Review Article CODEN: IJPRNK Impact Factor: 5.567 ISSN: 2277-8713
Mohit Vij, IJPRBS, 2016; Volume 5(3): 236-265 IJPRBS

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

# CURRENT AND FUTURE TRENDS OF CYCLODEXTRIN COMPLEXATION; A SUPERIOR TECHNIQUE FOR IMPROVING ORAL BIOAVAILABILITY OF POORLY SOLUBLE DRUGS

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**Accepted Date: 24/06/2016; Published Date: 27/06/2016**

**Abstract:** Aqueous solubility is one of the most influencing and primary factor when it comes to bioavailability of the drugs. Recently 40% of the drugs are poorly water soluble which limit formulation approaches, therapeutics effect and marketability due to their low dissolution and bioavailability. The aim of present study is to investigate towards improving solubilization and bioavailability of poorly soluble drugs by numerous strategies like physical, chemical and other modifications. Out of various techniques, complexation offers numerous routes to improve solubility and dissolution rate and complexation with cyclodextrin has been considered one of the major advancements in overcoming these issues with several successfully marketed products. This article covers the concept of cyclodextrin complexation with insoluble drugs and mechanism of drug release from this complex. Also considered within this review is the critical aspects and recent advances in preparation and characterization of cyclodextrin complexation as well as future visions and strategies for the solubilization of poorly water-soluble drugs with the help of cyclodextrins.

**Keywords:** Aqueous Solubility, Solubility enhancement, Cyclodextrins (CDs), Cyclodextrin complexation, Mechanism of drug release, Bioavailability.

*PAPER-QR CODE*

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Mohit Vij, IJPRBS, 2016; Volume 5(3): 236-265

## 1. INTRODUCTION

The poor aqueous solubility and dissolution rate of API is one of the biggest challenges in pharmaceutical development and is becoming more common among new drug candidates over the past two decades due to the use of high throughput and combinatorial screening tools during the drug discovery and selection phase (Lipinski et al., 2012; Gribbon et al., 2005). The biopharmaceutical classification system (BCS) categorizes oral medications into four groups on the basis of their solubility and permeability characteristics (Table 1). According to the Biopharmaceutics Classification System (BCS), a drug compound is poorly soluble if the highest dose strength is not soluble in 250 ml aqueous media over the pH ranges at 37°C (FDA, 2000). These compounds mostly belong to Class II, which are poorly soluble and highly permeable according to the pH of the gastrointestinal fluid and tend to present solubility or dissolution rate-limited absorption ( Amidon et al., 1995). Despite their high permeability, these drugs often have low oral bioavailability because of their slow and limited release of drug in gastrointestinal fluid. Therefore, one of the major challenges of the pharmaceutical industry is to apply strategies that improve the dissolution or apparent solubility of poorly soluble drugs to develop such problematic compounds into orally bioavailable and therapeutic effective drugs (Hart et al., 2013; Kawabata et al., 2011).

| BCS CLASS | SOLUBILITY | PERMEABILITY |
|---|---|---|
| **I** | HIGH | HIGH |
| **II** | LOW | HIGH |
| **III** | HIGH | LOW |
| **IV** | LOW | LOW |

**Table 1: Biopharmaceutics Classification System (BCS)**

## 2. TECHNIQUES OF SOLUBILITY AND BIOAVAILABILITY ENHANCEMENT

There are various approaches which can be used to overcome the poor aqueous solubility of drug candidates. Some of the approaches to improve the solubility are (Fig.1)

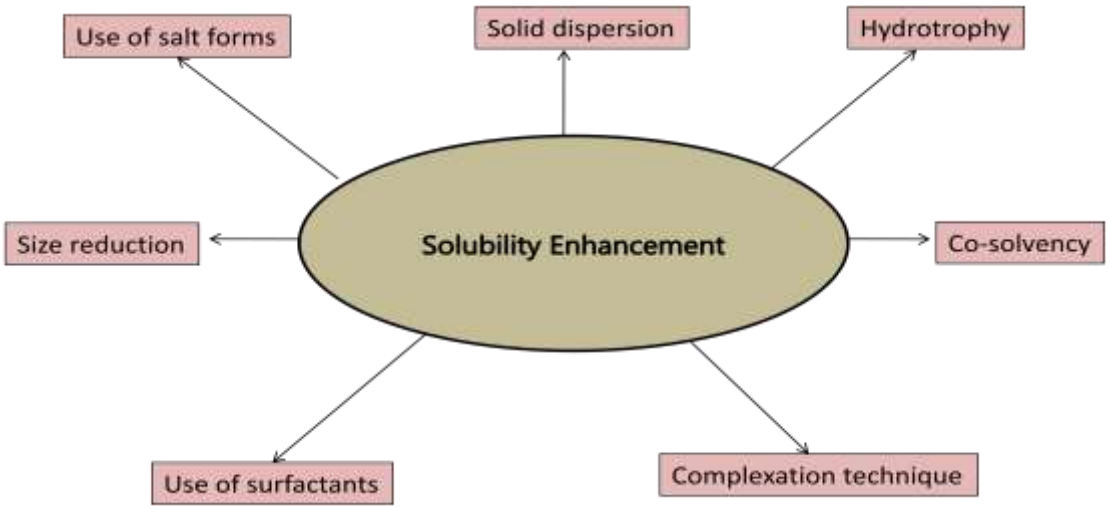


**Fig.1. Techniques of Solubility Enhancement**

**2.1 Size Reduction:**

The bioavailibility of low solubility drugs is often intrinsically related to drug particle size. By reducing particle size, the increased surface area may improve the dissoluition properties of the drug to allow a wider range of formulation approaches and delivery techniques (Adam et al., 2000).Recrystallization of poorly soluble materials using liquid solvents and antisolvents has also employed successfully to reduce particle size. The reliance upon organic solvents during processing often involves solvent extraction and handling procedures that may significantly increase complexity of manufacture. An increase in solubility at constant temperature and pressure can occur by increasing the free energy of the solid by physical means such as size reduction .

**2.2 Solid Dispersions:**

Solid dispersions represent a useful pharmaceutical technique for increasing the dissolution, absorption and therapeutic efficacy of drugs in dosage forms. The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The most commonly used hydrophilic carriers for solid dispersions include polyvinylpyrrolidone, polyethylene glycols, Plasdone-S630. Many times surfactants may also used in the formation of solid dispersion. Surfactants like Tween-80, Docusate sodium, Myrj-52, Pluronic-F68 and Sodium Lauryl Sulphate used. The solubility of few drugs can be improved by solid dispersion using suitable hydrophilic carriers. Griseofulvin dispersed in Polyethylene glycol 4000 and 6000 has shown to have a marked increase in dissolution rate. A solid dispersion of griseofulvin and polyethylene glycol 8000 (Gris-PEG®) is commercially available. Carbamazepine dispersed in PEG 6000 was also shown to produce a

faster dissolution rate (Doshi et al., 1997). Solid dispersion technique, which reduces the drug particle size and changes the microenvironment of drug particles, increases the rate of dissolution and absorption and thus changes the biopharmaceutical properties of poorly soluble drug.

There are several disadvantages and advantages to the use of solid dispersions.Limited commercial utilization of the method arises from major disadvantages related to physical and chemical instability of drug and vehicle, such as; changes in crystalline states and reduction in drug dissolution on aging. Moisture and temperature have more of a deteriorating effect on the solid dispersions than on the physical mixtures. Sticking of solid dispersions during processing may lead to problems. Two advantages are the chemicals needed for solid dispersions are already widely used in the pharmaceutical industry, so no extra toxicity studies are needed. Also, these approaches have a greater percentage than others of success in increasing solubility and the release rate. Solid dispersions are not commonly used because of the manufacturing, stability, and scale-up issues (Serajuddin et al., 1999).

**2.3 Surfactants:**

Surfactants aid in wetting of the particles by adsorbing onto the surfaces of hydrophobic drug particles and consequently increasing solubility of particulate agglomerates. Both facilitation of wetting through lowering of surface tension and solubility increase will aid dissolution of drug. There is also the possibility of solid solution formation at the crystal interface between drug and the surfactant. Surfactants like Spans, Polyglycolized glyceride, Tweens, Polyoxyethylene stearates and synthetic block copolymers like Poly (propylene oxide)-poly (ethylene oxide)- poly (propylene oxide) etc are very successful as excipient and carrier for dissolution enhancement. Seedhar et al. (2009) studied solubility improvement of enrofloxacine using a series of co-solvents and surfactants with solubility increase up to 26 times. The limiting factors in the use of surfactants as effective formulation aids may be mainly due to effectiveness of surfactants only below critical micellar concentration, adverse effects of the surfactants on the body, the concomitant solubilization of other ingredients such as preservatives, flavoring and coloring matter in the formulation, which may hamper the stability and effectiveness of formulations.

**2.4 Salt Formation:**

The use of salt forms is a well known technique to enhanced dissolution profiles. Salt formation is the most common and effective method of increasing solubility and dissolution rates of acidic and basic drugs like novobiocin and tolbutamide were observed lower absorption compared to their respective sodium salts . An alkaloid base is, generally, slightly soluble in water, but if the pH of medium is reduced by addition of acid, and the solubility of the base is increased as the pH continues to be reduced. The reason for this increase in solubility is that the base is

converted to a salt, which is relatively soluble in water. The solubility of slightly soluble acid increased as the pH is increased by addition of alkali, the reason being that a salt is formed (Nelson et al., 1958). The salt formation for neutral compounds is practically not feasible nor can appropriate salt forms be synthesized easily. Moreover sometime an increased dissolution rate may not be achieved in the GIT due to reconversion of salts into aggregates of their respective acids and base forms.

**2.5 Co-solvency**

Co-solvents are usually mixtures of miscible organic solvents such as ethanol, propylene glycol, PEG etc in aqueous vehicles routinely used as aids to the solubilisation of drugs. It is well-known that the addition of an organic co solvent to water can dramatically change the solubility of drugs. Most co solvents have hydrogen bond donor and/or acceptor groups as well as small hydrocarbon regions. Their hydrophilic hydrogen bonding groups ensure water miscibility, while their hydrophobic hydrocarbon regions interfere with waters hydrogen bonding network, reducing the overall intermolecular attraction of water. By disrupting waters self-association, co solvents reduce waters ability to squeeze out non-polar, hydrophobic compounds, thus increasing solubility (Vemula et al., 2010). Another advantage to co-solvency method is that high concentrations of the compound can be dissolved compared to other methods. The co-solvent method may be paired with other solubilization techniques such as pH-adjustment to increase solubility. The largest disadvantage of co-solvency is the toxicity of most water miscible solvents which includes its general toxicity, target organ toxicity, tissue irritation, or tonicity with respect to biological membranes that may limit or eliminate its use in drug formulations

**2.6 Hydrotrophy**

Hydrotrophy can be defined as the ability of a compound (hydrotropes) to increase the aqueous solubility of another a sparingly soluble compound. Basically hydrotrophes are ionic organic salts such as sodium benzoate, urea, sodium salicylate, nicotinamide, sodium acetate and sodium citrate that are increase solubility of poorly soluble drugs. This method does not require chemical modification, preparation of emulsion system, or use of organic solvents, which are the advantages of this method ( Vemula et al., 2010 ; Jain et al., 2010). In hydrotrophy process, a large amount of a hydrophobic drug is added to hydrotrophic agent in order to make the hydrophobic drug more soluble. The interactions between the hydrotropic agent and the drug are weak. Disadvantages of this method are that hydrotropes may self-assemble in solution and lose the ability to enhance drug's water solubility. Some advantages of hydrotrophy are; the mixing step is done only with the drug and hydrotrope in water, has high

selectivity and does not involve emulsification. Sodium benzoate in Carbamazepine and sodium salicylate in Paracetamol are few examples of drugs where hydrotropes have been used.

**2.7 Complexation technique**

There are numerous approaches available and reported in literature to enhance the solubility of poorly water soluble drug. The techniques are chosen on the basis of certain aspects such as properties of drug under consideration, nature of excipients to be selected and nature of intended dosage form. Among these approaches salt formation, solubulization with surfactants, particle size reduction, solid dispersion, and solvent deposition technique are most frequently used. But, there are practical limitations of these techniques (Wadke et al., 1989).Among all the solubility enhancement techniques, complexation technique has been employed more precisely to improve the aqueous solubility, dissolution rate, and bioavailability of poorly water soluble drugs. So this technique is considered as one of the most successful strategies to improve the dissolution profile of poorly soluble drugs (Patil et al., 2010 ; Loftsson et al., 1999).

Inclusion complexes are formed by the insertion of the nonpolar molecule or the nonpolar region of one molecule (known as guest) into the cavity of another molecule or group of molecules (known as host). Cyclodextrins is one of the best examples of the complexing agent and are most commonly used host molecules. Cyclodextrin based inclusion complexation is an important step towards improvement of solubility of variety of compounds, whereby undesirable properties of molecules may be disguised or altered and an improvement in the molecules physicochemical properties are achieved.

Numerous studies on Drug Cyclodextrin complexation have been published and have showed many advantageous properties of this complexation in improving the solubility and dissolution rate of poorly water soluble drugs (Loftsson et al., 1996 ; Rajewski et al., 1996 ; Uekama et al., 1999). These advantages include reducing particle size possibly to molecular level, enhancing wettability and porosity,as well as changing drug crystalline state preferably into amorphous state. Another significant advantage of complexation technique is that some commonly used complexing agents such as beta cyclodextrin ,hydroxy propyl beta cyclodextrin and sulfobutyl beta cyclodextrin are less toxic compared to other solublizing agents such as surfactant and co solvents.

Despite such high active research interests and attractive advantage of complexation, the number of marketed products arising from complexation approaches is disappointingly low due to certain reasons. First of all the compound has to be able to form complexes with selected ligand and the issues like potential toxicity, regulatory and quality control related to presence of ligand may add complication and cost to the development process .Only a few commercial products have been marketed during the past half-century. Therefore, in-depth knowledge

that has been acquired on various aspects of complexation such as cycodextrins properties, preparation methods, physicochemical characterization techniques as well as the pharmaceutical mechanism of complex formation and drug release are very important to ensure the preparation of a productive and marketable inclusion Complex formulation.

The aim of this review is to provide new knowledge from recent advances on cyclodextrins complexation areas to overcome some problems and issues that limit the marketability of inclusion complex products.In this review,we will focus on cyclodextrins used for oral delivery system followed by the discussion on mechanisms of drug release from inclusion complex,preparation methods and characterization techniques for inclusion complexs. Finally, the future perspectives and the possible reasons for cyclodextrin complexation to improve the bioavailability of oral administrated drugs will be explored, with emphasis on our contribution to this field.

## 3 Cyclodextrin

### 3.1 Structure and properties

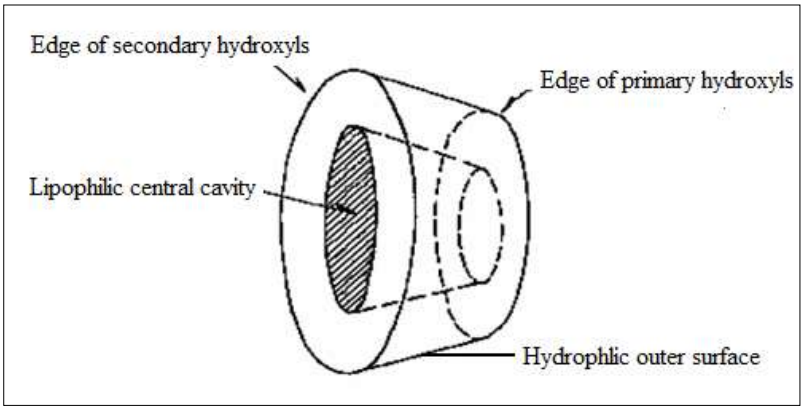


**Fig.2. Schematic drawing of the cyclodextrin molecule**

Cyclodextrins are belongs to the category of carbohydrates and are cyclic oligosaccharides.These cyclic torus-shaped molecules consist of (α-1,4-)-linked α-D-glucopyranose units with a lipophilic central cavity and a hydrophilic outer surface **(**Fig. 2). Due to the chair formation of the glucopyranose units, cyclodextrin molecules are shaped like cones with secondary hydroxy groups extending from the wider edge and the primary groups from the narrow edge. This gives cyclodextrin molecules a hydrophilic outer surface, whereas the lipophilicity of their central cavity is comparable to an aqueous ethanolic solution (Fromming et al., 1994). The most common natural cyclodextrins consist of six (α-cyclodextrin), seven (β-cyclodextrin) and eight (γ-cyclodextrin) glucopyranose units (Easton et al., 1999). Although the natural cyclodextrins and their complexes are hydrophilic, their aqueous solubility is rather

limited. This is thought to be due to relatively strong binding of the cyclodextrin molecules in the crystal state (i.e., relatively high crystal lattice energy). Random substitution of the hydroxy groups, even by hydrophobic moieties such as methoxy functions, will result in dramatic improvements in their solubility. The main reason for the solubility enhancement is that the random substitution transforms the crystalline cyclodextrins into amorphous mixtures of isomeric derivatives. Cyclodextrin derivatives of pharmaceutical interest include the hydroxypropyl derivatives of β- and γ-cyclodextrin, the randomly methylated β-cyclodextrin, sulfobutylether β-cyclodextrin, and the so-called branched cyclodextrins such as glucosyl-β-cyclodextrin. The natural α- and β-cyclodextrin, unlike γ-cyclodextrin, cannot be hydrolysed by human salivary and pancreatic amylases, but all three are subjected to fermentation by the intestinal microflora. Hydrophilic cyclodextrins are non-toxic at low to moderate oral dosages (Thompson et al., 1997). The natural cyclodextrins and their derivatives are used in topical and oral formulations, but only α-cyclodextrin and the hydrophilic derivatives of β- and γ-cyclodextrin can be used in parenteral formulations. γ-Cyclodextrin forms visible aggregates in aqueous solutions and, thus, is not well suited for parenteral formulations (Thompson et al., 1997). Due to its nephrotoxicity, β-cyclodextrin cannot be used in parenteral formulations. Presently, oral administration of methylated β-cyclodextrin is limited by its potential toxicity. Cyclodextrin monographs can be found in several Pharmacopoeias. For example, α-cyclodextrin and β-cyclodextrin are listed in the US Pharmacopeia, European Pharmacopeia and the Japanese Pharmacopeia. γ-Cyclodextrin will soon be included in the US Pharmacopeia and subsequently in the European Pharmacopeia as well. A monograph for 2-hydroxypropyl-β-cyclodextrin has recently appeared in the European Pharmacopeia. β-Cyclodextrin and γ-cyclodextrin are also listed in the 'generally regarded as safe' list of the FDA for use as food additives. More than 30 different pharmaceutical products containing cyclodextrins are now on the market worldwide. Some of orally administered products are listed in (Table 2). In the pharmaceutical industry, cyclodextrins have mainly been used as complexing agents to increase the aqueous solubility of poorly water-soluble drugs, and to increase their bioavailability and stability. In addition, cyclodextrins can be used to reduce or prevent gastrointestinal and ocular irritation, reduce or eliminate unpleasant smells or tastes, prevent drug-drug or drug-additive interactions, or to convert oils and liquid drugs into microcrystalline or amorphous powders.

| Drug | Type of cyclodextrin | Trade name | Formulation | Market |
|---|---|---|---|---|
| **Cefotiam hexetil HCl** | α-Cyclodextrin | Pansporin T | Tablet | Japan |
| **Oral prostaglandin E1 derivative** | α-Cyclodextrin | Opalmon | Tablet | Japan |
| **Benexate HCl** | β-Cyclodextrin | Ulgut, Lonmiel | Capsule | Japan |
| **Nimesulide** | β-Cyclodextrin | Nimedex, Mesulid | Tablet | Europe |
| **Omeprazol** | β-Cyclodextrin | Omebeta | Tablet | Europe |
| **Piroxicam** | β-Cyclodextrin | Brexin | Tablet | Europe |
| **Aceclofenac** | β-Cyclodextrin | Aceclofenac-B-Cyclodextrin | Tablet | India |
| **Betahistine** | β-Cyclodextrin | Betahist | Tablet | India |
| **Tiaprofenic acid** | β-Cyclodextrin | Surgamyl | Tablet | Europe |
| **Cetirizine** | β-Cyclodextrin | Zyrtec | Chewing tablet | Europe,USA |
| **Cephalosporin** | β-Cyclodextrin | Meiact | Tablet | Japan |
| **Chlordiazepoxide** | β-Cyclodextrin | Transillium | Tablet | USA |
| **Norfloxacin and Tinidazole** | β-Cyclodextrin | Entronor–TZ/ Noroxin | Tablet | India |
| **Refocoxib** | β-Cyclodextrin | Rofizgel | Tablet | India |
| **Nicotine** | β-Cyclodextrin | Nicorette | Sublingual tablets | Europe |
| **Nitroglycerin** | β-Cyclodextrin | Nitropen | Sublingual tablet | Japan |
| **Chlortheophyllin** | β-Cyclodextrin | Stada-Travel | Chewing tablet | Germany |
| **Iodine** | β-Cyclodextrin | Mena-Gargle | Solution | Japan |
| **Itraconazole** | 2-Hydroxypropyl-β-cyclodextrin | Sporanox | Tablet | Europe, USA |
| **Voriconazole** | 2-Hydroxypropyl-β-cyclodextrin | Vorzu | Tablet | India |
| **Hydrocortisone** | 2-Hydroxypropyl-β-cyclodextrin | Dexocort | Solution | Iceland |
| **Ziprazidone** | sulfobutylether β-cyclodextrin | Geodon | Capsule | USA |

**Table 2. List of few orally administrated marketed products containing cyclodextrins.**

### 3.2 Cyclodextrin complex mechanism

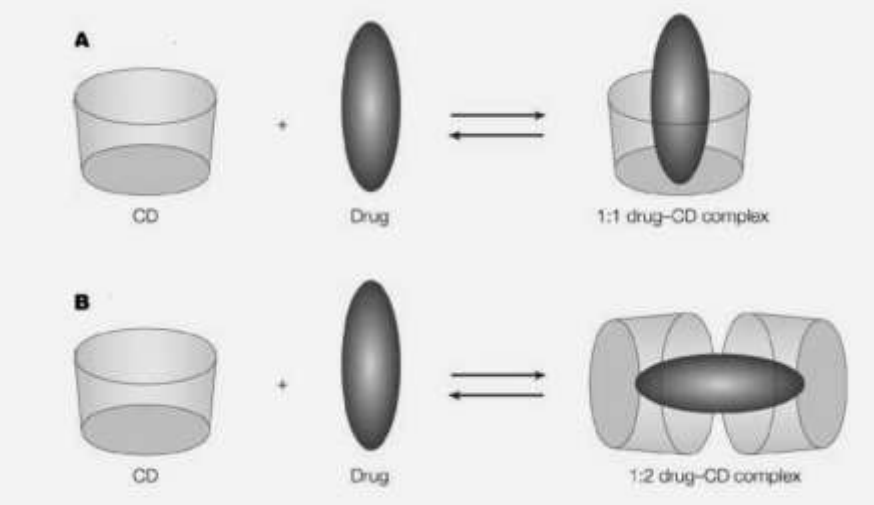


**Fig.3. Schematic overview when drug molecule forming complex with cyclodextrin molecule in 1:1 and 1:2 pattren.**

CDs are able to form dynamic molecular inclusion complexes with many drugs by incorporating the drug molecule, or more commonly a lipophilic moiety of the molecule, into the central cavity (Fig.3). No covalent bonds are formed or broken during the drug/cyclodextrin complex formation. The driving forces leading to the inclusion complex formation include release of enthalpy rich water molecules from the cavity, electrostatic interactions, van der Waals interactions, hydrophobic interactions, hydrogen bonding, release of conformational strains, and charge-transfer interaction (Dressman et al., 2001 ; Szejtli et al., 1988 ; Duchene et al., 1991). All these forces are relatively weak, allowing free drug molecules in solution to be in rapid equilibrium with drug molecules bound within the cyclodextrin cavity. The most widely used approach to study inclusion complexation (Fig. 4) is the phase solubility method described by Higuchi and Connors et al. (1965) which examines the effect of a solubilizer, i.e., CD or ligand, on the drug being solubilized, i.e, the substrate. Phase solubility diagrams are categorized into A and B types; A type curves indicate the formation of soluble inclusion complexes while B type suggest the formation of inclusion complexes with poor solubility. A $B_S$ type response denotes complexes of limited solubility and a $B_I$ curve indicates insoluble complexes. 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. β-CD often gives rise to B-type curves, whereas the chemically modified CDs like HP-β-CD and SBE-β-CD usually produce more soluble complexes and thus give A-type systems. The phase-solubility profiles only describe how the increasing cyclodextrin concentration influences the drug solubility. Most drug molecules (D) form 1:1 complexes with cyclodextrin molecules (CD) and using the following equations (equation I and II), one can

determine the equilibrium binding or stability constant, K, from the slope of the linear portion of the curve. The value of the stability constant (K 1:1) is most often between 100 to 20000$M^{-1}$ with a mean value of 129, 490 and 355 $mol^{-1}$ for α-, β-, and γ-CDs, respectively: (Stella et al., 1997 ; Connors et al., 1995 ; Connors et al., 1997 ; Rao et al., 2003).

$$D + CD \xrightleftharpoons{K_{1:1}} D/CD\ Complex \qquad (I)$$

$$K1{:}1 = [D/CD] / [D].[CD] \qquad (II)$$

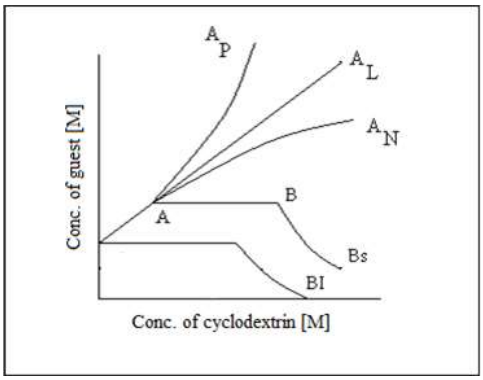


**Fig. 4. Phase solubility diagram.**

This is a somewhat oversimplified description of a much more complex mechanism (Loftsson et al., 2004), but is sufficient to explain the role of cyclodextrins in the oral drug delivery. 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 cyclodextrin). CDs encapsulation of a drug will change the drug's physicochemical properties, such as its aqueous solubility and chemical stability. The CD forms a hydrophilic shield around the applicable lipophilic moiety of the drug molecule. This will, in general, increase the apparent aqueous solubility of the drug. The CDs can also protect chemically labile drug molecules from potentially corrosive environments and, in this way, reduce or even prevent drug hydrolysis, oxidation, racemisation and enzymatic decomposition ( Loftsson et al., 1995).

### 3.3 Mechanisms of guest release from CD-complexes:

Complexation of the guest to cyclodextrin occurs through a non-covalent interaction between the molecule and the cyclodextrin (cavity). Complex formation is a dynamic process whereby the guest molecule continuously associates and dissociates from the host CD. In case of a 1:1 complex, the interaction is as follows equation III: (Astray et al., 2009).

$$G + H \underset{K_d}{\overset{K_a}{\rightleftharpoons}} GH \qquad \text{(III)}$$

Where, H is donated for cyclodextrin as a host, G is donated for drug as guest molecule, H-G is the inclusion complex, ka and kd are the assosiation constant and dissociation constant respectively. K=Ka/Kd, equilibrium constant is the important characteristic of this complexation. The larger is the guest molecule, the slower the formation and dissociation of the inclusion complex. Ionization decreases the rate of complex formation and dissociation. Dissociation due to dilution appears to be major drug release mechanism, although other factors such as competitive displacements of the drug from the complex, drug uptake by tissues, binding to protein, and ionic strength and temperature should also be considered to assess the stability and dissociation of CD-drug complex (Stella et al., 1999).

3.3.1 Dilution

Dissociation due to dilution appears to be a major release mechanism for the guest molecules from cyclodextrin complexes ( Piel et al., 1998). Guo et al. (1991) developed a novel drug dosage form of amphotericin B, a potential fungicidal agent that forms a very tight complex with sodium cholesteryl sulfate, and it does not readily dissociate after i.v. injection, although most of the CD-complexes dissociate upon dilution in the blood. In case of oral drug delivery, complexes also dissociate rapidly upon dilution in the stomach and intestinal contents and it is believed that only the drug, and not the complex, is absorbed. Dilution is minimal when a drug-CD complex is administered by ophthalmic, transmucosal, and transdermal routes. After oral administration, some dilution is likely to occur but dilution alone is probably insufficient to account for the relative good absorption of drugs as administered as CD-complexes (Tong et al., 2008).

3.3.2 Competitive displacement

Competitive displacement of drugs from cyclodextrin complexes probably plays a significant role in physiological environment. The beta-cyclodextrin complex of a poorly water-soluble drug, cinnarizine, is more soluble *in vitro* than cinnarizine alone. Oral administration of the complex showed less bioavailability, and it was suggested that cinnarizine was too strongly bound to the cyclodextrin to dissociate and this was limiting the bioavailability of cinnarizine. Co-administration of phenylalanine (displacing agent) improved the availability of cinnarizine from the complex in comparison to that from the conventional tablets of cinnarizine (Tokomura et al., 1985 ; Tokomura et al., 1986). Sometime addition of preservatives such as parabens to parenterals preparations not only leads to decreased antimicrobial activity of parabens due to complexation, but often cause displacement of the drug from complex leading to decreased

solubility of active drug. Van et al. (1996) showed that alcohols can displace 2-naphthol from β-cyclodextrin complexes.

3.3.3 Drug uptake by tissue

A potential mechanism for drug release from cyclodextrin is drug uptake by tissues. If the nature of the drug is lipophilic and has access to tissue, the tissue then acts as a sink causing dissociation of the complex based on simple mass action principles. This mechanism may become most relevant for strongly bound drugs or when the complex is administered at a site (e.g., ocular, nasal, sublingual, pulmonary, dermal or rectal) where dilution is minimal (Uekama et al., 1994 ; Jarvinen et al., 1994). In ophthalmic delivery, cyclodextrins have been used to increase the solubility and stability of poorly water soluble drugs in the tear fluids and in some cases to reduce irritation (Jarvinen et al., 1994 ; Davies et al., 1997).Due to solubility increase, complexation also results in a significant increase in drug bioavailability compared to suspensions (common ophthalmic formulations for low water soluble drugs)

3.3.4 Protein binding

As the strength of binding between drug and cyclodextrin increases instead of dilution another mechanism called drug binding to plasma proteins play a vital role in the release of drugs from their cyclodextrin complexes. Frijnik et al. (1991) studied the effect of cyclodextrin (HP-β-CD) on the displacement of flurbiprofen from plasma binding sites in vitro.In this case drug is given as HP-β-CD complex and as plasma solutions. After parenteral administration there was higher tissue levels from the cyclodextrin solutions, meaning that more drug was free to distribute to the tissues than from the plasma solution suggesting that drug from the cyclodextrin solutions were actually more readily available compared to the plasma solutions. The purpose of the Frijlink study was to show that the cyclodextrin could displace flurbiprofen from protein binding sites by competitive binding.

3.3.5 Change in ionic strength and temperature

For most molecules of the ionized or charged form has poorer binding to cyclodextrins compared to that of the non-ionized or neutral form. Okimoto et al. (1996) and Krishnamoorthy et al. (1996), demonstrated decreased complexation of molecules with HP-β-CD as the substrate becomes ionized. This decrease in stability was attributed to the overall increased hydrophilicity of the substrate upon ionization which reduces the substrates interaction with the more hydrophobic cavity of the cyclodextrin. Binding of substrates to cyclodextrins has been shown to be an exothermic process. Hence, any increase in temperature results in weakening of the complex. So most of the drug-CD complexes are usually prepared and stored at/or below room temperature. Since, normal body tissue temperatures can be as high as 37°C;

this temperature condition may be the contributing factor to drug dissociation, in physiological environment (Stella et al., 1999).

**4. Different Preparation methods and their efficiency in inclusion complex formation**

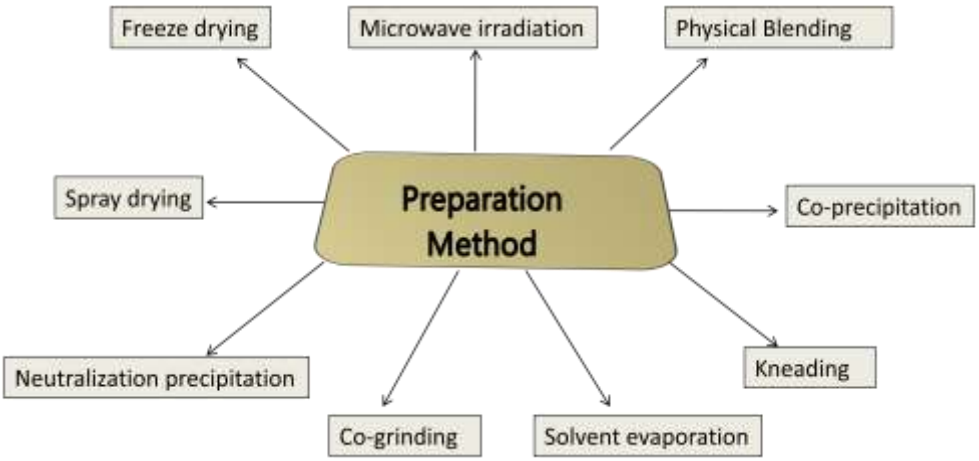


**Fig .5. Preparation methods for cyclodextrin complexation**

**4.1 Physical blending method**

A solid physical mixture of drug and CDs are prepared simply by mechanical trituration. In laboratory scale CDs and drug are mixed together thoroughly by trituration in a mortar and passes through appropriate sieve to get the desired particle size in the final product. In industry scale, the preparation of physical mixtures is based on extensive blending of the drug with CDs in a rapid mass granulator usually for 30 minutes. These powdered physical mixtures are then stored in the room at controlled temperatures and humidity conditions. This method is just one step process as well as neither water nor heat are necessary. Greice et al. (2009) suggested that physical mixture method is an adequate alternative for the preparation of quercetin/ β-cyclodextrin solid complex.With physical mixture method quercetin showed 2.2 fold increase in solubility where as with spray drying method it showed 2.5 fold increase in solubility.

**4.2 Co-precipitation**

The co-precipitation method is the most widely used method in the laboratory. Sapkal et al. (2007) have prepared inclusion complex of guest molecule of poor aqueous solubility with β-CD by co-precipitation method . CD is dissolved in water and the guest is added while stirring the CD solution. By heating, more CD can be dissolved (20%) if the guest can tolerate the higher temperature. The CD and guest solution must be cooled under stirring before a precipitate is formed. The precipitate can be collected by decanting, centrifugation or filtration and washed. Farcasa et al. (2006) successfully prepared the inclusion complex of diuretic furosemide drug

with β-cyclodextrin (β-CD) by co-precipitation method in a molar ratio of 1:1. The complex formation and its molecular/supramolecular structure have been investigated by Proton Nuclear Magnetic Resonance ($^1$HNMR) Spectroscopy, Fourier transform infrared spectroscopy (FT-IR), Differential scanning calorimetry (DSC), Powder x-ray diffraction (PXRD) and Scanning electron microscopy (SEM) measurements. The obtained complex exhibits high aqueous solubility than pure drug. Main disadvantage of this method lies in the scale-up (Shimpi et al., 2005), but the co-precipitation method yields a highly pure and crystalline inclusion complex (Miller et al., 2007).

**4.3 Kneading method**

In this method drug and CD were accurately weighed, placed in the mortar and triturated for 20 min. The mixture was then kneaded with 66% alcohol for 45 min and resulting paste was kept in vacuum desiccator overnight. The dry mass so obtained was powdered and passed through sieve no.80. In laboratory scale, kneading can be achieved by using a mortar and pestle. In large scale, kneading can be done by utilizing the extruders and other machines. This is the most common and simple method used to prepare the inclusion complexes and it presents very low cost of production. Aleem et al. (2008) concluded that the aqueous solubility and dissolution rate of CEF (cefdinir) can be significantly increased by forming an inclusion complex with CDs using kneading method.

**4.4 Solution/solvent evaporation method**

In this method organic solvents are used and therefore residual solvents need to be removed. Osadebe et al. (2008) have prepared complex of piroxicam and β-CD by solvent evaporation method. This method involves dissolving of the drug and CDs separately in to two mutually miscible solvents, mixing of both solutions to get molecular dispersion of drug and complexing agents and finally evaporating the solvent under vacuum to obtain solid powdered inclusion compound. Generally, the aqueous solution of CDs is simply added to the alcoholic solution of drugs. The resulting mixture is stirred for 24 hours and evaporated under vaccum at 45 ºC. The dried mass was pulverized and passed through a 60-mess sieve. Racecadotril - β-cyclodextrin complex was prepared by Semalty et al. (2014) by two different methods, solvent evaporation and kneading method to improve its solubility and dissolution. It was concluded that the complex prepared by the solvent evaporation method showed better solubility and the dissolution due to better amorphization of the drug. This method is quite simple and economic both on laboratory and large scale production and is considered alternative to the spray drying technique.

**4.5 Milling/Co-grinding technique**

A solid binary inclusion compounds can be prepared by grinding and milling of the drug and CDs with the help of mechanical devices. Drug and CDs are mixed intimately and the physical mixture is introduced in an oscillatory mill and grinded for suitable time. Alternatively, the ball milling process can also be utilized for preparation of the drug-CD binary system. The ball mill containing balls of varied size is operated at a specified speed for a predetermined time, and then it is unloaded, sieved through a 60-mesh sieve. This technique is superior to other approaches from economic as well as environmental stand point in that unlike similar methods it does not require any toxic organic solvents (Friedrich et al., 2005). This method differs from the physical mixture method where simple blending is sufficient and in co-grinding it requires to achieve extensive combined attrition and impact effect on powder blend. Abou-Taleb et al. (2006) studied that Co-grinding method led to enhancement of Rofecoxib (ROF) dissolution rate in comparison to the other preparation methods as Co-grinding technique form partial inclusion complex between ROF and HP-β-cyclodextrin as revealed by DSC, XRD and IR studies.

**4.6 Neutralization precipitation method**

This method is based on the precipitation of inclusion compounds by neutralization technique and consists of dissolving the drug in alkaline solutions like sodium/ammonium hydroxide and mixing with an aqueous solution of CDs. The resultant clear solution is then neutralized under agitation using hydrochloric acid solution till reaching the equivalence point. A white precipitate is being formed at this moment, corresponding to the formation of the inclusion compound. This precipitate is filtered and dried. Doijad et al. (2007) have studied the enhancement of solubility of piroxicam by complexation with beta-cyclodextrin using Neutralization precipitation. Acid and alkaline susceptible drugs can undergo degradation during this process is the limitation associated with this method. Terfenadine has relatively low bioavailability after oral administration due to its limited solubility in water. Choi et al. (2001) prepared the terfenadine-β-cyclodextrin (1:2) inclusion complex by the neutralization method to enhance the antihistaminic activity of terfenadine following the enhanced solubility and dissolution of terfenadine.

**4.7 Spray drying**

Spray drying process involves completely dissolving the cyclodextrin and drug in an aqueous solution, but solution is atomized into a drying chamber (75˚C) and collected as a dried solid material. The sufficient and efficient interaction between drug and CDs to form a perfect complex is the added advantage of atomization/spray drying method .The main disadvantages of spray drying processes are that the equipment is expensive, can occupy a considerable amount of laboratory space, and require specialized technical knowledge and low yield of the

final product.Aiman et al. (2009) sugestted that Spray drying was found most effective method to prepare inclusion complex for meloxicam and β-CD as spray dried complex showed the strongest interaction.

**4.8 Lyophilization/ Freeze drying technique**

In order to get a porous, amorphous powder with high degree of interaction between drug & CD, lyophilization/ freeze drying technique is considered as a suitable (Cao et al., 2005). In this technique, the solvent system from the solution is eliminated through a primary freezing and subsequent drying of the solution containing both drug & CD at reduced pressure. Thermolabile substances can be successfully made into complex form by this method. The limitations of this technique are long time process and yield poor flowing powdered product. Lyophilization/ freeze drying technique is considered as an alternative to solvent evaporation and involve molecular mixing of drugand carrier in a common solvent. The amorphous inclusion complexes of acetaminophen and B-cyclodextrin were successfully obtained by Suporn et al. (2004) with freeze drying method. This conclusion was supported by XRD, DSC and IR spectroscopy. Solid inclusion complexe of poorly soluble Cefpodoxime proxetil with BCD were prepared by using methods such as physical mixture, solvent evaporation and freeze drying. Prepared complexes were characterized by FTIR, DSC, PXRD and SEM. Bharvaga et al. (2008) revealed that the complex prepared by freeze drying showed the highest dissolution rate.

**4.9 Microwave irradiation method**

The methods widely utilized to prepare inclusion complexes are co-precipitation, kneading, freeze drying and co-grinding. They often involve time-consuming manufacturing processes and generally require large amounts of solvents. There is therefore a need for faster and more convenient processes. Microwave irradiation (MWI), a method recently used to prepare CD inclusion complexes, has the major advantages of shorter reaction times and higher yields of products (Zhao et al., 2003). In pharmaceutical technology, MW has been used because of its thermal effect in drying processes (granules or crystals), and for the sterilization of injections and infusions. This technique involves the microwave irradiation reaction between drug and complexing agent using a microwave oven. The drug and CD in definite molar ratio are dissolved in a mixture of water and organic solvent in a specified proportion into a round-bottom flask. The mixture is reacted for short time of about one to two minutes at 60°C in the microwave oven. After the reaction completes, adequate amount of solvent mixture is added to the above reaction mixture to remove the residual uncomplexed free drug and CD. The precipitate so obtained is separated using whatman filter paper, and dried in vacuum oven at 40°C.Inclusion complex of Cefdinir with *β*-CD and HP-*β*-CD prepared under microwave irradiation was studied by Vij et al. (2010) and Vij et al. (2011).They concluded that MWI

method can be used efficiently to prepare CEF–CD complexes with better dissolution behaviour and higher yield on industrial scale as compare to methods like kneading (KN), co-evaporation (CE), spray drying (SD).The structure of these inclusion complexes were inferred from the techniques like DSC,FTIR,SEM,XRD and NMR .

The preparation techniques for production of complex between drug and cyclodextrin is showed in (**Fig. 5)**. In fact, the optimal method and technique for complexation preparation would based on the physicochemical properties of drugs and CDs. In general, kneading, spray drying and freeze drying are most commonly used due to their high scalability and applicability.

## 5 Characterization of physicochemical properties

The dissolution enhancement of poorly water-soluble drugs in complex can be proven by the standard dissolution methods. Other properties of complex such as the physical states of drugs, the drug carrier interaction and the physical and chemical stability of drugs should also be evaluated. Consequently, many instrumental and analytical techniques are applied to measure these properties. The crystalline state of drugs and the degree of crystallinity are importantly characterized. The amount of drugs existing in amorphous state can be calculated indirectly from the extent of crystallinity in the sample. The crystalline state of drugs is commonly characterized by the following techniques: thermoanalytical techniques such as Differential Scanning Calorimetry (DSC) and Modulated Differential Scanning Calorimetry (MDSC); powder X-ray diffraction (PXRD). Other instrumental techniques such as Fourier Transformed Infrared spectroscopy (FTIR), solid state nuclear magnetic resonance, Thermal Gravimetry Analysis (TGA) are used to investigate the chemical stability and molecular interaction of the drug and cyclodextrin. Microscopy techniques such as optical microscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM) are also used to qualitatively characterize the crystalline states of drug, the molecular miscibility and surface morphology of complex.

Physicochemical characterization of inclusion complex is essential to evaluate the pharmaceutical applicability of complex and thoroughly understand the pharmaceutical mechanisms of drug dissolution enhancement and physicochemical stability. Therefore, an ongoing development of new and advanced characterization techniques in this area is very necessary.

### 5.1 Nuclear magnetic resonance (NMR)

The most direct evidence for the inclusion of a guest into a cyclodextrin cavity is NMR spectroscopy. Along with proving that a complex is formed, NMR studies may also be used to determine the direction of penetration of guest molecules into the cyclodextrin cavity (Jadhav

et al., 1983 ; Uekama et al., 1993). A shift in the peaks can be observed for both the guest and CD (Hedges et al., 1998). Proton and 13C-NMR have been used to determine the formation of inclusion complexes and to give an idea of how the guest substrate is positioned in the cyclodextrin cavity. 13C-NMR spectroscopy is utilized in determining the stoichiometry of inclusion complexes and the technique have wide applicability because it can be used on solid samples or samples dissolved in aqueous medium (Lin et al., 1991). NMR spectroscopy is applicable to calculate the stability constant. In addition to quantitative and qualitative information about complex formation, NMR can be used to probe the solution geometry of CD-based complexes as well as give kinetic information about their association and dissociation (Brewster et al., 2007).

**5.2 Powder X-ray diffraction (PXRD)**

PXRD is the most widely used method to identify and characterize the crystalline state of drugs in inclusion complex. When the guest molecules are liquid since liquid have no diffraction pattern of their own, then the diffraction pattern of a newly formed substance clearly differs from that of uncomplexed cyclodextrin. This difference of diffraction pattern indicates the complex formation. (Lee et al., 2006).When the guest compound is a solid substance, a comparison has to be made underidentical conditions between the diffractogram of the assumed complex and that of the mechanical mixture of the guest and cyclodextrin molecules . A diffraction pattern of a physical mixture is often the sum of those of each component, while the diffraction pattern of cyclodextrin complexes are apparently different from each constituent and lead to a “new” solid phase with different diffractograms .This method can detect material with long-range order as well as expose sharp diffraction peaks that indicate crystalline compound with characteristic fingerprint region. Thanks to the specificity of the fingerprint, the drug crystallinity can be separately identified from the carrier crystallinity and thus can differentiate the amorphous state and crystalline state of drugs in complexes. However, the crystallinities under 5–10% fraction may not be detected by PXRD (Uekama et al., 1982).

**5.3 Fourier Transform Infrared spectroscopy (FTIR)**

The infrared spectra of the complexes were analyzed and compared with the spectra of the pure compounds and their physical mixtures respectively. Due to complexation of the guest, shifts or changes in the spectrum occur (Hedges et al., 1998). Bratu et al. (2005) prepared the inclusion complexes of β-cyclodextrin with fenbufen and ibuprofen by the two different methods such as co-precipitation and the freeze-drying methods and they used FTIR spectroscopy to characterize the inclusion complexes. They found the fundamental changes which appear in the FTIR spectra of inclusion complexes of fenbufen and ibuprofen are mainly

in the C=O stretching region. These changes suggest drug-CD complex formation. Shifts or changes in the spectrum occur due to the complexation (Hedges et al., 1998).

**5.4 Ultraviolet-visible (UV-Vis) spectroscopy**

Spectrophotometric methods are useful to determine the value of stability constant, The complexation causes a change in the absorption spectrum of a guest molecule (Marzouqi et al., 2006). During the spectral changes, the chromophore of the guest is transferred from an aqueous medium to the non-polar cyclodextrin. These changes in the compound spectra generally reflect an alteration in the microenvironment of the drug. Hypsochromic or bathochromic shift or changes in the absorptivity without change in the λmax have been considered as evidence for interaction between cyclodextrin and the drug in the formation of the complex.Inclusion complexes shows both Conditions of an increase or decrease in the absorption intensity of UV band without change in its λmax . For example, the absorption intensity was increased when Bropiramine was complexed with β-cyclodextrin and decreased in the case of purine nucleosides when complexed with β cyclodextrin (Xiang et al., 1990).

**5.5 Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA)**

Thermal analyses (DSC and TGA) are useful for determining whether the product of the complexation protocol is true complex or not (Moriwaki et al., 2008). In DSC, The samples (10 mg) are placed in aluminum pans and the experiments run in a calorimeter at a 10 $^0$C/min heating rate over a wide range (0-450 $^0$C). An empty pan served as reference and indium is used to calibrate the temperature. Thermograms are determined for the samples: CD, guest or drug, physical mixture of guest/CD and solid complex guest-CD. DSC analysis gives supporting evidences for the complexation of guest or drug with CD. Araujo et al. (2008) worked on the development and pharmacological evaluation of ropivacaine-2-hydroxypropyl-β-cyclodxtrin inclusion complex and they studied DSC thermograms of HP-β-CD, Ropivacaine (RVC), RVC/HP-β-CD 1:1 physical mixture and RVC/HP-β-CD 1:1 complex. They reported that HP-β-CD and RVC gives a characteristic endothermic peak at 336 $^0$C and 117.6 $^0$C respectively corresponding to their melting point and HP-β-CD also gives a peak at 50 $^0$C due to loss of water molecule. Thermogram of the RVC/HP-β-CD physical mixture (1:1) shows two endothermic peaks at 246.5 $^0$C and 116.0 $^0$C whereas the solid complex of RVC/HP-β-CD presents only a broaden peak at 248.2 $^0$C. The absence of fusion peak of pure RVC at 117.6 $^0$C in the thermogram and the shift of endo or exothermic peaks of drugs is a clear indication of the complexation phenomenon.

TGA is a method of thermal analysis that measures the weight change as a function of time and temperature, thereby providing information about the stability of a material and the compatibility of different materials in a solid dispersion mixture. This method can provide useful information about the stability of drugs and carriers as well as the chemical and physical

processes in solid dispersions to decide the preparation method and the processing parameters for solid dispersion preparation. Other common applications in the pharmaceutical sciences include the determination of moisture and solvent content as well as decomposition, vaporization or sublimation temperatures (Stodghill et al., 2010).

**5.6 Scanning Electron Microscopy (SEM)**

Scanning Electron Microscopy is used to study the microscopic aspects of the raw material (cyclodextrin and the guest substances, respectively) and the product obtained by various complexation techniques (Vij et al., 2010 ; Vij et al., 2011).The difference in crystallization state of the raw material and the product seen under electron microscope indicates the formation of the inclusion complexes, if there is a clear difference in crystallization state of the raw material and the product obtained by different complexation methods then this method is adequate to affirm inclusion complex formation (Franco et al., 2009).

The particle morphology of Ketoprofen, βCD, HP-β-CD, their physical mixtures and solid complexes were evaluated by SEM photographs. Ketoprofen appeared as plate like crystals,tending to form aggregates. HP-β-CD consisted of shrunken, cylindrical particles, whereas βCD appeared as irregularly shaped crystals. The physical mixtures showed particles of HP-β-CD, βCD embedded with Ketoprofen particles and a comparable morphology with pure compounds, taken separately. In contrast, a drastic change in the morphology and change in the crystalline nature was observed in 1:1 freeze-dried, coprecipitated and kneaded products of both HP-β-CD and βCD, it was revealed that there was an apparent interaction in the solid state (Tayade et al., 2006) .

SEM analysis was performed to investigate the morphologies of pure drug and carriers and their combinations. The results showed that the typical polyhedron-shaped drug crystals of Ketoprofen were recognizable in physical mixtures but such polyhedronshaped drug crystals were no longer recognizable in the complexes of Ketoprofen with βCD and HP-β-CD prepared by co-evaporation and sealed-heating methods which demonstrated the formation of amorphous aggregates (Maestrelli et al., 2005).

**5.7 Thin Layer Chromatography (TLC)**

In Thin Layer Chromatography, the Rf values of a guest molecule diminishes to considerable extent and this helps in identifying the complex formation between guest and host molecule (Bekers et al., 1991).Inclusion complexation between guest and host molecules is a reversible process. Consequently, the complex may separate completely in guest and host molecules during the chromatographic process and only the spots of the guest and host molecules are found on the TLC-plate (Aithal et al., 1995). The Rf value of Diclofenac sodium and

Indomethacin were different from those of the complex formed using βCD on concurrent crystallization from water/organic solvent system (Aithal et al., 1995).

Physicochemical characterization of inclusion complexes is essential to evaluate the pharmaceutical applicability of complexes and thoroughly understand the pharmaceutical mechanisms of drug dissolution enhancement and physicochemical stability. Therefore, an ongoing development of new and advanced characterization techniques in this area is very necessary.

## 6 Future perspective and strategies

Out of various techniques inclusion complexation have generated much interest from pharmaceutical scientists because of the increasing number of drug candidates which is poorly water soluble and the recent advances on this area. Although inclusion complex have been investigated for such a long time, some novel carriers, additives and new preparation methods, characterization techniques have just been applied in recent years. This brings new aspiration to scientists to develop more such products in the future.

Several studies have introduced new carriers while many other studies utilize more than two polymers as carriers to combine the advantages of each polymer. Some novel carriers have been used with binary inclusion complexes in recent years like soluplus,lecithin and polyvinylpyrrolidone . Thomas et al. (2013) investigated the impact of third polymer soluplus on the solubility of binary inclusion complex of itraconazole with cyclodextrin.It was concluded that the bioavailability of itraconazole is likely to be increased after oral administration of ternary complex formulations as compared to binary inclusion complexes. Dan wang et al. (2013) found that the soybean lecithin was found to be the most promising third component in terms of solubility enhancement of Dihydroartemisinin. The dissolution rate of the solid ternary system was much faster than that of binary system of drug with HP-β-CD .

Paola et al. (2001) studied combined effect of hydroxypropyl-β-cyclodextrin (HP-β-CD) and polyvinylpyrrolidone (PVP) polymer on the solubility of drug naproxen (NAP) by formation of a NAP–HP-β-CD–PVP ternary complex. As compared to NAP–HP-β-CD binary systems ,the combined use of PVP and HP-β-CD resulted in a synergistic increasing effect on the aqueous solubility of naproxen which was 120 times that of the pure drug.

Laura et al. (2003) suggested a significant improvement on the complexation efficiency between drug vinpocetine and CDs by addition of small amounts of water-soluble polymers like PVP and HPMC.

Trichard et al. (2007) investigated the potential of novel lipid-carrier “beads” consisting of minispheres made of alpha cyclodextrin and soybean oil for the encapsulation and the oral

delivery of drugs. These beads constitute a novel and efficient system for encapsulation and oral delivery of Isotretinoin, which is a lipophilic drug. This study clearly demonstrates that α-cyclodextrin/oil beads are also able to dramatically increase oral bioavailability of poorly soluble drugs.

Ali et al. (2009) introduced a solid inclusion technique using supercritical carbon dioxide carrier for obtaining solid inclusion complexes between cyclodextrin and few antifungal drugs like itraconazole, econazole, and fluconazole. This method constitutes one of the most innovators methods to prepare the inclusion complex of drug with CD in solid state. This is a non-toxic method as it is not utilizing any organic solvent and products obtained by this method were among the ones showing the highest interaction between the drug and the CD (Bandi et al., 2004).Therefore, a solid inclusion method using supercritical CO2 carrier proved to be a novel and useful complexation method for antifungal drugs into βCD.This indicated that inclusion formation was influenced by the preparation technique too.

Various novel characterization techniques have also been introduced to the area of inclusion complex and showed many advantages. High performance DSC (HDSC), Project Rapid Heat/Cool (RHC) and chip calorimetry have been applied in this area to overcome some shortages of DSC relating to slow cooling/heating scan which can cause sample degradation and undesired transformation to a different solid state form (Guns et al., 2010). The application of modern microscopy tools like Scanning Tunneling Microscopy (STM) and atomic force microscopy promotes the investigation on cyclodextrin complexation. STM imaging allows the two-dimensional (2D) visualization of the arrangements, orientations, and even inner structures of molecules in air, in ultrahigh vacuum (UHV), and in solution (Yifeng et al., 2008 ; Ohira et al., 2003). In contrast, AFM is a relatively novel technique with which three-dimensional (3D) images can be obtained with better resolution. With such convenient microscopy approach it is easy to characterize various types of aggregates and inclusion complexes with size from less than 1 nm to about several micrometers (Yifeng et al., 2008 ; Botella et al., 1996). Fluorescent microscopy in the near-infrared between 950 and 1600 nm has been developed as a novel method of imaging and studying singlewalled carbon nanotubes (SWNTs) in a variety of environments.So this new technique offers an important tool for the observation and characterization of cyclodextrin based nanotubes and complexes (Wu et al., 2006). Molecular modeling is also a novel technique to know about interaction between drug and cyclodextrins by investigating the dominant driving force for the complexation process. Aiman et al. (2009) has used molecular modelling to investigate the interaction between meloxicam and βCD and concluded that dominant driving force for this complexation was evidently Van der Waals force with very little electrostatic contribution. The development of new characterization techniques will help to discover the thermodynamic property and mechanism of inclusion complex

formation therefore, more strategies will be applied to overcome the remaining problems regarding inclusion complex formation in the future.

## 7 CONCLUSION

Among the emerging new chemical entities, most are poorly water soluble drugs putting impact on their bioavailability and therapeutic effect. The solubility enhancement techniques also play an important role in getting the excellent dissolution properties of poorly soluble drugs. Successful improvement in aqueous solubility is mainly depends on the method which we choose. Complexation of poorly soluble drugs with cyclodrins is currently considered one of the most effective and attractive methods to solve the low bioavailability problem of poorly water-soluble drugs. Because CDs, act as safer solubilizing agents with bio adaptability and having versatile Characteristics like non-toxic, non-irritant and helps in taste masking. This review is mainly explains the various preparative methods for inclusion complex and characterization. Although all the methods mentioned above could enhance the solubility. But each method has its advantages and disadvantages, which is important when deciding the appropriate method for the drug selection. It is imperative that the most efficient method is chosen in order to decrease the possibility of errors and to get best results. Solid binary system of drug and CDs are capable to modify the physicochemical properties of drugs such as solubility, particle size, crystal habit, thermal behavior and there by forming a highly water soluble form of insoluble drug. These physicochemical modifications can judge by various characterization techniques mentioned in this review. The CDs, due to their extreme high aqueous solubility, they became capable to enhance the dissolution rate and overcome the bio-availability problem of the poorly soluble drugs. The permeation of insoluble drugs through various biological membranes can also be enhanced by preparing drug- CD inclusion compounds.

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