Review Article

Cyclodextrines: An Emerging Paradigm

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ABSTRACT

Cyclodextrins are a family of cyclic oligosaccharides composed of (1, 4) linked glucopyranose subunits. Cyclodextrins are useful molecular chelating agents. They possess a cage-like supramolecular structure, which is the same as the structures formed from cryptands, calixarenes, cyclophanes, spherands and crown ethers. These compounds having supramolecular structures carry out chemical reactions that involve intramolecular interactions where covalent bonds are not formed between interacting molecules, ions or radicals. The majority of all these reactions are of 'host–guest' type. Compared to all the supramolecular hosts mentioned above, cyclodextrins are most important. Because of their inclusion complex forming capability, the properties of the materials with which they complex can be modified significantly. As a result of molecular complexation phenomena CDs are widely used in many industrial products, technologies and analytical methods. The negligible cytotoxic effects of CDs are an important attribute in applications such as rug carrier, food and flavours, cosmetics, packing, textiles, separation processes, environment protection, fermentation and catalysis.

Keywords: Cyclodextrins, Inclusion complex, Equilibrium, Complexation techniques.

INTRODUCTION

Carbohydrates, such as cellulose, starch and sucrose, are probably the most abundant organic substances in nature and form very ancient time they have been used for shelter, clothing and food. For thousands of years humans have processed carbohydrates through fermentation and observed their enzymatic degradation. It is now known that these processes lead to formation of mixtures of monosaccharides, disaccharides and various oligosaccharides, such as linear and branched dextrins and that, under certain conditions, small amounts of cyclic dextrins or cyclodextrins are also being formed during these degradation processes. Technological advances of the 19th century laid the foundation of carbohydrate chemistry and by the middle of the century a number of relatively pure carbohydrates such as sucrose, cellulose from cotton, starch, glucose, fructose, mannose, and lactose were known to chemists in Europe (Robyt, 1998). A short chronological summary on the development of carbohydrate chemistry, with a special emphasis on cyclodextrins.

Native cyclodextrins are polysaccharides made up of six to eight cyclic linked oligosaccharides of D-glucopyranose monomers connected by α-1, 4- indican bonds. These compounds form cone-shaped molecules with primary hydroxyl groups (6-OH) arranged in an inner hydrophobic cavity of 5.7, 7.8 and 9.5 Å respectively for α-, β-, γ-cyclodextrins, and secondary hydroxyl groups (2- and 3-OH) rendering external walls hydrophilic[1,28]. These two microenvironments confer to the molecule the ability of forming inclusion complex with guest molecules. Cyclodextrins act as molecular hosts toward various, poorly water-soluble drugs, ranging from ion, very polar molecules to non-polar molecules, affecting advantageously their physicochemical properties. Thus, they have found extensive applications in chromatography, catalysis, asymmetric reactions, food, cosmetic, pharmaceutical technology[2,3,4] and medicinal applications. Partially or entirely encapsulation occurs by the intermediate of hydrophobic forces and van der Waals interactions, ion pairing, hydrogen bonding participating in improving, through complexation, the aqueous solubility and stability of drugs, vitamins and food colorant, preventing molecules self-aggregation, ameliorating dissolution rate, bioavailability of the hydrophobic drugs, decreasing toxicity and controlling drug releasing[5,6,7,8].

History

Isolation of substance recognizable as Cyclodextrins was made in 1891 by Villers.He isolated crystalline substance from a culture medium of Bacillus amylobactor, grown on a medium containing starch. This crystalline substance was named as "Cellulosine" because of similarity with cellulose. In between 1903-1911 further progresion in Cyclodextrin chemistry was made by Schardinger. He characterised the crystalline substance as mixture of two cyclic oligosacharides which he named crystalline dextrin B, a detailed description for preperation and isolation of these cyclic oligosacharides were published and known as Schardinger dextrins, cycloamyloses or cycloglucans. Now these compounds are commonly called cyclodextrins (i.e. α-Cyclodextrin (α-CD) and β-cyclodextrin (β-CD)) or less commonly cyclomaltodextrins (i.e. cyclomaltohexaose and cyclomaltoheptaose) or cycloamyloses (i.e. cyclohexaamylose and cycloheptaamylose). γ-Cyclodextrin (γ-CD; cyclomaltooctaose or cyclooctaamylose) was first described in 1935 by Freudenberg and Jacobi (K. Freudenberg, et al 1935).[1,5,19] Inclusion compounds were first observed by Mylius in 1886. It is a unique type of chemical complex in which one molecule is enclosed within other lattice structure for stable arrangement. Freudenberg; Cramer and Plieninger studied about complexation in between 1935 to 1953, and then onwards its complexation parameter and their physicochemical properties were studied. First application of molecular complexation between bio-organic and carbohydrate derivatives and these derivative was Cyclodextrin and opened up new field of application. It was (1953) patent by Freudenbery, Cramer and Plieninger, described specific effects that could be achieved by complexing drugs with Cyclodextrins. Then upto mid 1980 more than 70 patent were dedicated. These compounds studied simultaneously in Budapast,Stullgart, and Baltimore and found to be best complexing agent. The world's first CD-containing pharmaceutical product, prostaglandin E2/βCD (Prostarmon E™sublingual tablets), was marketed in Japan in 1976. Twelve years later, the first European CD-based pharmaceutical product, piroxicam/βCD (Brexin® tablets), was marketed and in 1997, the first US-approved product, itraconazole/2-hydroxypropyl-βCD oral solution (Sporanox ®) was introduced[8].

Need

Cyclodextrins is needful in following difficulties;

- Solubility limited poor bioavailability.
- The drug is soluble only in such organic solvents, which cannot be injected thus formulation of parenteral preparation is not feasible.
- The drug is irritating to mucous membranes, tissues or skin
- The drug is very bitter, astringent tasting.
- The drug is sensitive to destructing factors, like oxygen, light, water, etc.
- The drug is a liquid, volatile and/or sublimable, bad smelling or a hygroscopic solid.
- The drug is sticky, lipid like consistence or incompatible with formulation components[6].

Limitations

All the categories of drugs are not suitable substrates for CD complexation. Drug molecule to be complexed with CD should have certain characteristics explained below.

- More than five atoms (C, P, S, and N) form the skeleton of the drug molecule.
- Melting point temperature of the substance is below 250 ^{0}C.
- Solubility in water is less than 10 mg/mL.
- The guest molecule consists of less than five condensed rings.
- Molecular weight between 100 and 400.

Cyclodextrin production

Native corn starch (amylum) and soluble potato starch (Poland) were used as substrates for cyclodextrin production. Substrate solutions were prepared in phosphate buffer, pH=7.0, in a manner allowing the desired concentration to be reached after the addition of the dissolved enzyme. Both types of starches were dissolved in steam water bath. The resultant substrate solutions were cooled to 45 °C and the necessary amount of CGTase was added. The enzyme reaction was conducted in 100-mL Erlenmeyer flasks containing 50 mL of reaction medium, at 45 °C on a reciprocal shaker for 20 h. Samples were taken at certain intervals and CGTase was inactivated by boiling for 10 min in a water bath. The cyclodextrin content of the reaction mixture was determined.

Generally two different types of cyclodextrin production processes are used; these are solvent process, and non-solvent process.

Fig.1 represents the production of cyclodextrin from starch using solvent process. Solvent process requires an organic solvent mainly toluene, ethyl alcohol or acetone that acts as a complexing agent which extracts one type of cyclodextrin selectively and thus directs the enzyme reaction to produce particular type of cyclodextrin of interest. Non-solvent process does not require complexing agents and produces a mixture of cyclodextrins. The preparation process of cyclodextrins consists of four principal steps: (i) culturing of the microorganism that produces the cyclodextrin glucosyl tranferase (CGTase); (ii) separation, concentration and purification of the enzyme from the fermentation medium; (iii) enzymatical conversation of prehydrolyzed starch in mixture of cyclic and acyclic dextrins; and (iv) separation of cyclodextrins from the mixture, their purification and crystallization. Cyclodextrin glucosyl tranferase (CGTase) enzymes degrade the starch and produce intramolecular reactions without the participation of water producing cyclic and acyclic dextrins. Two ends of acyclic dextrins make a link through the glycosidic oxygen bridges by α (1, 4) bonds (Fig.1) in solvent process, the first step are to liquefy starch (starch concentration 20-30%). Using α-amylase, acids and mechanical disintegration, starch is liquefied to make it suitable for its incubation with CGTase at low temperature. In industry liquefaction of starch is generally carried out by α-amylase treatment and jet cooking. Next, the liquefied starch is treated with CGTase and complexing agent (CA) under controlled temperature and pH. Thus cyclodextrins are produced by enzymatic conversion. A particular type of CD forms a complex with complexing agent (CA) and then it is precipitated. Then CD-CA complex is separated from the reaction solution either by centrifugation or filtration. The separated complex is then washed. Next, the separated complex is decomplexed and filtered. The filtrate is either steam distilled or extracted to recover CA. Recovered CA is reused. Then product solution is concentrated under vacuum distillation and treated with activated carbon and then CD is crystallized and filtered. The CD crystals are washed and dried in non-solvent process, β-CD production starts with starch liquefaction followed by enzymatic conversion identical to that used in solvent process but no complexing agent is added to this process. Cyclodextrins produced by non-solvent process can be applied in food without restriction, in contrast to cyclodextrins produced in solvent processes[5,15,19].

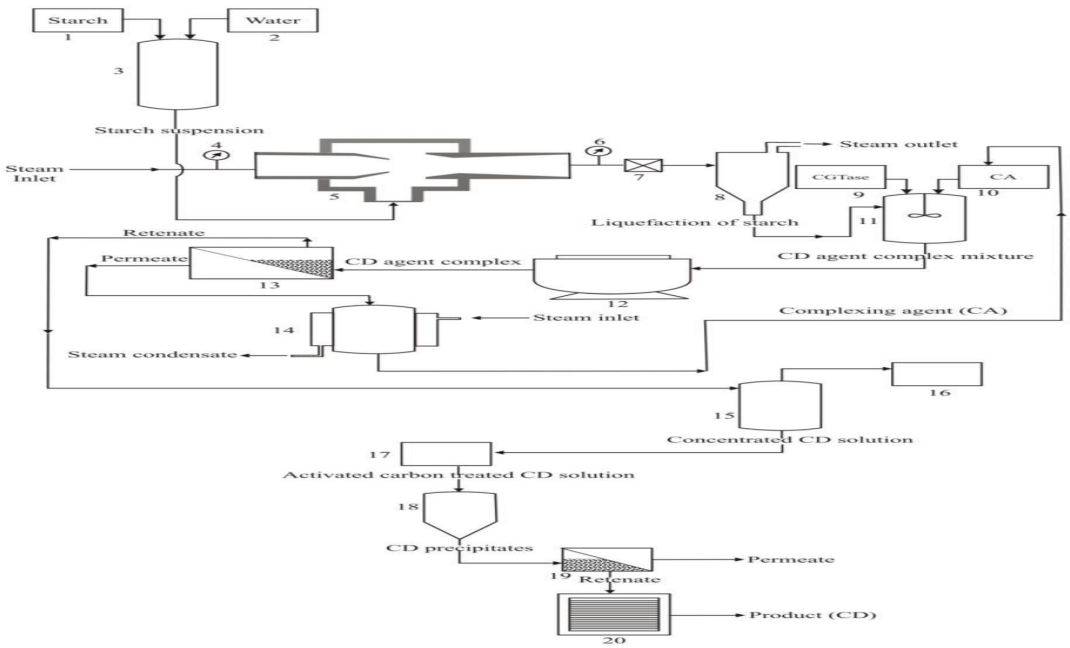

Fig.1: Schematic representation of cyclodextrin production from starch using solvent process Abbreviations: 1, 2, 9 and 10: Storage tanks; 3: Vessel; 4 and 6: Pressure indicators; 5: Hydroheater; 7: Back pressure valve; 8: Cooked starch collection vessel; 11: Bio-reactor; 12: Centrifuge; 13 and 19: Filtration unit; 14: Steam distillation apparatus; 15: Vacuum distillation unit; 16: Barometric condenser; 17: Activated carbon treatment unit; 18: Crystallizer; 20: Dryer.

Cyclodextrins and their derivatives shown in table 1.[21, 24]

Table.1: Cyclodextrins and their derivatives

S. No.	Cyclodextrin(CD)	Abbreviation	R	N
1	α-cyclodextrin	α-CD	H	4
2	β- cyclodextrin	β-CD	H	5
3	γ- cyclodextrin	γ-CD	H	6
4	Carboxymethyl β- cyclodextrin	CM-β-CD	CH_2COOH	5
5	Carboxymethyl ethyl β- cyclodextrin	CME-β-CD	$CH_2COOH.CH_2$ CH_3OR H	5
6	Diethyl β- cyclodextrin	DE-β-CD	CH_2CH_3ORH	5
7	Dimethyl β- cyclodextrin	DM-β-CD	CH_3 or H	5
8	Methyl β- cyclodextrin	M-β-CD	CH_3 or H	5
9	Random Methyl β- cyclodextrin	RM-β-CD	CH_3 or H	5
10	Glucosyl β- cyclodextrin	G1 β-CD	Glucosylor H	5
11	Maltosyl β- cyclodextrin	G2 β-CD	Maltosyl or H	5
12	Hydroxyethyl β- cyclodextrin	HE β-CD	CH_2 CH_2 OH H	5
13	Hydroxypropyl β- cyclodextrin	HP β-CD	CH_2 $CHOHCH_3$ Or H	5
14	Sulfobutyl ether β- cyclodextrin	SBE β-CD	(CH_2) SO_3Na	5

Cyclodextrins and Their Inclusion Complexes

Structural features

Cyclodextrins comprise a family of three well-known industrially produced major, and several rare, minor cyclic oligosaccharides. The three major CDs are crystalline, homogeneous, nonhygroscopic substances, which are torus-like macro-rings built up from glucopyranose units. The α-cyclodextrin (Schardinger's α-dextrin, cyclomaltohexaose, cyclohexaglucan, cyclohexaamylose, αCD, ACD, C6A) comprises six glucopyranose units, βCD (Schardinger's β-dextrin, cyclomaltoheptaose, cycloheptaglucan, cycloheptaamylose, βCD, BCD, C7A) comprises seven such units and γCD (Schardinger's γ-dextrin, cyclomaltooctaose, cyclooctaglucan, cyclooctaamylose, γCD, GCD, C8A) comprises eight such units.

As a consequence of the $^4C_1$ conformation of the glucopyranose units, all secondary hydroxyl groups are situated on one of the two edges of the ring, whereas all the primary ones are placed on the other edge. The ring, in reality, is a cylinder, or better said a conical cylinder, which is frequently characterized as a doughnut or wreath-shaped truncated cone. The cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges, respectively. The nonbonding electron pairs of the glycosidic-oxygen bridges are directed toward the inside of the cavity, producing a high electron density there and lending to it some Lewis-base character[5, 6].

The C-2-OH group of one glucopyranoside unit can form a hydrogen bond with the C-3-OH group of the adjacent glucopyranose unit. In the βCD molecule, a complete secondary belt is formed by these H bonds; therefore, the βCD is a rather rigid structure. This intramolecular H-bond formation is probably the explanation for the observation that βCD has the lowest water solubility of all CDs. The H-bond belt is incomplete in the αCD molecule, because one glucopyranose unit is in a distorted position[12]. Consequently, instead of the six possible H bonds, only four can be established simultaneously.The γCD is a non-coplanar, more flexible structure; therefore, it is the most soluble of the three CDs. Chemical structure of β-cyclodextrin as shown in figure 2.

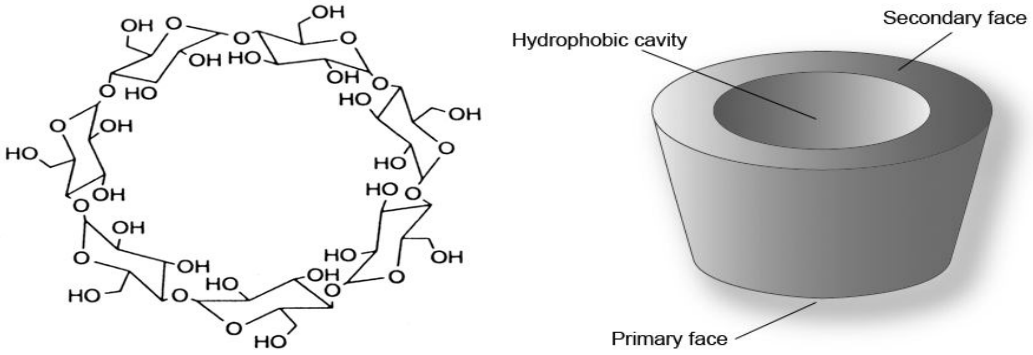

Fig. 2: Chemical structure of β-cyclodextrin

Properties

Cyclodextrins are of three types: α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin, referred to as first generation or parent cyclodextrins. α-, β- and γ-cyclodextrins are composed of six, seven and eight α-(1,4)-linked glycosyl units, respectively. β-Cyclodextrin is the most accessible, the lowest-priced and generally the most useful. Cyclodextrins crystallise in two main types of crystal packing, channel structures and cage structures, depending on the type of cyclodextrin and guest compound. These crystal structures show that cyclodextrins in complexes adopt the expected 'round' structure with all glucopyranose units in the $^4C_1$ chair conformation[1,19]. Furthermore, studies with linear maltohexaoses, which form an antipar-allel double helix, indicate that α-cyclodextrin is the form in which the steric strain due to cyclization is least whileγ-cyclodextrin is most strained. Apart from these naturally occurring cyclodextrins, many cyclodextrin derivatives have been synthesised. These derivatives usually are produced by aminations, esterifications or etherifications of primary and secondary hydroxyl groups of the cyclodextrins. Depending on the substituent, the solubility of the cyclodextrin derivatives is usually different from that of their parent cyclodextrins. Virtually all derivatives have a changed hydrophobic cavity volume and also these modifications can improve solubility, stability against light or oxygen and help control the chemical activity of guest molecules. Cyclodextrins are frequently used as building blocks. Up to 20 substituents have been linked to cyclodextrin in a regioselective manner. The synthesis of uniform cyclodextrin derivatives requires regioselective reagents, optimisation of reaction conditions and a good separation of products. The most frequently studied reaction is an electrophilic attack at the OH-groups, the formation of ethers and esters by alkyl halides, epoxides, acyl derivatives, isocyanates, and by inorganic acid derivatives as sulphonic acid chloride cleavage of C–OH bonds has also been studied frequently, involving a nucleophilic attack by compounds such as azide ions, halide ions, thiols, thiourea, and amines; this requires activation of the oxygen atom by an electron-withdrawing group. Because of their ability to link covalently or noncovalently specifically to other cyclodextrins, cyclodextrins can be used as building blocks for the construction of supramolecular[9,10]. Different physicochemical properties of cyclodextrins described in table 2.

Table 2: Physicochemical Properties of Different Cyclodextrins

S. No.	Property	α- CD	β- CD	γ- CD	DMβ-CD	HPβ-CD
1	No.of glucose units	6	7	8	7	7
2	Cavity dimention(A^0) Inside cavity dimeter Outer dimeter Height	 5.3 146 7.9	 6.5 154 7.9	 8.3 174 7.9	 6 -- 10	 6 -- --
3	Molecular weight	973	1135	1297	1366	1540
4	Aqueous solubility in gm/100 ml	14.5	1.85	23.2	5	>50
5	Melting point	275	280	275	295-300	--
6	**pKa	12.3	12.2	12.1	--	--
7	***Half life of ring opening (hr.)	6.2	5.4	3.0	8.5	--
8	Specific rotation	+150.5	+162.5	+177.4	+160-165	--
9	****Enzymatic hydrolysis	Negligible	slow	Rapid	--	--
10	Colour of Iodine complex	Blue	Yellow	Yellowish	--	--
11	Surface tention (mN/m)	71	71	71	60	52-69

DMβ-CD:-Heptakis-2,6-Di-O-Methyl β-Cyclodextrin.
HPβ-CD:-Hydroxyl propyl β-Cyclodextrin.
**:-pKa by potentiometry at 25^0
***:-Half life of ring opening in 1.0 N HCL at 60^0
****:-BynAspargillus oryzae α-amylase.

Toxicological considerations

The safety profiles of the three most common natural cyclodextrins and some of their derivatives have recently been reviewed. In general, the natural cyclodextrins and their hydrophilic derivatives are only able to permeate lipophilic biological membranes, such as the eye cornea, with considerable difficulty. Even the somewhat lipophilic randomly methylated α-cyclodextrin does not readily permeate lipophilic membranes, although it interacts more readily with membranes than the hydrophilic cyclodextrin derivatives. All toxicity studies have demonstrated that orally administered cyclodextrins are practically non-toxic, due to lack of absorption from the gastrointestinal tract. Furthermore, a number of safety evaluations have shown that β-cyclodextrin, 2-hydroxypropyl-*β*-cyclodextrin, sulphobutylether β-cyclodextrin, sulphated β-cyclodextrin and maltosyl β-cyclodextrin appear to be safe even

when administered parenterally. However, toxicological studies have also shown that the parent α- and β-cyclodextrin and the methylated β-cyclodextrins are not suitable for parenteral administration[5,8].

α-Cyclodextrin

The main properties are: relatively irritating after i.m. injection; binds some lipids; some eye irritation; between 2 and 3% absorption after oral administration to rats; no metabolism in the upper intestinal tract; cleavage only by the intestinal flora of caecum and colon. Excretion after oral administration to rats were: 60% as CO2 (no CO2 exhalation after oral administration to germ-free rats), 26–33% as metabolite incorporation and 7–14% as metabolites in faeces and urine, mainly excreted unchanged by the renal route after i.v. injections with *t*1/2 = 25 min in rats, LD50 oral, rat >10,000 mg/kg, LD50 i.v., rat: between 500 and 750 mg/kg.

β-Cyclodextrin

The main properties are: less irritating than α-cyclodextrin after i.m. injection; binds cholesterol; very small amounts (1–2%) absorbed in the upper intestinal tract after oral administration; no metabolism in the upper intestinal tract; metabolised by bacteria in caecum and colon; currently the most common cyclodextrin in pharmaceutical formulations and, thus, probably the best studied cyclodextrin in humans. Application of high doses may be harmful and is not recommended; bacterial degradation and fermentation in the colon may lead to gas production and diarrhoea, LD50 oral, rat >5000 mg/kg, LD50 i.v., rat: between 450 and 790 mg/kg.

γ-Cyclodextrin

The main properties are: insignificant irritation after i.m. injection; rapidly and completely degraded to glucose in the upper intestinal tract by intestinal enzymes (even at high daily dosages, e.g. 10–20 g/kg); almost no (0.1%) absorption (of intact γ-cyclodextrin) after oral administration; practically no metabolism after i.v. administration; probably the least toxic cyclodextrin, at least of the three natural cyclodextrins. Actively promoted as food additive by its main manufactures; complexing abilities, in general, less than those of β-cyclodextrin and the water soluble β-cyclodextrin derivatives; its complexes frequently have limited solubility in aqueous solutions and tend to aggregate in aqueous solutions, which makes the solution unclear (opalescence), LD50 oral, rat 8000 mg/kg, LD50 i.v., rat: about 4000 mg/kg[9,14, 27].

Modified cyclodextrins

The aqueous solubility of natural CDs is lower due to the relatively strong intramolecular hydrogen bonding in the crystal lattice. Particularly β-cyclodextrin shows low aqueous solubility among all naturally occurring cyclodextrin. Hydroxylation or methylation of the hydroxyl groups of β-cyclodextrin enhances solubility and inclusion capacity of parent CDs . The functionality of cyclodextrins is greatly increased by chemical modification because of the availability of multiple reactive hydroxyl groups[19].

Techniques for CD-complexes[1,9,16,19,20,22]

The uniqueness of molecular geometry of a cyclodextrin enabled a variety of guest compounds to be accommodated in the inner hydrophobic cavity to form molecular inclusion complexes of guest-host. A variety of non-covalent forces, Van der Waals forces, hydrophobic interactions and other forces are responsible for the formation of stable CD-complex that protects the guest molecule against the attack by various reactive molecules and thus, it reduces the rate of hydrolysis, oxidation, steric rearrangement, racemization and even enzymatic decomposition. Depending on the molecular dimensions of cyclodextrins, α-cyclodextrin can typically complex low molecular weight molecules or compounds with aliphatic side chains, β-cyclodextrin will complex aromatics and heterocycles and γ-cyclodextrin can accommodate lager molecules for e.g. macrocycles and steroids. Complexes can be formed either in solution or in the crystalline state. Water is typically the solvent of choice for inclusion complexation but complexation can be accomplished in a co-solvent system and in the presence of any non-aqueous solvents. Generally, guest /cyclodextrin complexes have a molar ratio, [guest]/[cyclodextrin] of 1:1. The molar ratio can be higher or lower and this depends on the size of the guest molecule and the identity of the cyclodextrin. Many techniques which are used to form CD-complexes are kneading, co-precipitation, dry mixing, sealing, slurry complexation, neutralization, spray drying, freeze-drying, and solvent evaporation.

1) Kneading

The kneading process is similar to the wet granulation process and requires conventional kneaders (e.g., low and high shear mixers). The inclusion complex of guest molecule with

CD is prepared in the laboratory by wetting the physical mixture in a mortar with a minimum volume of water and subsequently kneading thoroughly with a pestle-mortar to obtain a paste which is then dried under vacuum at room temperature and sieved through appropriate sieve and is stored in a desccicator until further evaluation.

2) Co-precipitation

The co-precipitation method is the most widely used method in the laboratory. Sapkal et al. have prepared inclusion complex of guest molecule of poor aqueous solubility with β-CD by co-precipitation method. At first guest molecule is dissolved in minimum quantity organic solvent like acetone and is added drop wise to the β-CD in minimum quantity of water previously maintained at 75^{o}C while stirring. Stirring is maintained for 1 h at 75^{o}C. Then gradually it is cooled to room temperature while stirring. The precipitates is then filtered, dried and stored at ~25° ± 2.0°C and relative humidity of 40-50%. Some time the precipitate may be washed with a small amount of water or other water-miscible solvent such as methanol, ethyl alcohol, or acetone. Unfortunately, the use of organic solvents as precipitants can interfere with complexation and therefore this approach is less attractive than the kneading method. Another disadvantage of this method lies in the scale-up but the co-precipitation method yields a highly pure and crystalline inclusion complex.

3) Dry mixing

In dry mixing, guests are added to the CD and simply mixing them together results complexation. Parlati et al. have prepared solid complexes with a modified dry mixing method by milling the reactants in the molar ratio 2:1 for 3 days. This method works best with oils or liquid guests. The main advantage of this method is that no water needs to be added, unless a washing step is required. The disadvantages of this method are the risk of caking on scale-up, insufficient mixing that leads to incomplete complexation, and prolonged mixing time. The duration of mixing is variable and depends on the guest.

4) Sealing

The solid guest-cyclodextrin complexes can be formed by grinding definite amount of physical mixtures of guest and cyclodextrin, sealing the mixture in a glass container and keeping in a temperature range of 60 to 90°C. Wang et al., prepared the inclusion complex of paeonol with β-cyclodextrin by a simple, quick 'Sealed-control temperature method'. The complex formation was confirmed by infrared (IR) spectrum and powder X-ray diffraction. The study also revealed that the inclusion complex formation by this method was affected by heating temperature, heating time, and crystallinity of β-cyclodextrin.

5) Slurry-complexation

In this method, cyclodextrin is suspended in water up to a 40-45% w/w concentration and stirred in a reactor. Cyclodextrin which is in solution forms complexes with the guest and the complex precipitates. Generally ambient temperatures are required for slurry complexation. With many guests, some heat may be applied to increase the rate of complexation, but care must be applied as too much heat can destabilize the complex. The amount of time required to complete the complexation depends on the particular characteristics of guest and the r.p.m. of stirring. The complex can be collected in the same manner as with the co-precipitation method. The main advantage of this method is the reduction of the amount of water required and the size of the reactor.

6) Neutralization

Solid complexes of ionizable guests can be prepared by neutralization method, wherein the guest is dissolved in an acidic (for basic guests) or basic (for acidic guests) aqueous CD solution. Then the solubility of the guest is lowered by appropriate pH adjustments to force the complex out of solution. Terfenadine has relatively low bioavailability after oral administration due to its limited solubility in water. Choi et al., prepared the terfenadine-β-cyclodextrin (1:2) inclusion complex by the neutralization method to enhance the antihistaminic activity of terfenadine. The formation constant of inclusion complex was higher at lower pH, while its formation ratio was 1:2 irrespective of pH. They concluded that this inclusion complex enhanced the antihistaminic activity of terfenadine following the enhanced solubility and dissolution of terfenadine.

7) Spray drying

In spray drying, cyclodextrin is dissolved in 200 ml of a solution previously alkalinized with 25% aqueous ammonia (final pH 9.5). The guest is dissolved in 100 ml of 96%

ethyl alcohol. Both solutions are mixed and sonicated; the final solution is spray-dried to get the complexes.

8) **Freeze-drying (lyophylization)**
In freeze-drying, physical mixture of guest and cyclodextrin is wetted with a small amount of buffer solution and is kneaded forming a homogeneous suspension which is then freeze-dried. The final complexes are pulverized and sieved through appropriate sieve. Freeze-drying is an industrially applicable method for heat labile guests, but large amount of water, if it is used as solvent, and excessive CD would be required because of the low solubility of hydrophobic guest in aqueous solution and makes the process time consuming.

9) **Solvent evaporation**
In this method organic solvents are used and therefore residual solvents need to be removed. Osadebe et al. have prepared solid dispersion (SD) of piroxicam and β-CD by solvent evaporation method. At first appropriate amounts of piroxicam and β-CD are dissolved in a common solvent, methanol and stirred for 24 h at 28°C to prepare inclusion complex with 1:1 and 1:2 molar ratios. After that the mixture is concentrated under vacuum, filtered, and dried under vacuum at 25°C for 24 h to get the complex.

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 Where, CD is the cyclodextrin, G is the guest molecule, CD-G is the inclusion complex, kR and kD are the recombination constant and dissociation constant respectively. K, equilibrium constant is the important characteristic of this association. 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[1,19].

1) **Dilution**
Dissociation due to dilution appears to be a major release mechanism for the guest molecules from cyclodextrin complexes. Guo et al.,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 here also dilution is probably insufficient to account for the relative good absorption of drugs as administered as CD-complexes.

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

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. In ophthalmic delivery, cyclodextrins have

been used to increase the solubility and/ or stability of poorly water soluble drugs in the tear fluids and in some cases to reduce irritation.

4) **Protein binding**
Drug binding to plasma proteins may be a vital mechanism by which the drug may be released from drug-CD complex. Frijnik et al.,studied the effect of cyclodextrin (HPβCD) on the displacement of both naproxen and flurbiprofen from plasma binding sites in vitro. After parenteral administration they estimated, after 10 and 60 minutes, the tissue distribution of both naproxen and flurbiprofen either as HPβCD complex or as plasma solutions and concluded that more drugs was free from cyclodextrin solution to distribute to tissues than from the plasma solutions.

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. 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.

Application

1) **Cyclodextrins in pharmaceutical industry**
The uses and benefits of cyclodextrin complexation are well recognized in pharmaceutical industries that were evidenced by several reviews in the past years. These benefits are bioavailability enhancement, active stabilization, odors or taste masking, irritation reduction and material handling benefits. Practical use of natural cyclodextrins as drug carriers is restricted owing to their low aqueous solubility. The β-cyclodextrin is essentially nontoxic when given orally but it cannot be given in parenteral preparation owing to its low aqueous solubility and nephrotoxicity. Rate of metabolism of α-cyclodextrin is slower and that of γ-cyclodextrin is much faster than that of β-cyclodextrin. Cyclodextrins are metabolized in the colon. CD derivatives (hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin) have been widely investigated for parenteral drug delivery. These have several advantages: enhancement of drug's aqueous solubility, minimization of toxic effect, reduction of tissue irritation and lesser or no precipitation of drug in the physiological pH.

2) **Drug solubility and dissolution**
Inclusion complexation or solid dispersion with cyclodextrins can improve drug solubility or dissolution of poorly water-soluble drugs. In case of drugs with inadequate molecular characteristics for complexation cyclodextrin act as hydrophilic carriers, or as tablet dissolution enhancers for drugs with high dose (with which use of a drug/CD complex is difficult) e.g., paracetamol. The magnitude of apparent stability constant for several drug/CD complexes, K in M-1, ranges from 0 to 1,000. Out of various commercially available CDs, methylated CDs with a relatively low molar substitution appear to be the most powerful solubilizers. Reduction of drug crystallinity on complexation or solid dispersion with CDs also contributes to the CD increased apparent drug solubility and dissolution rate. CDs can enhance drug dissolution even whenthere is no complexation. CDs can also act as release enhancers, for example β-CD enhanced the release rate of poorly soluble naproxen and ketoprofen from inert acrylic resins and hydrophilic swellable (high-viscosity hydroxypropyl methyl cellulose [HPMC]) tableted matrices. β-CD also enhanced the release of theophylline from HPMC matrix by increasing the apparent solubility and dissolution rate of the drug[6].

3) **Drug absorption/bioavailability**
In case of hydrophobic drugs, CDs increase the permeability by increasing drug solubility, dissolution and thus making the drug available at the surface of the biological barrier, from where it partitions into the membrane without disrupting the lipid layers of the barrier. In such cases it is important to use just enough CD to solubilize the drug in the aqueous vehicle since excess may decrease the drug availability. There are four possible mechanisms affecting the absorption and thus enhancing bioavailability of drugs by various administration routes complexed with hydrophilic CDs which have been extensively studied and are summarized as follows: (a) CDs increase the solubility, dissolution rate, and wettability of poorly water-soluble drugs; (b) CDs prevent the

degradation or disposition of chemically unstable drugs in gastrointestinal tracts as well as during storage; (c) CDs perturb the membrane fluidity to lower the barrier function, which consequently enhances the absorption of drugs including peptide and protein drugs through the nasal and rectal mucosa; and (d) competitive inclusion complexation with third components (bile acid, cholesterol, lipids, etc.) to release the included drug[6].

4) **Control of drug release**

There are two types of control on drug release via oral delivery i.e., rate-controlled release and the time-controlled release. The hydrophobic CDs such as ethylated and acylated CDs with low aqueous solubility are known to work as prolonged-release carriers of water-soluble drugs. Among the various acylated CDs, per-Obutanoyl-β-CDs (TB-β-CDs) has the prominent retarding effect for water soluble drugs, owing to the mucoadhesive property and appropriate hydrophobicity that differ from those of other derivatives having shorter or longer chains. The gel forming property of 2-hydroxypropyl-β-CDs (HP-β-CDs) is also useful to design the prolonged release of water-soluble drugs. Moreover, sulfobutyl ether β-CDs (SBE7-β-CDs) can serve as both a solubility modulating and an osmotic pumping agent for the controlled-porosity osmotic pump tablets, from which the release rate of both highly and poorly water-soluble drugs can be controlled precisely. The combined use of CDs complex and CDs conjugate will be useful for designing various kinds of time-controlled type oral drug delivery preparations. The release of drug from the drug/CDs conjugate after oral administration shows a typical delayed release behavior. Therefore, when the CDs conjugates are combined with other different release preparations, we can obtain more advanced and optimized drug release system, securing balanced oral bioavailability, and prominent therapeutic efficacy. For example, a repeated-release preparation may be designed by combining the CDs conjugate with a fast releasing fraction, while a combined preparation of the conjugate with a slow-releasing fraction may provide a prolonged release preparation. These modified-releases by means of the combination were demonstrated using the ketoprofen/ a-CDs conjugate.

5) **Site-specific drug delivery**

Drug targeting to specific organ or tissues by drug/CD complex are sometimes disadvantageous because the complex dissociates before it reaches targeting site. Such problem can be surmounted by binding a drug covalently to CDs. CDs are known to be scarcely capable of being hydrolyzed and only slightly absorbed in passage through the stomach and small intestine; however, they are fermented to small saccharides by colonic microflora and thus absorbed as maltose or glucose in the large intestine. This biological property of CDs can be exploited for site-specific delivery of drugs to colon. Kihara et al. have recently demonstrated that Starburst PAMAM dendrimer (generation 2 or 3) conjugate with a-CDs (a-CDE conjugates) in the molar ratio of 1:1 can be utilized as a novel non-viral vector for gene and siRNA delivery in vitro and in vivo. These in vitro and in vivo results highlight the potential use of CDs, CDs conjugates and CDs polymers for gene, antisense and siRNA therapies. A number of bioadaptable CDs derivatives and polymers have been designed and evaluated for practical uses in pharmaceutical field in the form of complex or conjugate[6].

6) **Drug safety**

CDs have been used to ameliorate the irritation caused by drugs. The increased drug efficacy and potency (i.e., reduction of the dose required for optimum therapeutic activity), caused by CD-increased drug solubility, may reduce drug toxicity by making the drug effective at lower doses. β-CD enhanced the antiviral activity of ganciclovir on human cytomegalovirus clinical strains and the resultant increase in the drug potency reduced the drug toxicity. The toxicities associated with crystallization of poorly water soluble drugs in parenteral formulations can often be reduced by formation of soluble drug:CD complexes[6].

7) **Drug stability**

Cyclodextrin complexation provides molecular shielding by encapsulating labile drugs molecules at molecular level. Thus insulate them against various degradation processes and increase the shelf life of drugs. CD-induced enhancement of drug stability is result of inhibition of drug interaction with vehicles and/or inhibition of drug bioconversion at the absorption site e.g., photostability of promethazine on complexing with HP-β-CD, DM-β-CD,

stability against hydrolysis of Melphalan and Carmustine by complexation with SBE-b-CD, and HP-β-CD etc. SBE-β-CD showed greater stability enhancement of many chemically unstable drugs than other CDs. The stabilizing effect of CDs depends on the nature and effect of the included functional group on the drug stability and the nature of the vehicle. HP-β-CD significantly reduced the photodegradation of 2-ethyl hexyl p-dimethyl aminobenzoate in solution than in emulsion vehicle. CDs improved the photostability of trimeprazine (when the solution pH is reduced) and promethazine. CDs also enhanced the solid state stability and shelf life of drugs. CDs were reported to enhance the physical stability of viral vectors for gene therapy, and the formulations containing sucrose and CDs were stable for 2 years when stored at 20^0C. Since the hydrolysis of drugs encapsulated in CDs is slower than that of free drugs, the stability of the drug/CD complex, i.e., the magnitude of the complex stability constant, plays a significant role in determining the extent of protection. The effect of complexation on drug stability can be represented by the following equation.

$$\frac{1}{K_0 - K_{obs}} = \frac{1}{K_c(K_0-K_C)\,[CD]} + \frac{1}{(K_0-K_C)}$$

Where,

K0 is the degradation rate constant of free drug,

K_{obs} is the observed degradation rate constant in the presence of CD,

Kc is the stability constant for the complex, and [CD] is the concentration of CD[19,26]

8) Cyclodextrins in food industry

Cyclodextrins have been used in food processes with a variety of objectives: (i) to protect lipophilic food components that are sensitive to oxygen and light or heat; (ii) to stabilize fragrances, flavors, vitamins, and essential oils against degradation; (iii) to suppress unpleasant tastes and odors (iv) to convert liquid food ingredient to solid powder; (v) to solubilize vitamins and food color; (vi) to control the release of certain food ingredients and to remove undesirable component; and (vii) to maintain food quality during storage by improved packaging technology. There are various regulatory authorities that control the use of various cyclodextrins in terms of safety (GRAS, Generally Recognized as Safe), limit of addition, acceptable daily intake etc. These are: The Joint FAO/WHO Expert Committee on Food Additives (JECFA), USFDA (US Food and Drug Administration) and U.S. Environmental Protection agency (EPA). The maximum level of β-CDs recommended in foods is 5 mg/kg per day. For α-CD and γ-CDs no Acceptable Daily Intake (ADI) was mentioned owing to their toxicological profiles. Molecular encapsulation of flavor components within the cavity of cyclodextrin has been proven to be most effective method of stabilizing flavor in food item and thus it provides protection against heat and evaporation. The taste and flavor of spray-dried powdered products are the most important quality factors. To prevent the loss of a hydrophobic flavor compound (*l*-menthol) during the drying of a droplet, Liu *et al.*, adopted complexation technique. They found β-cyclodextrin appeared to be a better host for menthol than α- and γ-CD. Flavors are generally volatile in nature which deteriorates readily and cyclodextrin form stable dry complexes with flavor and remain stable for longer periods without any further protection at room temperature. Fresh citrus juice is not bitter initially but turns bitter in the course of storage at a rate that depends on the pH and storage temperature. Therefore, CDs can be used for the removal or masking of undesirable components. Some marketed food products based on cyclodextrin described in table 3[5,19].

Table 3: Examples of some marketed food products based on cyclodextrin

Trade name	Type of food product	Role of CDs used	Country
Cyroma-line	Flavored sugar for baking	To preserve flavor on heating	Hungary
Natual	Low cholesterol cheese	To reduce cholesterol	France
Balade	Low cholesterol butter	To reduce cholesterol	Belgium
Simply eggs	Low cholesterol eggs	To reduce cholesterol	USA
Flavono	Chewing gum	To stabilize flavor	Japan
Stick lemon	Instant tea drink	To preserve flavor	Japan
Poder Tea	Instant green tea	For stabilization of color	Japan
FlavorAktiv Standard Kit	Beer flavor standards	To preserve flavor standards	Great Britain

9) Cyclodextrins in biotechnological field

The applications of cyclodextrin in biotechnological field began only in the 1980s and the majority of biotechnology processes mean an enzyme-catalyzed transformation of a substrate in an aqueous medium. CDs and its derivatives enhance the solubility of complexed substrates in aqueous media, and reduce their toxicity without damaging the microbial cells or the enzymes. Hence, the enzymatic conversion of lipophilic substrates can be intensified, the yield of the product-inhibited fermentation can be improved, organic toxic compounds are tolerated and metabolized by microbial cells at higher concentrations, and the compounds in small amounts can be isolated simply and more economically from complicated mixtures. Kumar et al., investigated a novel one step microbial transformation process for the production of testosterone from cholesterol by Lactobacillus bulgaricus and found that biotransformation of cholesterol was significantly increased in presence of cyclodextrin in the fermentation medium. Prabhu and Ramadoss reported enhancement of rate of production of benzyl penicillin when both phenylacetic acid and 6-aminopenicillanic acid were complexed with beta-methyl-cyclodextrin (β-m-CD) and γ-CD respectively and condensed in presence of catalyst penicillin acylase (EC 3.5.1.11).

10) Cyclodextrins in chemical industry

Cyclodextrins and their derivatives in the chemical industry are used as catalysts to improve the selectivity of reactions, as reaction inhibitors, as well as for the purification and separation of industrial-scale products. Cyclodextrins are extensively used in separations due to their unique property to form inclusion complexes with other smaller hydrophobic molecules. Most of the derivatized and all non-derivatized cyclodextrins are soluble in water; they are often used in aqueous environments as solubilizers of lipophilic compounds via inclusion complex formation and this property makes them potentially useful agents for various types of separations. CD has been extensively used as chiral selector for enantiomer separation and as an additive for improving the separation of some cyclic compounds. CDs tend to form inclusion complexes with certain compounds whose molecular size and structure match with the CD's cavities by hydrophobic interaction. Wang and Ren predict that there is a certain interaction with CD as the cyclic size of thymine is smaller than that of adenine and they explore the possibility of improving the separations of purines and pyrimidines by using β-CD as an additive.

11) Cyclodextrins in cosmetics, personal care and toiletries

A large number of cosmetic components are nearly insoluble in water and all these chemical substances are able to form inclusion complexes with cyclodextrins and results more soluble compared to the pure compounds. In many personal care products triclosan acts as a topical antiseptic and disinfectant and it is nearly insoluble in water, moderately soluble in alkaline solutions, and quite soluble in organic solvents. The cyclodextrin complex of triclosan is soluble in water and gives a clear solution. Cyclodextrins can be used in deodorant sticks and the cyclodextrins are able to complex perspiration malodors. Perfuming cosmetic products is an important part of meeting consumer requirements and the essential functions of fragrance materials are to provide a pleasant odor, to mask the bad smell of the product, and to give the product an identity. The amount of fragrance materials in the product rapidly decreases during storage because of their volatility and poor stability. In cosmetic preparations, CDs are mainly used (i) to increase the water solubility of lipophilic guests; (ii) to convert the liquid or oily guests to powder form; (iii) to increase the physical and chemical stability of guest molecules by protecting against decomposition, oxidation, hydrolysis, or loss by evaporation; (iv) to provide the controlled release of guest; (v) to minimize or prevent skin irritation; (vi) to prevent interactions between various formulation ingredients; (vii) to increase or decrease the absorption of various compounds into skin; (viii) to stabilize emulsions and suspensions; and (ix) to reduce or eliminate undesired odors . In some cosmetic preparations, more than one benefit is obtained by complexation with cyclodextrin. Cyclodextrins used in silica-based toothpastes increase the availability of triclosan, an antimicrobial agent, by cyclodextrin complexation results almost threefold enhancement of triclosan availability. Table 4 lists the applications of cyclodextrin in cosmetics and personal care items. Various methyl ether derivatives are proposed on the market: dimethyl and trimethyl CDs and particularly methylated and randomly methylated β-CDs. Glucosyl and maltosyl CDs are marketed and are highly water-soluble. Hydroxyethyl cyclodextrins are not easily available in the market. Finally, CD polymers with low molecular weights are water-soluble,

whereas high-molecular-weight products are water-insoluble but capable of swelling[23].

Table 4: Application of cyclodextrin in cosmetics and personal care items

Cosmetics and personal care items	Role of CDs used	Country
Powdered hair bleach	Stability	UK, Belgium, USA
Cold cream	Solubility	USA
Skin cleanser	Tocopherol carrier	Italy

Cyclodextrins are suitable for skin treatment products and for makeup cosmetics, which are more durable on the surface of the skin. The use of cyclodextrin can prevent the growth of microorganism and improve the antimicrobial efficacy of the talc powders. A solution of iodine-cyclodextrin can be used as deodorant for the body, for bath etc. Most perfume concentrates (rose oil, citral, and citronellal), aromatic essential oils can be stabilized by complex formation with CD and can be used in solid preparations, such as powdered detergents, perfumed tablet that dissolve easily in bath water. In toothpaste preparation, the inclusion of a fragrance in a cyclodextrin is worthwhile simply for better stability, but it can also be a good indicator of duration of tooth brushing. Fragrance compounds can be stabilized by cyclodextrin and coated with oils and incorporated into soap[19].

Utilization of cyclodextrins depicted in table 5[8].

Table 5: Utilization of cyclodextrins

Drug/cyclodextrin	Trade name	Formulation	Company(country)
α-Cyclodextrin (αCD)			
Alprostadil	Caverject Dual	i.v.solution	Pfizer(Europe)
Cefotiam-hexetil HCL	Pansporin T	Tablet	Takeda(Japan)
OP-1206	Opalmon	Tablet	Ono(Japan)
PEG_1	Prostavastin	Parenteral solutions	Ono(Japan) Schwarz(Europe)
β-Cyclodextrin (βCD)			
Benexate HCl	Ulgut, Lonmiel	Capsule	Teikoku(Japan) Shionogi(Japan)
Cephalosporin	Meiact	Tablet	Meiji Seika(Japan)
Cetrizine	Cetrizin	Chewing Tablet	Losan Pharma (Germany)
Chlordiazepoxide	Transillium	Tablet	Gador(Argentina)
Dexamethasone	Glymesason	Ointment, Tablet	Fujinaga(Japan)
Dextromethorphan	Rynathisol		Synthelabo(Europe)
Diphenhydramin and chlortheophyllin	Stada-Travel	Chewing Tablet	Stada(Europe)
Iodine	Mena-Gargle	Solution	Kyushin(Japan)
Meloxicam	Mobitil	Tablet and Suppository	Medical Union Pharmaceuticals(Egypt)
Nicotine	Nicorette	Sublingual Tablet	Pfizer(Europe)
Nimesulide	Nimedex	Tablet	Novartis(Europe)
Nitroglycerin	Nitropen	Sublingual Tablet	Nihon Kayaku(Japan)
Omeprazole	Omebeta	Tablet	Betafarm(Europe)
PEG_2	Prostarmon E	Sublingual Tablet	Ono(Japan)
Piroxicam	Brexin, Flogene, Cicladon	Tablet, Suppository	Chiesi(Europe) Ache(Brazil)
Tiaprofenic acid	Surgamyl	Tablet	Roussel-Maestrelli(Europe)
2-Hydroxypropyl-β-cyclodextrin (HPβCD)			
Alfaxalone			
Cisapride	Propulsid	Suppository	Janseen(Europe)
Hydrocortisone	Dexocort	Solution	Actavis(Europe)
Indomethacin	Indocid	Eye drop solution	Chauvin(Europe)
Itraconazole	Sporanox	Oral and i.v. solution	Janseen(Europe,USE)
Mitomysin	MitoExtra, Mitozytrex	i.v.infusion	Novartis(Europe)

Sulfobutylether β-cyclodextrin sodium salt (SBEβCD)			
Aripiprazol	Abilify	i.m.solution	Bristol-Myers Squibb(USA);Otsuka Pharm.(USA)
Maropitant	Cerenia	Parenteral Solution	Pfizer Animal Health(USA)
Voriconazole	Vfend	i.v.solution	Pfizer(USA, Europe,Japan)
Ziprasidone mesylate	Geodon, Zeldox	i.m.solution	Pfizer(USA,Europe)
Randomly methylated β-cyclodextrin (RMβCD)			
17β-Estradiol	Aerodiol	Nasal Spray	Servier(Europe)
Cloramphenicol	Clorocil	Eye drop solution	Oftalder(Europe)
Insulin		Nasal spray	Spain
2-Hydroxypropyl-γ-cyclodextrin (HPγCD)			
Diclofenac Sodium salt	Voltaren	Eye drop solution	Novartis(Europe)
Tc-99 Teoboroxime	Cardio Tec	i.v.solution	Bracco(USA)

Determination of CD-complexes

There are several instrumental techniques available to characterize the complex formation are discussed below,[5, 8, 11, 13, 19]

1) Phase–solubility analysis

Phase–solubility analysis of the effect of complexing agents on the compound being solubilized is a traditional approach to determine not only the value of the stability constant but also to give insight into the stoichiometry of the equilibrium. Experimentally, an excess of a poorly water-soluble drug (i.e. a substrate or drug, D) is introduced into several vials to which a constant volume of an aqueous vehicle containing successively larger concentrations of the CD are added. The need for excess drug is based on the desired to maintain as high a thermodynamic activity of the drug as possible. The vials are shaken or otherwise agitated at constant temperature until equilibrium is established. The suspensions are then filtered and the total concentration of the drug (Dt) determined based on appropriate analytical techniques (UV spectrophotometry, HPLC, etc). The phase–solubility profile is then constructed by assessing the effect of the CD on the apparent solubility of the drug (D). The practical and phenomenological implications of phase–solubility analysis were developed by Higuchi and Connons in their pioneering work published in 1964 and as later reviewed by Connons. Based on the shape of the generated phase–solubility relationships, several types of behaviors can be identified.

2) High performance liquid chromatography (HPLC)

Carolina et al., prepared an inclusion complex between lidocaine (LDC) and hydroxypropyl-β-CD (HP-β-CD) and characterized the inclusion complex by thermal analysis (DSC), UV absorption and HPLC. They found that the rate of LDC release decreased after complexation and thermodynamic parameters from the HPLC studies revealed that a stable complex was formed.

3) Circular dichroism

Marconi et al., worked on the conformational and circular dichroism studies on cyclodextrin inclusion complexes. They presented the structure of inclusion complexes between cyclodextrins and several chromophores of photochemical interest. The method proposed by them proves to be a suitable enough to elucidate different geometrical configurations and to gain insight into the relationship between structural and dynamic properties of the complexes.

4) Nuclear magnetic resonance (NMR)

Only NMR studies prove that a complex is formed. A shift in the peaks can be observed for both the guest and CD .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. 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[17,18,29].

5) **X-ray powder diffraction analysis (XRPD)**
Prepared the inclusion complexes of piroxicam and beta-cyclodextrin by different methods such as physical mixture, kneading, coprecipitationevaporation and heating under reflux. XRPD analysis of different 1:1 complexes showed that there was increase in the halo of the diffractograms seen between the arbitrary units of 0 and 0.25 (y-axis) formed on mixing of two compounds and the reduction of intensity of the prominent piroxicam peak at 2θ=8.7o and resulted in increase in the volume of the beta-cyclodextrin due to inclusion of piroxicam.

6) **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. In DSC, The samples (10 mg) are placed in aluminum pans and the experiments run in a calorimeter at a 10°C/min heating rate over a wide range (0-450°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., 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°C respectively corresponding to their melting point and HP-β-CD also gives a peak at 50°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 °C and 116.0°C whereas the solid complex of RVC/HP-β-CD presents only a broaden peak at 248.2°C. The absence of fusion peak of pure RVC at 117.6°C in the thermogram and the shift of endo or exothermic peaks of drugs is a clear indication of the complexation phenomenon. In DSC or TGA, the guest must have a melting or boiling temperature below about 300°C, the temperature at which cyclodextrins decomposes. In DSC, no energy absorption is observed at the melting temperature of the guest when the guest is complexed. With both DSC and TGA, an increase in the boiling temperature (about 10°C) is observed because of interaction of the guest with cyclodextrin provides a higher energy barrier to overcome for volatilization[22].

7) **Ultraviolet-visible (UV-Vis) spectroscopy**
Spectrophotometric methods are useful to determine the value of stability constant, if the complexation events induce changes in the compound spectra as a function of the guest-host interaction. These changes in the compound spectra generally reflect an alteration in the microenvironment of the drug. The changes observed in UV and related processes are similar to those associated with dissolution of the drug in a solvent of decreased polarity.

8) **Differential solubility**
Determined the concentration of free and bound piroxicam from piroxicam-β-cyclodextrin complex using differential solubility method and compared these results with the results of conventional DSC. In this method, a mixed solvent system of water-acetonitrile (1:1, v/v) is used to measure the total drug concentration in complex form and free drug in solution.

9) **FTIR and FT-Raman spectroscopy**
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. Bratu et al.,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.

FT-Raman spectroscopy has also been used to characterize the inclusion complexes. Shifts or changes in the spectrum occur due to the Complexation.

Regulatory status

The regulatory status ofCDs continues to evolve. βCD is listed in a number of pharmacopoeia sources including the US Pharmacopoeia/National Formulary (USP/NF), European Pharmacopoeia (Ph.Eur.) and Japanese Pharmaceutical Codex (JPC). αCD is similarly listed in the Ph.Eur., USP/NF and JPC and γCD is referenced in the JPC and will soon be included in the Ph.Eur. andUSP/NF.Amonograph forHPβCDis available in the Ph.Eur. and a draft has been circulated for the USP/NF. Other derivatives are not yet compendial but efforts are underway for their inclusion. αCD, βCD and γCD were also introduced into the generally regarded as safe (GRAS) list of the FDA for use as a food additive in 2004, 2001 and 2000, respectively, and HPβCD is cited in the FDA's list of Inactive Pharmaceutical Ingredients. SBEβCD is also available in various dosage forms and is also listed in the FDA's compilation of Inactive Pharmaceutical Ingredients. Consensus seems to be building among regulators that CDs are excipients and not part of the drug substance although various opinion have been given and interpretation related to this point can be division and product-specific[8].

CONCLUSION

The ability of cyclodextrins to form inclusion complexes with many guest molecules by taking up a whole molecule or some part of it, into the cavity place cyclodextrins is a unique class of encapsulation technique. This type of molecular encapsulation will affect many of the physicochemical properties of the guest molecules. The ability of cyclodextrins to form complexes with a wide variety of organic compounds helps to alter the apparent solubility of the molecule, to increase the stability of compound in the presence of light, heat and oxidising conditions and to decrease volatility of compound. In conclusion, due to the unique architecture and the chelating properties, cyclodextrins are becoming an important part of the biotechnologist options in the horizons of biocatalysts, encapsulation and control release and in many pharmaceutical applications.

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