicity) or by difficulty in breaking apart molecules in the solid state (high lattice energy)[19].
1.7 Regulatory requirements to liquid dosage form [20]

Regulatory agency may ask for different studies at the time of review of dossier of liquid solution in comparison of available solid dosage form are as below:

1. Use of surfactant:
   - Micelle size
   - % drug release from micelle
   - Bioequivalence study with reference listed drug product

2. Use of Non aqueous solvent:
   - Toxicity of solvent
   - Daily intake limit or maximum allowable dose of particular solvent
   - Animal Toxicity data
   - Pharmacokinetic study
   - Local tolerability study

Patent aspect of development of liquid formulation:
   - It should be novel formulation.
   - If a patent non-infringing formulation is required, change in ratio of solvent or use of other solvent as per daily intake, use of buffer system, change in pH of finished product etc.
1.8 General scheme for preparation of liquid formulation

- Purified water or water for injection taken in S.S. vessel of total batch size.

- Sparging of any inert gas.

- Addition of excipient one by one in purified water or water for injection, as per development or optimization trials.

- Addition of preservative/antioxidant to above solution.

- Addition of active pharmaceutical ingredient to above solution.

- Proper mixing of solution during each step and note down each critical process parameters at each steps.

- Final bulk solution preparation.

- Clarification or sterile filtration.

- Filling of filtered solution in desire container closure system.

- Autoclave or terminal sterilization of finished product in final container.

- Labelling & Packaging.
Table 1.3: Examples of currently available liquid formulation of poorly water soluble drug

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Composition</th>
<th>Merit/Demerits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drotaverine Hydrochloride Injection</td>
<td>Use of organic solvent. Use of propylene glycol</td>
<td>Pain at site of injection due to high osmolality of injection.</td>
</tr>
<tr>
<td>Docetaxel inj</td>
<td>Alcohol &amp; polysorbate -80</td>
<td>Organic solvent used in higher concentration.</td>
</tr>
<tr>
<td>Temsirolimus inj</td>
<td>Non aqueous injection</td>
<td>Organic solvent used in higher concentration.</td>
</tr>
<tr>
<td>Difluprednate ophthalmic emulsion</td>
<td>Oil based milky emulsion, castor oil, Poly-80, glycerin</td>
<td>Require special technology for compounding.</td>
</tr>
<tr>
<td>Nepafenac ophthalmic sus.</td>
<td>Micronized API in Carbopol base</td>
<td>Highly thermal instable.</td>
</tr>
<tr>
<td>Cyclosporine oral solution</td>
<td>Use of phospholipids</td>
<td>Side effects.</td>
</tr>
<tr>
<td>Busulfan Injection</td>
<td>Non aqueous injection</td>
<td>Organic solvent used in higher concentration.</td>
</tr>
<tr>
<td>Cabazitaxel Injection</td>
<td>Non aqueous injection</td>
<td>Organic solvent used in higher concentration.</td>
</tr>
<tr>
<td>Melphalan for Injecton</td>
<td>Lyophilized product</td>
<td>Diluent containing ethanol &amp; Propylene glycol</td>
</tr>
<tr>
<td>Gemcitabine Injection RTU</td>
<td>Non aqueous injection</td>
<td>Organic solvent used in higher concentration.</td>
</tr>
<tr>
<td>Paclitaxel Injection</td>
<td>Cremophore and non aqueous injection</td>
<td>Cremophore reported as a toxic agent.</td>
</tr>
</tbody>
</table>
1.9 Introduction to ophthalmic formulation: Ophthalmic drug delivery system [21,22]

A multitude of ocular dosage forms are available for delivery of drugs to the eye. These can be classified on the basis of their physical forms as follows:

1. LIQUIDS: Solutions, Suspensions, Sol to gel systems (in situ gel), Sprays
2. SOLIDS: Ocular inserts, Contact lenses, corneal shield Filter paper strips, artificial tear inserts.
3. SEMI-SOLIDS: Ointments, Gels

1.9.1 Liquids

Liquids are the most acceptable ophthalmic dosage form. This is because the drug absorption is rapid from solution.

- Solutions and Suspensions

Solutions are the pharmaceutical dosage forms most widely used to administer drugs that must be active either the eye surface or in the eye after passage through the cornea or the conjunctiva. The slow release of the drug from the suspension provides a sustained effect for a short duration of time.

Advantage:
- The drug in the solution is in the dissolved state and thus it is immediately active.

Limitations:
- The very short residence time at the eye surface,
- Poor bioavailability because almost 75% is lost via nasolacrimal drainage

Extensive work has been done to prolong ocular retention of drugs by altering the viscosity or the pH of the solution.

- Sol to gel Systems

In the early 1980s, the new concept of producing a gel in situ (eg. in the cul-de-sac of the eye) was suggested for the first time. It is widely accepted that increasing the viscosity of a drug formulation in the precorneal region will leads to an increased bioavailability, due to slower drainage from the corneal region of the eye. Various concepts for the in situ gelling systems have been investigated. These systems can be triggered by temperature, pH or by ion activation.
Sprays
This dosage form is not commonly used but some practitioners use mydriatics or cycloplegics alone or in combination in the form of eye spray. These sprays are used in the eye either for dilating the pupil or for cycloplegic examination.

1.9.2. Solids
The concept of using solids for the eye is designed on providing sustained release characteristics.

Ocular inserts
Ocular inserts are solid dosage form and can overcome the limitations reported with traditional ophthalmic systems like aqueous solutions, suspensions and ointments. The typical pulse entry type drug release is behavior observed with ocular aqueous solutions (eye drops), suspensions and ointments whereas, more controlled, sustained and continuous drug delivery is obtained by controlled release ocular drug delivery system.

Contact lenses
The water soluble drug can be soaked by contact lenses. These lenses saturated by drug are placed in the eye for releasing the drug for long period of time. The hydrophilic contact lenses can be used to sustain and prolong the ocular residence time of the drugs.

Corneal shield
Collagen shields are used as a corneal bandage which are with foetal calf skin tissue and originally developed. These devices form a thin film once softened by the tears, that confirms exactly to the corneal surface, and it undergoes dissolution up to 10, 24 or 72 hours. Due to its biological inertness, structural stability and also good biocompatibility collagen film proved as a promising carrier for ophthalmic drug delivery system.

Artificial tear inserts
The commercially available tear insert is fabricated with Hydroxypropyl Cellulose (HPC) without addition of preservative (Lacrisert). It is a rod shaped pellet. This device is for the treatment of dry eye disorders. In 1981, it was developed by Merck, Sharp and Dohme.

Filter paper strips
Sodium fluorescein and Rose Bengal dyes are available in the market as drug impregnated filter paper strips. These dyes are used diagnostically to examine corneal injuries and infections such as dry eye disorders and herpes simplex.
1.9.3 Semi-solids

A wide variety of semisolids vehicles are used for topical ocular delivery which are classified as: simple and compound bases.

Simple bases are referred to a single continuous system. These include lanolin, white petrolatum, and viscous gels prepared from polymers such as Polyvinyl Alcohol (PVA), Carbopol etc. Compound bases are usually of a biphasic type forming either water in oil (w/o) or oil in water (o/w) emulsions.

A drug in either a simple or a compound base increases the duration of action due to

- reduction in dilution by tears
- reduction in drainage by way of a sustained release effect
- prolonged corneal contact time.

Semi-solids dosage forms are applied once or twice a day which provide sustained release. The primary purpose of the ophthalmic ointment vehicle was to prolong drug contact time with the external ocular surface. But they present a limitation of matting of eyelids and blurring of vision. Ophthalmic gels are similar in viscosity to ophthalmic ointments.

1.9.4 Challenges to Ophthalmic drug delivery [23, 24, 25]

Ophthalmic drug delivery is a bothersome task due to various barriers.

- Topical application in the form of eye drops is the most common method used to treat outside of the eye, like dry eyes, and as well as to provide intraocular treatment with absorption through the cornea, like glaucoma.

- Contact time of the drug (1-2 min) is very short because due to lacrimal fluid production followed by drainage into either the nasolacrimal ducts or conjunctiva, and blinking, induce a rapid elimination of the topically applied drug solution.

- The absorption of drug is lowered by the three layered cornea, the epithelium limiting the absorption of hydrophilic drugs and the stroma limiting the absorption of lipophilic drugs. Hydrophilic layer over the tears is formed by mucin secreted to protect the ocular surface.
• Tight junction formed between blood-retinal barriers and blood-aqueous limit drug penetration from the systemic bloodstream into the intraocular environment. Thus, in most cases, drugs administered by systemic route are not be able to reach therapeutic levels in the eye; However, orally administered drugs will not reach therapeutic levels in the eye unless given in very high dose. These high doses could result in systemic side effects.

• Periocular and intravitreal administration have become increasingly more common since they partly overcome the inefficient drug delivery related to topical and systemic dosing to the posterior segment of the eye. However these routes are not very patient compliant and may result in tedious side effects.

1.10 Introduction to QbD (Quality by Design): [26, 27]

Quality-by-design (QbD) is a systematic approach to develop drug, which begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and risk management. The concept of QbD can be extended to analytical methods. QbD mandates the definition of a goal for the method, and emphasizes thorough evaluation and scouting of alternative methods in a systematic way to obtain optimal method performance. Candidate methods are then carefully assessed in a structured manner for risks, and are challenged to determine if robustness and ruggedness criteria are satisfied. As a result of these studies, the method performance can be understood and improved if necessary, and a control strategy can be defined to manage risk and ensure the method performs as desired when validated and deployed.

There are several design available to carry out DoE like response surface methodology (RSM), Central Composite Design, Box-Behnken Design, One Factor RSM Design, RSM Optimal Design, 3-Level Factorial Design, Hexagonal Design, Pentagonal Design, Hybrid Design, Distance Based Design, User Defined Design and Mixture Design etc.

In statistics, response surface methodology (RSM) explores the relationships between several explanatory variables and one or more response variables. The method was introduced by G. E. P. Box and K. B. Wilson in 1951. The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response. Box and Wilson suggest using a second-degree polynomial model to do this. They
acknowledge that this model is only an approximation, but use it because such a model is easy to estimate and apply, even when little is known about the process. For formulation optimization study we have used design expert software (version 8.0.7.1), in which response surface method Box Behnken model was used for further evaluation for finalized the formula.

**Basic approach of response surface methodology**

An easy way to estimate a first-degree polynomial model is to use a factorial experiment or a fractional factorial designs. This is sufficient to determine which explanatory variables have an impact on the response variable(s) of interest. Once it is suspected that only significant explanatory variables are left, then a more complicated design, such as a central composite design can be implemented to estimate a second-degree polynomial model, which is still only an approximation at best. However, the second-degree model can be used to optimize (maximize, minimize, or attain a specific target for) a response.

**Special geometries**

**Simplex geometry and mixture experiments**

Mixture experiments are discussed in many books on the design of experiments, and in the response-surface methodology textbooks of Box and Draper and of Atkinson, Donev and Tobias. An extensive discussion and survey appears in the advanced textbook by John Cornell.

In statistics, **Box–Behnken designs** are experimental designs for response surface methodology, devised by George E. P. Box and Donald Behnken in 1960, to achieve the following goals:

- Each factor, or independent variable, is placed at one of three equally spaced values. (At least three levels are needed for the following goal.)
- The design should be sufficient to fit a quadratic model, that is, one containing squared terms and products of two factors.
- The ratio of the number of experimental points to the number of coefficients in the quadratic model should be reasonable (in fact, their designs kept it in the range of 1.5 to 2.6).

The estimation variance should more or less depend only on the distance from the centre (this is achieved exactly for the designs with 4 and 7 factors), and should not vary too much inside the smallest (hyper) cube containing the experimental points 2 level, 3
variable Full factorial study – Total 14 experiments (Considering 2 center points per blocks).

Graphs of DOE

1. Main effect plot: They are also called as mean plot. A main effect plot describes the effect of an independent variable on a dependent variable on averaging across the levels of any other independent variables.

2. Pareto charts: A Pareto chart, named after Italian engineer, sociologist, economist, political scientist, and philosopher Vilfredo Pareto, is a type of chart that contains both bars and a line graph, where individual values are represented in descending order by bars, and the cumulative total is represented by the line.

3. Interaction plot: An interaction plot displays the impact that changing the settings of one factor has on another factor. Because an interaction can enhance or diminish main effects, evaluating interactions is extremely important.

4. Surface plot: Three-dimensional surface plots help to understand the potential relationship between three variables. Independent variables are mapped on the x- and y-scales, and the response variable (z) is represented by a smooth surface (surface plot) or a grid (wireframe plot).

5. Contour plot: A contour plot is a graphical representation of a 3-dimensional surface by plotting constant z slices, called contours, on a 2-dimensional format. That is, given a value for z, lines are drawn for connecting the x and y coordinates where that z value occurs. The contour plot is formed by: Vertical and horizontal axis: Independent variables are plotted on x and y axis. Lines: iso-response values are obtained. The independent variables of the experiment are usually restricted to a regular grid.

1.11 Preformulation study \([29, 30]\)

Every drug has intrinsic chemical and physical properties which has been consider before development of pharmaceutical formulation. This property provides the framework for drug’s combination with pharmaceutical ingredients in the fabrication of dosage form. Objective of preformulation study is to develop the elegant (stable, effective and safe) dosage form by establishing kinetic rate profile, compatibility with the other ingredients and establish physico-chemical parameter of new drug substance. Among these properties, drug solubility, partition coefficient, dissolution rate, polymorphic forms and stability are plays important role in preformulation study. Polymorphism having crystal
and amorphous forms shows different chemical physical and therapeutic description of the drug molecule.

1.1.1 Major area of preformulation research

A. Bulk characterization:
   1. Crystallinity & polymorphism
   2. Hygroscopicity
   3. Fine particle characterization
   4. Powder flow properties

B. Solubility analysis:
   1. Ionization constant -Pka
   2. pH solubility profile
   3. Common ion effect -Ksp
   4. Solubilization
   5. Dissolution
   6. Partition co-efficient

C. Stability analysis:
   1. Solution stability
   2. pH rate profile
   3. Solid state stability
   4. Bulk stability
   5. Stability in toxicology formulation

- Crystallinity & Polymorphism

Crystal habit & internal structure of drug can affect physico-chemical properties which range from flow ability to chemical stability. Habit means the description of outer appearance of a crystal. While internal structure describes the molecular arrangement within the solid, changes in internal structure usually alter crystal habit.
Hygroscopicity

A substance which absorbs sufficient moisture from the atmosphere is called hygroscopic. Most pharmaceutical compounds lose or gain water from the atmosphere depending on the relative humidity (RH). Materials unaffected by RH are termed as non hygroscopic.

- Amorphous compounds may take up water and re-crystallize & or degrade anhydrous material may hydrate and become less soluble.
- The weight change with sorption may cause errors in potency.
- The volume changes associated with water gain and loss may compromise dosage form integrity. Changing the solid state form in the dosage form requires regulatory approval.
- Different forms may have different compaction, flow and charging characteristics.

Following points should be considered for prevention of hygroscopicity,

- Good packaging (air tight)
- Use of foil blisters
- Use of desiccants.

1.11.2 Solubility study [28]

The solubility of drug is an important physicochemical property because it effects the bioavailability of the drug, the rate of drug release into dissolution medium and consequently, the therapeutic efficiency of the pharmaceutical product. The solubility of the molecules in various solvents is determined as a first step. This information is valuable is developing a formulation. Solubility is usually determined in variety of commonly used solvents and some oils if the molecule is lipophilic. The solubility of material is usually determined by the equilibrium solubility method, which employs a saturated solution of the material, obtained by stirring an excess of material in the solvent for a prolonged until equilibrium achieved. The approximate solubilities of Pharmacopoeial and national formulary substances are indicated by the descriptive terms in accompanying table.
Table 1.4 Solubility descriptions as per pharmacopeia
(Description and solubility appendix 5.30)

<table>
<thead>
<tr>
<th>Descriptive Terms</th>
<th>Part of Solvents Required for 1 Part of Solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Soluble</td>
<td>Less than 1</td>
</tr>
<tr>
<td>Freely soluble</td>
<td>From 1 to 10</td>
</tr>
<tr>
<td>Soluble</td>
<td>From 10 to 30</td>
</tr>
<tr>
<td>Sparingly Soluble</td>
<td>From 30 to 100</td>
</tr>
<tr>
<td>Slightly Soluble</td>
<td>From 100 to 1000</td>
</tr>
<tr>
<td>Very slightly</td>
<td>From 1000 to Soluble 10000</td>
</tr>
<tr>
<td>Insoluble</td>
<td>From 10000 to over</td>
</tr>
</tbody>
</table>

1.11.3 Drug-excipient compatibility study

In the typical drug/excipient compatibility testing program, binary (1:1 or customized) powder mixes are prepared by triturating API with the individual excipients. These powder samples, usually with or without added water and occasionally compacted or prepared as slurries, are stored under accelerated conditions and analysed by stability-indicating methodology, e.g. HPLC. (The water slurry approach allows the pH of the drug-excipient blend and the role of moisture to be investigated.) Alternatively, binary samples can be screened using thermal methods, such as DSC/TGA. No need for stability set-downs; hence cycle times and sample consumption are reduced. However, the data obtained are difficult to interpret and may be misleading; false positives and negatives are routinely encountered. Also it is sensitive to sample preparation. However, the binary mix approach takes time and resources and it is well known that the chemical compatibility of an API in a binary mixture may differ completely from a multi-component prototype formulation.

Drug-excipient interactions can be studied using both approaches in a complementary fashion. The first tier approach is to conduct short-term (1-3m) stability studies using generic prototype formulations under stressed conditions, with binary systems as diagnostic back-up:

Chemical stability measured by chromatographic methods.

Physical stability measured by microscopic, particle analysis, in vitro dissolution methods, etc.
The idea is to diagnose any observed incompatibility from the prototype formulation work then hopefully identifies the “culprit” excipients from the binary mixture data. Hopefully, a prototype formulation can then be taken forward as a foundation for product development. Application of statistical models (e.g. $2^n$ factorial design) to determine the chemical interactions in more complex systems such as prototype formulations, with a view towards establishing which excipients cause incompatibility within a given mixture.

1.12 Stability study [31, 32]

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions. The choice of test conditions defined in this guideline is based on an analysis of the effects of climatic conditions in the three regions of the EC, Japan and the United States. The mean kinetic temperature in any part of the world can be derived from climatic data, and the world can be divided into four climatic zones, I-IV. This guideline addresses climatic zones I and II. The principle has been established that stability information generated in any one of the three regions of the EC, Japan and the United States would be mutually acceptable to the other two regions, provided the information is consistent with this guideline and the labeling is in accord with national/regional requirements.

1.12.1 Force degradation:

Forced degradation (FD) study is a process in which the natural degradation rate of a pharmaceutical product is increased by the application of an additional stress. FD studies (i) help to identify reactions that cause degradation of pharmaceutical product, ii) are part of the development strategy and an integral component of validating analytical methods that indicate stability and detect impurities which are formed during manufacture, storage, or use and their properties are different from the desired product with respect to activity, efficacy and safety and iii) are designed to generate product-related variants and develop analytical methods to determine the degradation products formed during accelerated and long term stability studies. Any significant degradation product should be evaluated for characterization and quantization for its potential hazard.

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors.
such as temperature, humidity and light and others is to establish a retest period for the drug substance or a shelf life for the drug product and recommended storage conditions.

1.12.2 Accelerated stability study

Studies designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as part of the formal stability studies. Data from these studies, in addition to long term stability studies, can be used to assess longer term chemical effects at non-accelerated conditions and to evaluate the effect of short term excursions outside the label storage conditions such as might occur during shipping. Results from accelerated testing studies are not always predictive of physical changes.

Table 5: Stability conditions

<table>
<thead>
<tr>
<th>Study</th>
<th>Storage condition</th>
<th>Minimum time period covered by data at submission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long term*</td>
<td>25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH</td>
<td>12 months</td>
</tr>
<tr>
<td>Intermediate**</td>
<td>30°C ± 2°C/65% RH ± 5% RH</td>
<td>6 months</td>
</tr>
<tr>
<td>Accelerated</td>
<td>40°C ± 2°C/75% RH ± 5% RH</td>
<td>6 months</td>
</tr>
</tbody>
</table>

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

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

“Significant change” for a drug substance is defined as failure to meet its specification.

In general, “significant change” for a drug product is defined as:

- A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures;
- Any degradation product’s exceeding its acceptance criterion;
- Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., color, phase separation, resuspendibility, caking, hardness, dose delivery per actuation); however, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions;
- and, as appropriate for the dosage form:
• Failure to meet the acceptance criterion for pH; or
• Failure to meet the acceptance criteria for dissolution for 12 dosage units.

1.12.3 Real time stability study
Stability studies under the recommended storage condition for the re-test period or shelf life proposed (or approved) for labeling.
1.13 Sterilization methods used for pharmaceutical products [33-34]
Sterilization is a term referring to any process that eliminates (removes) or kills all forms of life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Sterilization can be achieved by applying heat, chemicals, irradiation, high pressure and filtration or combinations thereof.

![Figure 1.3: Different methods of sterilization](image)

The effectiveness of every sterilization method depends on some factors like the type and the number of microorganisms, the type and amount of organic material that protect the microorganisms, the number and the size of cracks on the product or instrument that might be present during the sterilization of microorganisms.

1.13.1 Sterilization of API
Drug substances are usually sterilized by radiation. The safety of irradiation facilities is regulated by the United Nations International Atomic Energy Agency and monitored by the different national Nuclear Regulatory Commissions. The incidents that have occurred in the past are documented by the agency and thoroughly analyzed to determine root cause and improvement potential. Such improvements are then mandated to retrofit existing facilities and future design.

Radiation have effects on cells and microorganisms depending on the effects of wave-length, dose rate and exposure time. Irradiation of the particles with gamma rays or X-rays does not induce materials or products to turn into a radioactive form. Only irradiation of the products with particles may cause formation of radioactive form depending on the energy, type of the particle and the type of the target material. Some of the high energetic, high penetrating
particles and neutrons may cause this effect. The mechanism of the effect of radiation on the microorganisms can be direct or indirect. Direct effect is the ionization of the molecule by absorbing the radioactive energy directly. The major target is the water molecule in the product that causes the production of H3O\(^+\) and OH\(^-\) radicals as the radiolysis products. Hydroxyl radicals are responsible from 90% of DNA damages and they have a strong oxidant effect. The presence of O\(_2\) molecules in the product may cause the effect of the radiation to the product.

![Illustration of the penetration properties of the different radiation technologies](image)

**Figure 1.4**: Illustration of the penetration properties of the different radiation technologies (electron beam, X-ray, gamma rays)

Gamma rays are formed with the self disintegration of Cobalt-60 (60Co) or Cesium-137 (137Cs). It is a high penetrating and commonly used sterilization method. It is generally used for the sterilization of gaseous, liquid, solid materials, homogeneous and heterogeneous systems and disposable medical equipment, such as syringes, needles, cannulas, density materials, cosmetics and i.v. sets. It can easily be applied on many materials but is incompatible with polyvinyl chloride (PVC), acetal and polytetrafluoroethylene (PTFE). It is a continuous or batch process. Complete penetration can be achieved depending on the thickness of the material. It supplies energy saving and it needs no chemical or heat dependence. Depending on the radiation protection rules, the main radioactive source has to be shielded for the safety of the operators. Storage of is needed depending on emitting gamma rays continuously. Immediate (dosimetric) release can be done because it needs no sterilization testing after the completion of the process. Another advantage is it has no residue after the sterilization process.

It is commonly used for the sterilization of medical devices like gamma radiation sterilization. E-beam sterilization can be generally made by the use of e-beams that are obtained from the accelerator and by isotope method. Its advantage is the need of very short exposition time depending on the 10 MeV of very high electron energy. This high energy is fundamental for an effective sterilization, while 15 min. is sufficient for the accelerator
method, isotope method requires 24 hours. $^{60}$Co isotope source is generally used for the isotope method. The energy of the produced and accelerated electrons is increased by specially designed machines. An on-off technology that operates with electrical energy is used. It is a continuous process. It can be applied to many materials depending on its penetration. Immediate release can be done because it needs no sterilization testing after the completion of the process. The most important advantage about e-beam radiation is its having much higher dosing rate than gamma or X-rays. Another advantage is that having no residue after sterilization process. The use of higher dose rate causes less exposure time and reduced potential degradation to polymers.

**X-rays**

Large packages and loads of medical devices can be sterilized with high-energy X-rays that are a form of ionizing energy called bremsstrahlung. X-rays can effectively be used for the sterilization of multiple pallet loads of low-density packages with very good dose uniformity ratios. It is an electricity based process and it does not require any chemical or radio-active material. Presently, it is not an official sterilization method for drugs and medical devices.

**UV light irradiation**

It operates as a germicidal lamp and is only used for the sterilization of surfaces and some transparent objects. But, it is not used for the sterilization of contaminated areas and plastics and is not an official technique for drugs and medical devices.

### 1.13.2. Sterilization of Drug product

#### Steam sterilization

Steam destroys organisms by coagulating the cell protein. Poaching an egg is an everyday example of protein being coagulated. In order to destroy all microbes, the steam must be able to come into contact with all surfaces. Steam can only sterilize the surfaces it can touch. For this reason, air pockets are the greatest enemy of the steam process since they can prevent the steam from touching all surfaces. Air pockets can occur as a result of improper packing assembly and loading. There are two types of steam cycles commonly used: gravity displacement and dynamic air removal, which includes the prevacuum and steam flush pressure pulse (SFPP) cycles. Gravity displacement steam sterilization was the first type of cycle introduced to hospitals. Operating rooms still use this type of cycle for “flash” sterilization. Gravity is used to displace the air as steam enters the chamber. A prevacuum cycle removes the air mechanically, which is more efficient. The SFPP cycle uses mechanical air removal above atmospheric pressure. These cycles have three phases:
1. Conditioning phase: The air is removed, steam enters the chamber and the load is heated to a set temperature.

2. Exposure phase: The duration of this phase is scientifically determined. It consists of heating time, the actual kill time, plus a safety factor equal to 50% of the kill time.

3. Exhaust phase: After the exposure phase is completed, the steam is replaced with air, and the chamber returns to atmospheric pressure.

**Filtration**

Fluids that would be damaged by heat (such as those containing proteins like large molecule drug products, but also wine and beer) irradiation or chemical sterilization, can be only sterilized by microfiltration using membrane filters. This method is commonly used for heat labile pharmaceuticals and protein solutions in medicinal drug processing. Usually, a filter with pore size 0.2 µm (microfiltration) will effectively remove microorganisms. In the processing of biologics, viruses must be removed or inactivated. Nanofilters with a smaller pore size of 20 -50 nm (nanofiltration) are used. The smaller the pore size the lower the flow rate. To achieve higher total throughput or to avoid premature blockage, pre-filters might be used to protect small pore membrane filters.

Membrane filters used in production processes are commonly made from materials such as Nylon, Polyvinylidene fluoride (PVDF); Glass sintered and mix ester cellulose or polyethersulfone etc. (PES). The filtration equipment and the filters themselves may be purchased as pre-sterilized disposable units in sealed packaging, or must be sterilized by the user, generally by autoclaving at a temperature that does not damage the fragile filter membranes. To ensure proper functioning of the filter, the membrane filters are integrity tested post-use and in occasions pre-use. The non-destructive integrity test assures the filter is undamaged, it also is a regulatory requirement enforced by agencies like FDA, EMA etc. For best results, final or terminal pharmaceutical sterile filtration is performed in clean room.
1.14 References:

7. Jans et al. USO05759580A, Compositions containing micronized nebivolol.


32. ICH harmonized tripartite guideline, stability testing of new drug substances and products, Q1A(R2)
