CHAPTER – 1

INTRODUCTION
1.1 INTRODUCTION OF HIV INFECTION AND AIDS

The human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS), commonly referred to as HIV/AIDS, constitutes one of the most serious infectious disease challenges to public health globally since few decades. AIDS is the result of damage to the immune system after infection with the most advanced stage of HIV and requires a treatment for life time. AIDS involves the infection and destruction of critical immune cells (especially the CD4 cells) which causes the diminished function of the immune system. CD4 cells are the part of immune system and are the cells that the body uses to defend itself.

HIV (Figure 1.1) belongs to the retrovirus subfamily lentivirus that can be subdivided into HIV-1 and HIV-2 subspecies. HIV-1 has a high virulence and transmissibility which accounts for the epidemic of HIV transmission all over the world. In contrast, HIV-2 has less virulence and transmissibility which is more prevalent in West Africa and takes a longer time to develop into immunodeficiency from infection than HIV-1\(^1,2\). Both types of viruses cause AIDS which is defined as a “clinical condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections”. About 90% of deaths related to HIV infection and AIDS are caused by opportunistic infections (e.g., herpes viruses, fungal infections).
1.2 HIV/AIDS DRUG THERAPY AND ITS CURRENT LIMITATIONS

The current scheme for the treatment of HIV infection is called highly active antiretroviral therapy (HAART). It is based on a combination (or ‘cocktails’) of drugs belonging to at least two types, or "classes" of antiretroviral agents which are currently approved by FDA. The key factor in the HAART is to disrupt HIV at different stages in its replication. HAART considerably reduced the incidence of opportunistic infections and death in AIDS patients in recent years.

In spite of the huge advances made in antiretroviral (ARV) drug therapy in disease management, its current use is associated with
several disadvantages and inconveniences to the HIV/AIDS patient\textsuperscript{4,5} which are as follows,

- Many ARV drugs suffer extensive first pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability.
- Requires repeated administration of doses leading to decreased patient compliance.
- Therapeutic concentrations of majority of ARV drugs cannot be maintained for the necessary duration at the site of HIV localisation.
- ARV drugs suffer from many physicochemical problems such as poor solubility that may lead to formulation difficulties.
- They exhibit unacceptable toxicity or cause undesirable side-effects.

In the wake of all these facts, better drugs, safer and easier to administer, with more favourable pharmacologic properties and activity against drug resistant viruses are needed for HIV treatment. Hence, many approaches are currently being investigated to overcome these limitations that can improve the efficacy and quality of both existing as well as new ARV drugs\textsuperscript{6}. Majority of the currently marketed anti HIV agents are formulated either as solids (tablets, capsules for oral use) or liquid dosage forms (solution, suspension for oral and parenteral use). Generally, most ARV compounds are designed to interfere with different stages of the HIV life cycle. Therefore, they are
classified according to the stages of HIV life cycle. A number of anti-
HIV drugs approved by the FDA for HIV treatment are listed in Table
1.1.

**Table 1.1:** FDA approved anti-HIV drugs\(^7-9\)

<table>
<thead>
<tr>
<th><strong>Drug class</strong></th>
<th><strong>Drug (Brand name)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry/Fusion inhibitors</td>
<td>enfuvirtide (Fuzeon), maraviroc (Selzentry)</td>
</tr>
<tr>
<td>Reverse transcriptase inhibitors</td>
<td>delavirdine (Rescriptor), efavirenz (Sustiva), etravirine (Intelence) nevirapine (Viramune)</td>
</tr>
<tr>
<td>Non-nucleoside reverse transcriptase inhibitors (NNRTIs)</td>
<td>abacavir (Ziagen), tenofovir (Viread) didanosine (Videx), stavudine (Zerit) emtricitabine (Emtriva), zidovudine (Retrovir), zalcitabine (Hivid), lamivudine (Epivir)</td>
</tr>
<tr>
<td>Nucleoside reverse transcriptase inhibitors (NRTIs)</td>
<td>raltegravir (Isentress)</td>
</tr>
<tr>
<td>Integrase inhibitors</td>
<td></td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>amprenavir (Agenerase), atazanavir (Reyataz), darunavir (Prezista) fosamprenavir (Lexiva), indinavir (Crixivan), ritonavir (Norvir) nelfinavir (Viracept), saquinavir (Invirase), tipranavir (Aptivus)</td>
</tr>
<tr>
<td>Fixed-dose combination (Fixed-dose combination tablets contain two or more anti-HIV medications that can be from one or more drug classes)</td>
<td>lamivudine/zidovudine (Combivir) abacavir/lamivudine/zidovudine (Trizivir), lopinavir/ritonavir (Kaletra), tenofovir/emtricitabine (Truvada), efavirenz/emtricitabine/tenofovir DF (Atripla), abacavir/lamivudine (Epzicom)</td>
</tr>
</tbody>
</table>
**Entry and fusion inhibitors:** Fusion inhibitors work by blocking HIV from entering human cells. Other fusion inhibitors under investigation are AMDO70, PRO 140, TNX-355 and vicriviroc maleate.

**Non-nucleoside reverse transcriptase inhibitors (NNRTIs):** NNRTIs block reverse transcriptase, a protein that HIV needs to make more copies of itself. These are not active against HIV-2. The new molecule under investigation is rilpivirine (TMC278). NNRTIs are the most frequently used ARV drugs used in the treatment of HIV-1.10.

**Nucleoside reverse transcriptase inhibitors (NRTIs):** These are the first class of ARVs. All these compounds are prodrugs which need to be converted intracellularly in the cytoplasm to their active form before exerting their antiviral activity. The active forms of these drugs are substrates for reverse transcriptase enzyme, and they result in termination of DNA chain elongation of the retrovirus. A few other molecules under investigation are apricitabine (ATC), elvucitabine (ACH-126443), fosalvudine (HDP 99.0003), KP-1461 (SN1212) and racivir (RCV).

**Integrase inhibitors:** These work by blocking integrase, a protein that HIV needs to insert its viral DNA into the DNA of an infected cell. The new molecule under investigation is elvitegravir (GS 9137).

**Protease inhibitors (PIs):** PIs act by blocking protease, a protein that HIV needs to make more copies of itself. They act primarily at the end of the HIV life cycle to cause the formation of non-infectious immature
virions. A few newer molecules under investigation are tipranavir, DMP-450 and TMC 114.

1.3 DRUG SOLUBILITY AND DISSOLUTION RATE

The most challenging aspects in the pharma industry are related to strategies that improve the solubility of poorly soluble drugs. Solubility plays an important role in drug disposition, since the maximum rate of passive drug transport across a biological membrane, is the product of permeability and solubility. For drugs that have very poor aqueous solubility, the rate at which the drug dissolves (dissolution) is often the slowest step and therefore exhibits a rate limiting effect on drug bioavailability. Hence, great efforts have been made to improve oral bioavailability of poorly water soluble drugs by increasing their dissolution rate through various techniques. These include the formulation of amorphous solid form, nanoparticles, microemulsions, solid dispersions, melt extrusion, salt formation and formation of water-soluble complexes etc. Among them, the solid dispersion technique\textsuperscript{11,12} and the complexation with cyclodextrins are most frequently used\textsuperscript{13,14}.

Typical processes involved in the absorption of drugs given on oral solid dosage forms are disintegration, deaggregation, and dissolution before any substantial absorption takes place in GIT. The dissolution of the solid drug particles from a well-designed solid dosage form is one of the important rate-determining steps in drug absorption from GIT. Since, drug absorption requires that molecules be in solution at
the absorption site. There are many factors that affect drug dissolution from the dosage forms. In 1897, Noyes and Whitney\textsuperscript{15} described the quantitative analysis that correlated the amount of time it took to dissolve a drug from solid particles. The current version of the equation is slightly modified from the original but remains based on a diffusion layer model (Figure 1.2). This provides some hints as to how the dissolution rate of poorly soluble compounds might be improved to minimize the limitations to oral bioavailability, i.e.,

$$\frac{dc}{dt} = \frac{AD(Cs - Cb)}{hV}$$

Where,

$dc/dt =$ Rate of dissolution

$A =$ Surface area of drug exposed to dissolution (cm$^2$),

$D =$ Diffusion coefficient of the compound (cm$^2$/s),

$Cs =$ Concentration of a saturated solution of the drug in dissolution medium (M or mg/ml),

$Cb =$ Concentration of drug in the bulk medium (M or mg/ml) at any time $t$ (seconds),

$h =$ Thickness of the diffusion boundary layer adjacent to surface of the dissolving compound,

$V =$ Volume of dissolution media (ml)

The dissolution rate is proportional to both $A$ and $Cs$, and $Cs$ is influenced by the composition of the aqueous dissolution medium, including its pH. As a result, the drug solubility and dissolution rate are not constant throughout the GIT. According to the above equation,
for drugs with low aqueous solubility, particle size and the resulting surface area could have a significant effect on the rate of dissolution over the time interval during which gastrointestinal absorption occurs, and can affect the bioavailability\cite{16}. Hence to enhance the dissolution rate, one needs to increase the diffusion coefficient, increase the surface area, decrease the stagnant diffusion layer thickness, or increase the saturation solubility of the drug.

![Diagram](Fig: 1.2) Dissolution of drug particles according to diffusion layer model

### 1.3.1 Various approaches to improve the solubility\cite{17-21}

The improvement or enhancement of solubility plays an important role in formulation of pharmaceuticals because they define the rate of dissolution of drug. Different methods are available to enhance the solubility and hence dissolution rate of poorly water soluble drugs. They are summarized in Table 1.2.
**Table 1.2: Major formulation strategies for poorly soluble drugs**

| I. Physical modification | A. Particle size reduction | • Micronization  
| | | • Nanosuspensions  
| | | • Microemulsions  
| | | • Lyophilization  
| | • Polymorphs  
| | • Pseudo polymorphs (including solvates)  
| | • Nano-crystals  
| | • Co-crystals  
| | • Use of Cyclodextrins  
| | • Micellar/surfactant systems  
| | • Co-solvent systems  
| | • pH adjustment  
| | • Eutectic mixtures  
| | • Solid dispersions (non-molecular)  
| | • Solid solutions  
| | II. Chemical modification | • Soluble prodrugs  
| | | • Salt formation  
| | | • Covalent polymer drug conjugates  

1.4 SOLID DISPERSIONS (SDs)

The production of solid dispersion (SD) is commonly acknowledged as a method to enhance the aqueous solubility of poorly soluble drugs, thereby increasing the oral bioavailability. Hence, the formulation of poorly soluble drugs as SDs is a significant area of research aimed at improving their dissolution and bioavailability. Solid dispersion is a method of improving the dissolution rate of poorly soluble drugs which was first proposed by Sekiguchi K and Obi N\(^2\). It was suggested that the drug is either present in eutectic mixture or in a microcrystalline state. In some cases, all or certain fraction of drug may be molecularly dispersed in the matrix forming a solid solution.

Chiou and Riegelman\(^2\) defined the term solid dispersion as ‘the dispersion of one or more active ingredients in an inert carrier matrix at solid-state prepared by the melting (fusion), solvent or melting-solvent method’. While Corrigan\(^2\) suggested the definition of solid dispersion as a ‘product formed by converting a fluid drug-carrier combination to the solid state’. Solid dispersion technique is a promising approach to present a poorly soluble drug in an extremely fine state of subdivision which increases the surface area for dissolution in gastrointestinal fluids and has been widely employed to improve the solubility, dissolution rate and hence oral absorption of poorly water soluble drugs\(^25,26\). Figure 1.3 shows the technique of formation of solid dispersion of drug with an inert carrier.
**Fig: 1.3** Flow diagram showing the formation of SDs

### 1.4.1 Classification of SDs\(^{23,27}\)

On the basis of release mechanisms and molecular arrangement in the matrix, SDs are distinguished into following six types as shown in Figure 1.4.

**Fig: 1.4** Categories of SDs
1.4.2 Preparation techniques of SDs\textsuperscript{28-33}

The various preparation techniques of SDs are solvent evaporation method, melting (fusion method) and kneading method, melting solvent method (melt evaporation), spray drying, melt extrusion method, lyophilization, melt agglomeration process, supercritical fluid methods, co-precipitation method and spin-coated films. Both the carrier-drug combination as well as the method of manufacture has great impact on the type of SD formed. Some methods of preparation of SDs are described as follows,

**Melting or fusion method:** In this method, a physical mixture of the drug and the carrier is heated until it is melted. The melt is then cooled, and the resultant solid dispersion is pulverized and sieved.

**Solvent method:** This method includes dissolving the drug and the carrier in a common organic solvent, followed by evaporating the solvent at elevated temperature, under vacuum, or by freeze-drying or spray-drying the mixture.

**Melting-solvent method:** In this method, the drug is dissolved in a minimum amount of an organic solvent, and then it is added to the molten carrier. The melt is then cooled, and the resultant solid dispersion is pulverized and sieved.

**Hot-melt extrusion:** In this technique, the blend of drug and carrier is processed with a twin-screw extruder of the same type used in the
polymer industry. The blend is simultaneously melted, homogenized and then extruded and shaped as tablets, granules, pellets, sheets, sticks or powder. An important advantage of the hot melt extrusion method is that the drug-carrier mixture is only subjected to elevated temperatures for a few minutes, which enables both the drug and the carrier to remain thermally stable.

**Supercritical fluid process:** This process includes dissolving the drug and carrier in supercritical CO\textsubscript{2} under precise conditions of temperature and pressure, followed by rapid depressurization. Supercritical CO\textsubscript{2} is nontoxic and it has the potential as an alternative for organic solvents.

1.4.3 **Mechanism of drug release from SDs**: The possible mechanism of enhanced dissolution from SDs includes one or more of the following,

**Reduction in particle size and agglomeration:** Reduction in particle size and agglomeration increases the exposed surface area of the drug. Size reduction has been classically considered to be a result of eutectic or solid solution formation. This mechanism suggests an intrinsic link between solid state structure and release. Similarly it has been suggested that the presentation of particles to the dissolution medium as physically separate entities may reduce aggregation.
**Increased solubility or dissolution rate of the drug:** Many of the carriers used may increase the solubility of the drug. Similarly, the carrier and drug may form a soluble complex, and is well established for cyclodextrins. Also, changes to the physical properties of the drug such as degree of crystallinity and polymorphic form may also be considered under this category.

**Conversion of crystalline drug into amorphous form:** Since the amorphous form is the highest energy form of a pure compound, it produces faster dissolution.

**Particles with improved wettability:** Many of the carriers used for SDs may have some wetting properties. Hence it is reasonable to suggest that improved wetting may lead to reduced agglomeration and increased surface area. Strong contribution to the improvement of drug solubility is related to the drug wettability improvement in SDs. It was noticed that even carriers without any surface activity, such as urea improved drug wettability. In addition, many of the carriers with surface activity used for SDs, such as cholic acid and bile salts, can significantly increase the wettability properties of drugs. Furthermore, carriers can influence the drug dissolution profile by direct dissolution or co-solvent effects. Recently, the inclusion of surfactants in the third generation solid dispersions reinforced the importance of this property.
1.4.4 Carriers used in preparing SDs\textsuperscript{17,23,25}

Huge numbers of different materials have been examined as potential carriers for preparing SDs. The carriers under various categories are given in Table 1.3.

Table 1.3: Various carriers used in SDs

<table>
<thead>
<tr>
<th>Nature of the carrier</th>
<th>Name of the carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars</td>
<td>dextrose, sorbitol, sucrose, fructose, maltose, galactose, xylitol, mannitol and lactose.</td>
</tr>
<tr>
<td>Acids</td>
<td>citric acid, tartaric acid and succinic acid.</td>
</tr>
<tr>
<td>Polymers</td>
<td>Polyvinyl pyrrolidone, polyethylene glycols, hydroxy propyl methyl cellulose, guar gum, xanthan gum, sodium alginate, methyl cellulose, pectin, hydroxy ethyl cellulose, hydroxy propyl cellulose, and cyclodextrins.</td>
</tr>
<tr>
<td>Insoluble or enteric polymer</td>
<td>Hydroxy propyl methylcellulose phthalate, eudragit RL, eudragit RS, eudragit L100, eudragit S100.</td>
</tr>
<tr>
<td>Surfactants</td>
<td>Polyethylene stearate, deoxycholic acid, poloxamer 188, tweens and spans.</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Nicotinic acid, succinamide, gelatin, polyvinyl alcohol, urea, skimmed milk, penta erythritol, penta erythyl tetracetate, urea, urethane, hydroxy alkyl xanthines etc.</td>
</tr>
</tbody>
</table>
1.4.5 Natural polysaccharides

The natural polymers i.e., the polysaccharides, from algal origin (e.g. alginates), plant origin (e.g. pectin, guar gum) microbial origin (e.g. dextran, xanthan gum) and animal origin (chitosan, chondroitin) are now extensively used for the development of pharmaceutical dosage forms. Polysaccharides are usually nontoxic, biocompatible, and demonstrate several peculiar physicochemical properties that make them suitable for different applications in drug delivery systems\textsuperscript{34}. They can also be modified chemically and biochemically and are very stable, safe, nontoxic, hydrophilic and in addition biodegradable, which suggests their use in the design of pharmaceutical dosage forms.

**Dextrans (DEX)**

Dextrans (DEX) are a class of polysaccharides and is an $\alpha$-D-1,6-glucose-linked glucan with side-chains 1-3 linked to the backbone units of the dextran biopolymer. The degree of branching is approximately 5%. The $\alpha$-(1,6)-linkage is a common feature of dextran which imparts high water solubility to the molecules. These $\alpha$-D-glucans also possess side chains stemming from $\alpha$-(1,2), $\alpha$-(1,3), or $\alpha$-(1,4) branch linkages\textsuperscript{35}. Dextrans are synthesized naturally from fermentation of sucrose by dextransucrase enzymes, secreted from various strains of *Leuconostoc mesenteroides* B512F, *Streptococcus* and *Lactobacillus* species\textsuperscript{36,37}. These bacteria growing in sugar juices
produce dextran. Besides, dextrans can be synthesised from maltodextrins by dextran dextrinase activity of certain *Gluconobacter* strains and the chemical synthesis of an essentially unbranched dextran has also been reported\textsuperscript{38}. Dextrans of pharmaceutical interest are derived from *Leuconostoc mesenteroides* NRRL B-152. The product of microbiological synthesis is called as ‘native dextran’, having molecular weight ranging between $10^7$ to $10^8$. Dextran fractions obtained from the enzymatic hydrolysis of native dextrans are supplied in molecular weights from 1000 to 2000000 daltons.

The dextran can be used as a promising model carrier for a wide variety of therapeutic agents due to its excellent physicochemical characteristics such as high aqueous solubility with low toxicity and availability in a wide range of molecular masses\textsuperscript{39}. Dextran fulfils many of the ideal characteristics such as it is nontoxic, non-immunogenic and non-antigenic. In addition dextran polymers are biodegradable and stable under mild acidic and basic conditions. They can be autoclaved and also stable to chemical manipulations.

Naessens M and his co-authors\textsuperscript{40} reviewed various chemical and physical properties of different dextrans, together with the characteristics and molecular mode of action of dextranucrase. Also valuable applications of dextran and some problems associated with undesirable formation of dextran are outlined. Dhaneshwar SS and his group\textsuperscript{41} reviewed various features of dextran carriers like its
source, structural and physicochemical characteristics, pharmacokinetic fate and its applications as macromolecular carrier.

Since last three decades, dextran and its derivatives have been investigated extensively for delivery of various drugs, proteins and imaging agents\textsuperscript{42}. Recently Gil EC and his research group\textsuperscript{43} suggested that, fractions of dextran with molecular weight between 43000 and 170000 could be more suitable for immediate release.

1.4.6 Methods of characterization of SDs\textsuperscript{12}

The SDs can be studied and characterized in two ways as follows,

**In solution state:** Solubility studies, dissolution studies, UV-spectral studies, and \textsuperscript{1}H NMR studies.

**In solid state:** Microscopic methods including polarization microscopy and scanning electron microscopy (SEM), thermoanalytical methods which includes differential scanning calorimetry (DSC) and hot stage microscopy, thermogravimetric analysis (TGA), spectroscopic methods i.e., Fourier Transform Infrared Spectrometry (FT-IR) and powder X-ray diffractometry (PXRD).
1.5 INCLUSION COMPLEXES

Complexation is a result of association between two or more molecules to form a nonbonded entity with a well defined stoichiometry. Inclusion complexation is a term used to describe the specific nonbonded interaction of a drug and a ligand. Inclusion complexation with cyclodextrin is like a “host-guest interaction”. In this interaction ‘cyclodextrin’ act as host molecule and the ‘drug molecule’ to be entrapped in host cavity act as guest molecule.

Cyclodextrins (CDs)

Cyclodextrins (CDs) are a group of structurally related natural products formed during bacterial digestion of cellulose and belongs to a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. The chemical structure and toroidal or cone shapes of cyclodextrin are shown in Figure 1.5. Their molecules are relatively large with a number of hydrogen donors and acceptors and, thus, in general they do not permeate lipophilic membranes. Due to their molecular structure and shape, CDs have a unique ability to act as molecular containers by entrapping guest molecules in their internal cavity. Hence, they are widely used as “molecular cages” in the pharmaceutical industry, as complexing agents to increase the aqueous solubility of poorly soluble drugs and to increase their bioavailability and stability.
CDs are cyclic \( \alpha \)-(1,4)-linked oligomers of D-glucopyranose. The three naturally occurring CDs are \( \alpha \), \( \beta \) and \( \gamma \) types containing 6, 7 and 8 D-glucopyranonsyl units respectively differing in their ring size and solubility. The physical and molecular properties of \( \alpha \), \( \beta \) and \( \gamma \) CD are summarized in Table 1.4. The hydrophobic and hydrophilic regions of CDs are shown in Figure 1.6. The safety, complexation efficiency, cost, and acceptance in pharmacopoeias are some important factors to be considered in selecting a CD for drug complexation. Hydrophilic CDs are considered as nontoxic at low to moderate oral dosages\(^{45} \). In addition complexation is determined by the CDs inner cavity and by the appropriate size of the guest molecule. Only those guest molecules with suitable shape and size can be incorporated into the CDs inner cavity to form inclusion complexes. \( \beta \)-CD is the most widely used in research and manufacturing due to its cost and cavity size for most drug molecules.
Table 1.4: Molecular properties of α-, β- and γ-CD\textsuperscript{46,47}

<table>
<thead>
<tr>
<th>Characteristics of CD</th>
<th>Type of CD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α</td>
</tr>
<tr>
<td>Number of glucose units</td>
<td>6</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>972</td>
</tr>
<tr>
<td>Solubility (g/100 ml at 25°C)</td>
<td>129.5</td>
</tr>
<tr>
<td>pKa</td>
<td>12.33</td>
</tr>
<tr>
<td>Outer diameter (nm)</td>
<td>1.52</td>
</tr>
<tr>
<td>Inner diameter (nm)</td>
<td>0.45-0.57</td>
</tr>
<tr>
<td>Cavity height (nm)</td>
<td>0.79</td>
</tr>
<tr>
<td>Cavity volume (nm\textsuperscript{3})</td>
<td>174</td>
</tr>
</tbody>
</table>

Fig: 1.6 Hydrophilic and hydrophobic regions of the CD molecules (Courtesy)\textsuperscript{48}
1.5.1 Method of preparation of cyclodextrin inclusion complexes

Many techniques are used to form CD complexes, like milling/co-grinding\(^{49}\), co-evaporation\(^{50}\), neutralization method\(^{51}\), kneading method\(^{52,53}\), co-precipitation\(^{54}\), atomization (spray drying)\(^{55}\), lyophilisation (freeze drying)\(^{56}\), preparation in suspensions\(^{57}\), melting method\(^{58}\), supercritical fluid crystallization\(^{59,60}\), microwave irradiation\(^{61}\), and solvent evaporation method\(^{62}\). Some methods among them are described as follows,

**Milling/Co-grinding technique**: Cyclodextrin inclusion complexes can be prepared by grinding or milling the guest molecule with CD with the help of mechanical devices such as ball mill.

**Co-evaporated dispersion**: The drug is dissolved in ethanol and CD is either dissolved in alcoholic solution or dissolved separately in water or other suitable liquid medium. The CD solution is then added to the drug solution or vice-versa and stirred to attain equilibrium. The resulting solution is evaporated to dryness preferably under vacuum.

**Neutralization method**: Martin and Udupa\(^{51}\) reported this method for various fluoroquinolones. In this method equimolar concentration of drug and CD are separately dissolved in 0.1N NaOH, mixed and stirred for about half an hour, pH is recorded and 0.1N HCl is added dropwise with stirring until pH reaches 7.5, where upon complex
precipitates. The residue is filtered and then washed until it is free from Cl\(^-\). It is dried at 25\(^\circ\)C for 24 h and stored in a dessicator.

**Kneading method:** In this method CD is kneaded like a paste, with small amount of water or compatible solvent to which the drug component has been added. Drug molecule can be added without a solvent or in small amount of ethanol in which drug has been suspended. Several hours of grinding of paste in mortar, results in evaporation of solvent and formation of powder like complex.

**Co-precipitation method:** In this method the drug and CD are dispersed in water and the solution is heated to obtain concentrated, viscous and translucent liquid. The solution is left to give a precipitate of inclusion complex.

**Spray drying technique:** In this method first a monophasic solution of drug and CD is prepared using a suitable solvent (generally hydroalcoholic solutions are used). The solution is then stirred to attain equilibrium following which the solvent is removed by spray drying.

**Freeze drying technique:** This method is similar to spray drying method except that in this method, after attaining the equilibrium, the solvent is removed by freeze drying.

**Preparation in suspension:** This method involves simple stirring of drug in aqueous suspension of CD which can achieve complexation
within 2-24 h at ambient temperature. This is a recommended method for industrial application.

**Melting method:** Complexes can be prepared by simply melting the guest, mixed with finely powdered CD. In such cases, there has to be large excess of drug and after cooling this excess is removed by careful washing with a weak complex forming solvent or by vacuum sublimation.

**1.5.2 Mechanism of drug release from CD complexes**

Inclusion complexation with cyclodextrin is like a “host-guest interaction”. Cyclodextrin act as host molecule and the ‘drug molecule’ to be entrapped in host cavity act as guest molecule. The internal cavity, hydrophobic in nature, is a key feature of the CDs providing the ability to form complexes, which include a variety of guest molecules. CD inclusion is a stoichiometric molecular phenomenon in which usually only one drug molecule interacts with the cavity of the CD molecule to become entrapped. Inclusion complex formation can be regarded as ‘encapsulation’ of the drug molecule, or at least the labile part of the molecule. The physicochemical properties of free CD molecule differ from those in complex.

Complexation of the drug (D) to CD occurs through a non-covalent interaction between the molecule and the CD cavity. No covalent bonds are formed or broken during the drug/cyclodextrin complex formation. The driving forces leading to the inclusion complex
formation comprise release of enthalpy rich water molecules from the cavity, electrostatic interaction, van der Waals interaction, hydrophobic interaction, hydrogen bonding release of conformational strain, and charge-transfer interaction. This is a dynamic process whereby the drug molecule continuously associates and dissociates from the host CD. The strength of interaction between a drug and a CD is described by the fundamental property known as binding constant (or stability constant) K. The magnitude of the binding constant, K in M\(^{-1}\), between most pharmaceutical agents and CDs ranges from 0 to 100,000. Where, 0 being the value for a drug that is incapable of forming an inclusion complex and 100,000 M\(^{-1}\) being near the upper value observed experimentally for cyclodextrin complexes of drugs.

![Diagram of equilibrium binding of drug and CD to form a 1:1 complex](image)

**Fig: 1.7** Illustration of equilibrium binding of drug and CD to form a 1:1 complex (Courtesy)\(^{66}\)

The most common type of cyclodextrin complexes is the 1:1 drug/cyclodextrin (D/CD) complex where one drug molecule (D) forms a complex with one cyclodextrin (CD) molecule as shown in Figure
1.7. In a given aqueous complexation medium, saturated with the drug, the concentration of free drug (D) is constant and equal to the apparent intrinsic solubility of the drug in the aqueous medium (i.e., drug solubility in absence of CD). CDs show a remarkable ability to form inclusion complexes with various molecules that fit partially or entirely inside the cavity. CD encapsulation of a drug will change the drugs physicochemical properties, such as its aqueous solubility and chemical stability. The CD molecule forms a hydrophilic shield around applicable lipophilic moiety of the drug molecule. This will increase the apparent aqueous solubility of the drug. Decrease of drug crystallinity on complexation or solid dispersion with CDs also contributes to the CD increased apparent drug solubility and dissolution rate. CDs can increase drug dissolution even when there is no complexation.

1.5.3 Study of cyclodextrin complexation

The most widely used approach to study inclusion complexation is the phase solubility method described by Higuchi and Connors which examines the effect of a solubilizer (CD or ligand) on the drug being solubilized (the substrate). Phase solubility diagrams are characterized into ‘A’ and ‘B’ types as shown in Figure 1.8.

‘A’ type curves demonstrate the formation of soluble inclusion complexes while ‘B’ type suggests the formation of inclusion complexes with poor solubility. Bs type denotes the complexes of
limited solubility and a $B_i$ curve indicates insoluble complexes. Further, A-type curves are subdivided into $A_L$ (linear increases of drug solubility as a function of CD concentration), $A_P$ (positively deviating isotherms), and $A_N$ (negatively deviating isotherms) subtypes. βCD normally gives rise to B-type curves due to their poor water solubility whereas, the chemically modified CDs like HPβCD and SBE-βCD usually produce soluble complexes and thus give A-type systems.

![Graphical representation of phase solubility profiles](image)

**Fig: 1.8** Graphical representations of A and B-type phase solubility profiles with applicable subtypes ($A_P$, $A_L$, $A_N$ and $B_S$, $B_i$)

Most of the drug-cyclodextrin complexes are thought to be inclusion complexes, but cyclodextrins are also known to form non-inclusion complexes and the complex aggregates are capable of dissolving drugs through micelle-like structures\(^6^8\). The phase-solubility profiles only describe how the increasing cyclodextrin
concentration influences the drug solubility. In the case of a 1:1 complex, using the following equation one can determine the equilibrium binding or stability constant ‘K’, from the slope of the linear portion of the curve.

\[ K_{1:1} = \frac{\text{Slope}}{S_o(1\text{-Slope})} \]

Where, So is the intrinsic solubility of the drug studied under the conditions.

1.5.4 Factors influencing inclusion complex formation

Type of CD: Type of CD can influence the formation in addition to the performance of drug:CD complexes. For complexation, the cavity size of CD should be appropriate to accommodate a drug molecule of particular size. Compared to neutral CDs, complexation can be enhanced when the CD and the drug carry opposite charge but may decrease when they carry same charge.

Temperature: Temperature can affect drug/CD complexation. In most cases increasing the temperature decreased the magnitude of the apparent stability constant of drug/cyclodextrin complex and the effect was reported to be a result of possible reduction of drug:CD interaction forces, such as van der Waals and hydrophobic forces. However, temperature changes may have negligible effect when the drug:CD interaction is predominantly entropy driven i.e., resulting
from the release of water molecules hydrated around the charges of
guest and host molecules through inclusion complexation.

**Methods of preparation of inclusion complex:** Co-grinding,
kneading, solid dispersion, solvent evaporation, co-precipitation, spray
drying or freeze drying etc., can affect drug:CD complexation. The
effectiveness of a method depends on the nature of the drug and type
of cyclodextrin used. In many cases, spray drying\textsuperscript{71}, and freeze-
drying\textsuperscript{72} were found to be most effective methods for drug
complexation.

**Ion pairing agents:** When ion pairing agents added in small amounts,
enhance CD solubilizing effect by increasing the apparent complex
stability constant. The ion pairing agents due to their direct
participation in drug complexation improve both pharmaceutical and
biological properties of drug:CD complexes, independent of drugs
physiochemical properties\textsuperscript{73}.

**Additives:** Certain additives may compete with drug molecules for CD
cavities and thus decrease the apparent complex stability constant,
e.g. additives with positive and negative effect\textsuperscript{74}.

**Solvents:** Organic solvents typically tend to reduce the complexation
of the drug in the CD by competing for the hydrophobic cavity\textsuperscript{75}.
**Co-solubilizers:** Water soluble polymers, in low concentrations, have recently been shown to increase the complexing abilities of CDs and enhance the availability of drugs in aqueous CD solution\(^7^6\).

**1.5.5 CDs and biopharmaceutics classification system of drugs**

The solubility and permeability behaviour of drug molecules are the most important parameters which are extensively studied due to the significant impact on drug absorption. FDA and other drug regulatory organizations have defined a ‘BCS’ in which drugs are sorted into four classes based on their solubility and permeability\(^7^7\) as shown in Table 1.5.

**Table 1.5:** The biopharmaceutics classification system (BCS)

<table>
<thead>
<tr>
<th>Class I</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly soluble</td>
<td>Poorly soluble</td>
</tr>
<tr>
<td>Highly permeable</td>
<td>Highly permeable</td>
</tr>
<tr>
<td><strong>Class III</strong></td>
<td><strong>Class IV</strong></td>
</tr>
<tr>
<td>Highly soluble</td>
<td>Poorly soluble</td>
</tr>
<tr>
<td>Poorly permeable</td>
<td>Poorly permeable</td>
</tr>
</tbody>
</table>

Numerous drug candidates with great potency belong to either Class II or Class III with significant difficulties in low solubility or permeability. Class II consists of water-insoluble drugs that easily
permeate lipophilic biologic membranes once they are in solution, displaying dissolution-limited drug absorption after oral administration (low \( C_{Aq} \) and high \( P \)). Thus, low \( C_S \) hampers their dissolution. The drug permeation through the aqueous diffusion layer adjacent to the mucosal surface will also be slow as a result of low \( C_S \). Water-soluble cyclodextrin complexes of these drugs will enhance their apparent \( C_S \) value, enhance their diffusion to the mucosal surface and increase their \( C_{Aq} \) value, leading to enhanced oral bioavailability. CDs can increase the aqueous solubility of lipophilic drugs without changing their intrinsic ability to permeate biological membranes\(^{78} \). Thus, through CD complexation it is possible to move Class II drugs, and sometimes even Class IV drugs, into Class I.

1.5.6 Applications of CDs\(^{79-81} \)

The formation of inclusion complexes provides numerous advantages in pharmaceutical formulation development as shown in Figure 1.9. One major application of drug complexation with cyclodextrin is to enhance the apparent water solubility and permeability of insoluble hydrophobic drugs by increasing the amount of dissolved drug in bio-liquid and biological membranes, leading to the increase of bioavailability.

Because of multi-functional characteristics and bioadaptability, cyclodextrin are used in many drug delivery systems such as Oral Immediate release, Delayed release (pH-dependent release), Prolonged
release and Modified release, Site-specific release (Colon-targeting, Brain targeting)], Sublingual, Parenteral, Ophthalmic, Nasal, Transdermal, Rectal, Pulmonary, Peptide and Protein Delivery, Gene and Oligonucleotide Delivery, and Novel drug delivery (Liposomes, Microspheres, Microcapsules, Nanoparticles)

**Fig: 1.9** Schematic representations of applications of CD in pharmaceutical industries for improving the drug performance in formulations

- **Enhance bioavailability**
  - Increase solubility and dissolution rate
  - Avoid organic solvents

- **Reduce irritation**
  - Gastrointestinal
  - Dermal
  - Ocular

- **Simplify handling**
  - Reduce volatility
  - Convert oils/liquids to powders

- **Implements patience compliance**
  - Reduce unpleasant Odours
  - Taste masking

- **Stabilize activities**
  - Light, UV radiation
  - Temperature
  - Oxidation
  - Hydrolysis

- **Prevent ingredient interactions**
  - Drug-drug
  - Drug-additive
1.5.7 Choice of an ideal drug candidate for inclusion complexation\textsuperscript{82,83} 

Drug molecule to be complexed with CD should have certain ideal characteristics as given below,

- It should contain more than five atoms (C, P, S, and N) which form the skeleton of the drug molecule.
- Melting point temperature of the drug should be below 250°.
- Solubility of drug in water should be less than 10 mg/ml.
- The drug molecule should consist of less than five condensed rings.
- Molecular weight of drug should be between 100 and 400.

1.5.8 Methods for characterization of inclusion complexes\textsuperscript{84} 

The inclusion complexes can be studied and characterized in two ways as follows,

**In solid state:** The inclusion complexes can be studied and characterized by Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Differential scanning calorimetry (DSC), Thermogravimetric Analysis (TGA), Fourier transform infrared spectrometry (FTIR), Powder X-ray diffractometry (PXRD).

**In solution state:** Solubility studies, Dissolution studies, UV-spectral studies, \textsuperscript{1}H NMR studies and Thin Layer Chromatography (TLC).