CYCLODEXTRIN-BASED PHARMACEUTICS: PAST, PRESENT AND FUTURE

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Abstract | Cyclodextrins are cyclic oligomers of glucose that can form water-soluble inclusion complexes with small molecules and portions of large compounds. These biocompatible, cyclic oligosaccharides do not elicit immune responses and have low toxicities in animals and humans. Cyclodextrins are used in pharmaceutical applications for numerous purposes, including improving the bioavailability of drugs. Current cyclodextrin-based therapeutics are described and possible future applications discussed. Cyclodextrin-containing polymers are reviewed and their use in drug delivery presented. Of specific interest is the use of cyclodextrin-containing polymers to provide unique capabilities for the delivery of nucleic acids.

α-1,4- AND α-1,6- GLYCOSIDIC LINKAGES
The D-glucopyranoside unit contains six carbons and two of these units can be chemically linked from the 1-carbon of a unit to either the 4-carbon or the 6-carbon of the second unit.

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Cyclodextrins (CDs) comprise a family of cyclic oligosaccharides, and several members of this family are used industrially in pharmaceutical and allied applications. CDs are manufactured from starch, one of the two glucose-containing polymers produced by photosynthesis (the other is cellulose). Starch consists of D-glucopyranoside building blocks that have both α-1,4- AND α-1,6-GLYCOSIDIC LINKAGES. The degradation of starch (which is primarily derived from corn, but also from potatoes and other sources) by the enzyme glucosyltransferase generates, by chain splitting and intramolecular rearrangement, primary products that are cyclic oligomers of α-1,4-D-glucopyranoside, or CDs. FIGURE 1 shows several schematic representations of β-CD. CDs derive their system of nomenclature from the number of glucose residues in their structure, such that the glucose hexamer is referred to as α-CD, the heptamer as β-CD and the octomer as γ-CD (FIG. 2). There are literally thousands of variations of CDs that have variable ring size and random or site-specific chemical functionalization. A comprehensive overview of all aspects of CDs is available[1].

The earliest reference to a substance that was later recognized as a CD was published in 1891 (REF. 2). By 1953, Freudenberg *et al.*[3] had received the first patent on the use of CDs in drug formulations. This patent covered most of the important concepts that are used even today, including the improvement of drug properties such as increased aqueous solubility and increased stability towards drug oxidation. Currently, CDs have found uses in many applications, such as in agrochemicals, pharmaceuticals, fragrances, foods and so on. This review will concentrate on the pharmaceutical uses of CDs.

The basis of CDs as pharmaceutical excipients

The three-dimensional structure of CDs endows them with properties that are useful for pharmaceutical applications. Because of the large number of hydroxyl groups on CDs, they are water-soluble. The water solubilities of α-, β- and γ-CD at ambient conditions are approximately 13%, 2% and 26% (weight by weight (w/w)), respectively (for β-CD this is approximately 18.8 g per l or 16.6 mM)[1]. The lower solubility of β-CD compared with α-CD, even though the former contains a higher number of hydroxyl groups than the latter, is due to the formation of an internal hydrogen-bond network between the secondary hydroxyl groups. The disruption of hydrogen bonding via molecular manipulation gives rise to an increase in water solubility. For example, hydroxypropyl-β-CD (HPβCD) has an aqueous solubility of 60% (w/w) or more[4]. Although the entire CD molecule is water soluble, the

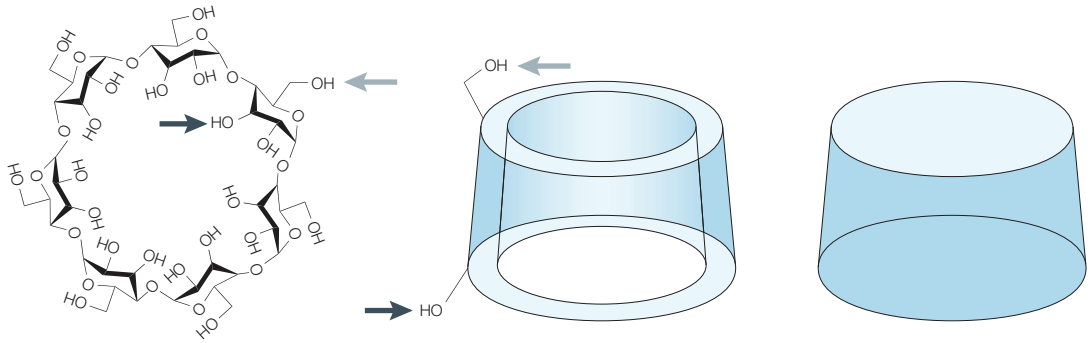


Figure 1 | **Schematic representations of β-cyclodextrin.** The open and closed arrows point to primary and secondary hydroxyl groups, respectively. The cyclodextrin (CD) architecture is a cup that is 0.79 ± 0.01 nm from top to bottom (primary OH face to secondary OH face), and is slightly larger on the face containing secondary hydroxyl groups. The cavity (0.47–0.53, 0.60–0.65 and 0.75–0.83 nm for α-, β- and γ-CD, respectively) and exterior diameters of the CDs (1.46 ± 0.04, 1.54 ± 0.04 and 1.75 ± 0.04 nm for α-, β- and γ-CD, respectively, for the faces containing secondary hydroxyl groups) expand as the number of glucopyranoside units increase[1].

interior of the cup is relatively apolar and creates a HYDROPHOBIC micro-environment. CDs therefore have HYDROPHILIC cavity exteriors and hydrophobic cavity interiors. These properties are responsible for their aqueous solubility and ability to encapsulate hydrophobic moieties within their cavities, and the incorporation of 'guest' molecules in CD inclusion complexes in aqueous media has been the basis for most pharmaceutical applications. A dynamic equilibrium between free CDs, free drug molecules and their formed inclusion complexes is established if drug molecules are of sufficient size and have appropriate properties for the formation of inclusion complexes. FIGURE 3 schematically illustrates this dynamic equilibrium for 1:1 and 1:2 drug–CD complexes. The formation of inclusion complexes is possible with the entire drug molecule or only a portion of it. FIGURE 3C presents models of how α-, β- and γ-CD can form inclusion complexes with prostaglandin E_2. Because of cavity size, α-CD complexes well with aliphatic chains and molecules such as polyethylene glycol (PEG), whereas β-CD is appropriate for aromatic rings, such as that in paclitaxel.

HYDROPHOBIC
An affinity for, and propensity to dissolve in, non-polar solvents such as hydrocarbons.

HYDROPHILIC
An affinity for, and propensity to dissolve in, water and other polar solvents.

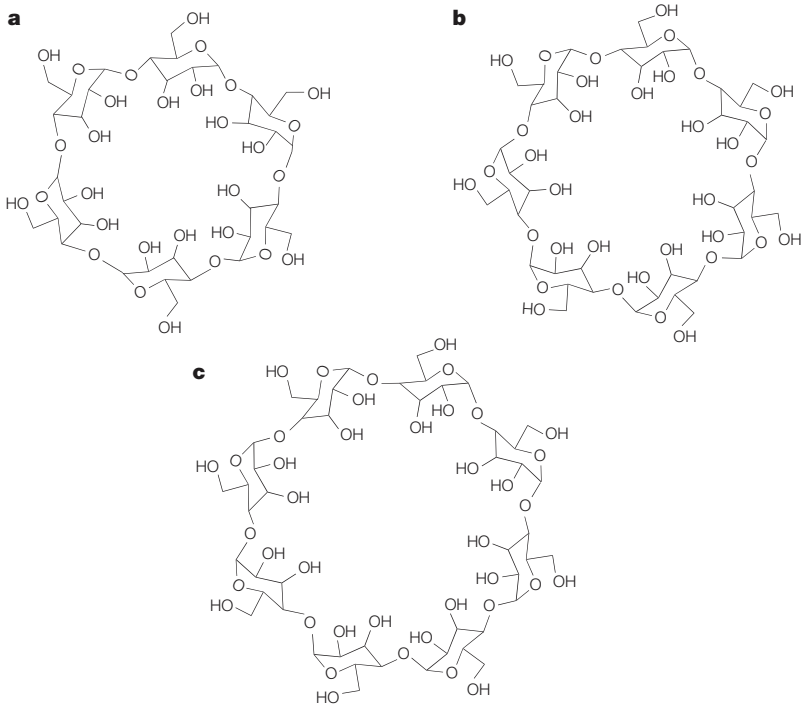


Figure 2 | **Schematic representations of cyclodextrins.** α-CD (**a**), β-CD (**b**) and γ-CD (**c**) contain 6, 7 and 8 glucopyranoside units, respectively. The molecular masses of α-, β- and γ-CD are 972, 1,135 and 1,297 Da, respectively.

For CDs to be pharmaceutically useful, they must be biocompatible. CDs show resistance to degradation by human enzymes; CDs injected intravenously into humans are therefore essentially excreted intact via the kidney. However, bacterial and fungal enzymes (amylases) can degrade CDs. Ingested CDs can therefore be metabolized in the colon prior to excretion. The toxicities of CDs are dependent on their route of administration. For example, the dose that causes 50% death (LD_{50}) values of α-, β- and γ-CD administered intravenously into mice are approximately 1.0 g per kg[5], 0.79 g per kg[5] and more than 4.0 g per kg[6], respectively. β-CD has an affinity for cholesterol and can extract it and other lipid membrane components from cells. At sufficiently high concentrations, β-CD can cause haemolysis of erythrocytes. Additionally, parenteral administration of β-CD is not possible because of its poor solubility (which leads to microcrystalline precipitation in the kidney), as well as the fact that it forms complexes with cholesterol that accumulate in the kidney and produce renal tubule damage. Functionalized β-CDs can mitigate these problems.

Chemically modified CDs result from etherification or the introduction of other functional groups at the 2-, 3- and 6-hydroxyl groups of the glucose residues. These changes improve solubility through two mechanisms: by breaking the 2-OH–3-OH hydrogen bonds, and by preventing crystallization due to creation of a statistically substituted material that is made up of many isomeric components and gives rise to an amorphous product.

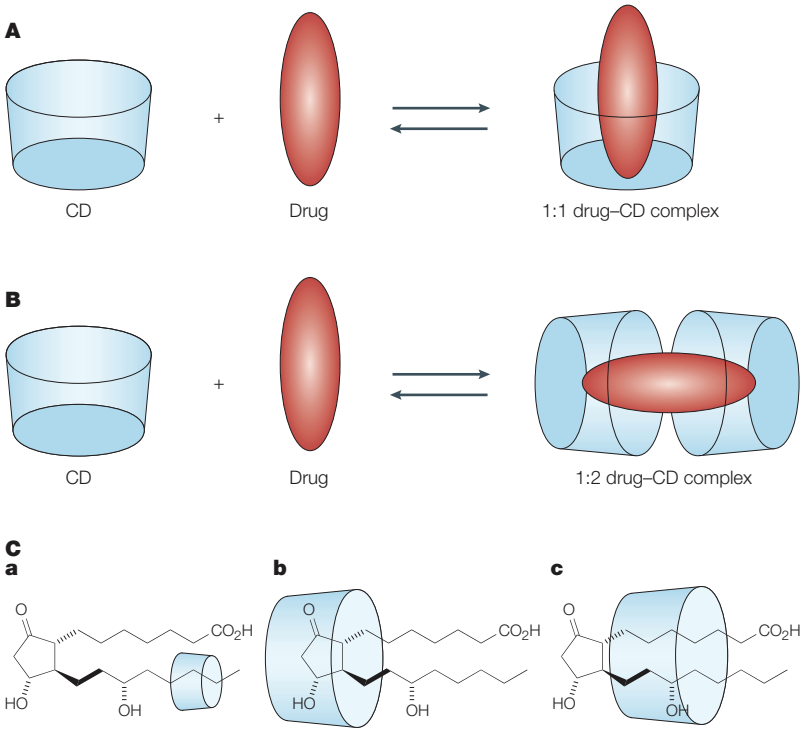


Figure 3 | **Schematic illustration of the association of free cyclodextrin (CD) and drug to form drug–CD complexes. A** | 1:1 drug–CD complex. **B** | 1:2 drug–CD complex. **C** | Proposed models of inclusion complexes between prostaglandin E_2 and (**a**) α-CD, (**b**) β-CD and (**c**) γ-CD. Adapted from REF. 1.

The complexity of these mixtures can be appreciated by considering β-CD. For this compound, there are 21 hydroxyl functional groups and therefore $2^{21}-1$ possible combinations for substitutions (that is, more than 2 million). If an optically active centre is introduced, as in the case of 2-hydroxypropylation, the number of geometrical and optical isomers is truly astronomical, given that the β-CD nucleus contains 28 chiral centres. It is conceivable that the pharmaceutical performance of these isomeric mixtures can change with the extent and degree of substitution, and so these factors have to be assessed and specified in the excipient. In practice, this is done by analogy with other chemically modified pharmaceutical starches and celluloses, such as hydroxypropyl cellulose and hydroxylpropylmethyl cellulose. Both the European Pharmacopeial monograph and the proposed United States Pharmocopeial monograph on HPβCD, for example, specify that the material should have a molar substitution (expressed as the number of hydroxypropyl groups per anhydroglucose unit) between 0.4 and 1.5; this means 2.8–10.5 hydroxypropyl functional groups per cyclodextrin molecule. They also specify that less than 1.5% unmodified β-CD should be present. The molar substitution can be determined using nuclear magnetic resonance and infra-red methods. Two functionalized CDs, hydroxypropyl β-CD (HPβCD) and sulphobutyl ether β-CD (SBEβCD), are available in FDA-approved products for human use (see below). In addition, CDs do not produce an immune response in mammals. Because of these highly desirable properties, CDs have found numerous pharmaceutical applications; reviews on the use of CDs in drug delivery are available[7–13].

Pharmaceutical applications of CDs

Current pharmaceutical research has a number of drivers, including the nature of the drugs being developed, the need for generating orally bioavailable dosage forms and the preparation of solubilized parenteral formulations. Drug discovery has evolved over the years to the point that high-throughput screening techniques have become routine. These approaches put significant evolutionary pressure on emerging drug candidates, and this has led to a systematic increase in molecular mass, lipophilicity and a decrease in water solubility for lead compounds over time[14,15]. This, in turn, has had a significant impact on what is required from drug delivery formulators, in that the number of formulation options has had to be increased to address the larger diversity of challenges presented.

For a drug to be orally available, the compound must dissolve and be absorbed through the gastrointestinal tract in such a way that it generates adequate drug levels at the pharmacologically active site to ensure that the desired action is obtained in a reproducible manner. Retrospective studies show that >40% of drug failures in development can be traced to poor biopharmaceutical properties, specifically poor dissolution or poor permeability[16]. In recognition of the importance of these factors, the FDA and other drug regulatory organizations have defined a Biopharmaceutical Classification System in which drugs are divided into four types on the basis of their solubility and permeability characteristics (FIG. 4)[17–19]. High-throughput drug discovery methodologies are increasingly selecting difficult Type II compounds, and CDs can be an important enabling technology for these compounds in particular[20,21]. By increasing the apparent water solubility of a drug candidate, formulations can be generated such that a Type II material behaves like a Type I compound, with a resulting increase in oral bioavailability[20,22] (BOX 1).

The reasons for the inclusion of CDs in a particular formulation can vary widely (BOX 2)[23], and are specific to the circumstance — that is, the specific physicochemical issues that have to be overcome and the administration route[24–26].

Initial applications of α- and β-CDs: prostaglandin and nonsteroidal anti-inflammatory agents. CDs first came to the fore in marketed products as drug delivery technologies that enabled the development of various prostaglandins[27,28]. One of first of these compounds, PGE_2, a substance with potent oxytocin-like effects, was of interest as a possible agent for the induction of labour in childbirth[29,30]. As with other members of the E-type prostoglandins, these compounds are highly unstable, and this feature complicated their formulation and development. β-CD complexes of PGE_2 resulted in a significant increase in their solid-state stability, and a product designed along these lines

WETTABILITY
The wettability of a liquid is defined as the contact angle between a droplet of the liquid in thermal equilibrium on a horizontal surface.

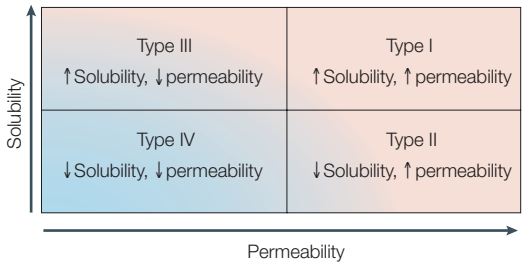


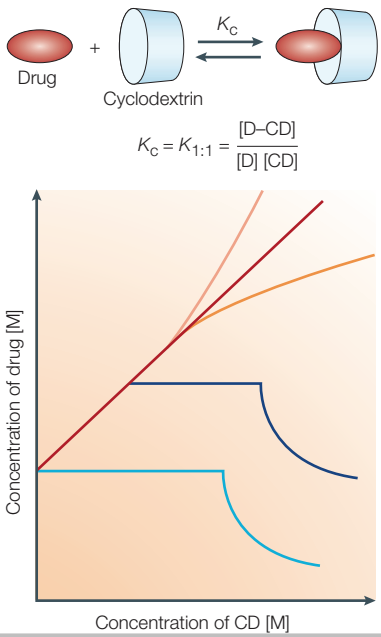
Figure 4 | **Biopharmaceutical Classification System (BCS) characterization of drugs based on solubility and permeability measures.** Drug solubility is defined by the dose/solubility ratio, with a soluble drug defined as one in which the highest dose intended for human use will dissolve in 250 ml of water (the so-called FDA glass of water). Permeability can be defined by various *in vivo* or *in vitro* assays, but a permeable drug is one associated with ≥90% oral bioavailability or ≥90% absorption as assessed by urinary excretion data.

(dinoprostone betadex; Prostarmon E; Ono) was approved for the Japanese market in 1976 (TABLE 1). Prostarmon E is highly effective and represented a significant medical advance, especially for the induction of labour in oxytocin-insensitive individuals, but also for its tendency to produce less bleeding after delivery.

The second prostaglandin marketed as a CD complex was PGE_1 (alprostadil alphadex; Prostavasin/Edex/Caverject/Prostandin; Schwarz Pharma). PGE_1 relaxes smooth muscle and increases blood flow, and was initially developed as a therapeutic to treat peripheral circulatory disorders. Given its limited metabolic stability, initial therapies with PGE_1 required intra-arterial administration to obtain useful clinical results.

Box 1 | **Solubilization with cyclodextrins**

Cyclodextrins (CDs) can enhance apparent water solubility by forming dynamic, non-covalent, water-soluble inclusion complexes as depicted in the figure. This interaction is an equilibrium governed by an equilibrium constant, K_c. The nature of the complex, as well as the numerical value of the equilibrium constant, can be derived from from measuring a particular property of the complex as a function of drug and CD concentrations. In phase-solubility analysis, the increased solubility is assessed as a function of CD concentration. As illustrated, a number of solubility profiles are possible, each giving insight into the type of complex formed, as well as its stoichiometry. An A-type profile (red line) represents the formation of soluble CD complexes, whereas B-type systems (blue line) indicate the formation of complexes of limited solubility.



On the basis of this administration route, the stabilizing effect of α-CD on PGE_1 and the suitability of this CD for parenteral use (unlike β-CD), formulations of PGE_1/α-CD complexes were developed. In 1979, alprostadil alphadex (Prostavasin) was approved for the treatment of peripheral vascular complications, including Buerger's disease[29,30]. The compound also showed activity against chronic arterial occlusions and arteriosclerosis.

Another vascular malady that can be treated with alprostadil alphadex is male erectile dysfunction, for which the complex is given by intracavernous injection[31–34]. The PGE_1–α-CD complex was found to be effective in subjects who were not responsive to sildenafil citrate (Viagra; Pfizer), an oral inhibitor of phosphodiesterase-5[35]. In a study of 67 patients that failed sildenafil citrate therapy at 50 and 100 mg, 85–90% reported improvements in erectile function when PGE_1–α-CD was self-administered after 'at-home' treatment. Caverject Impluse is approved for use in the United States on the basis of these medical needs.

The complexity of the administration route prompted the development of more convenient dosing options, including intravenous dosage forms. These drivers resulted in a modified formulation (Prostandin), which was approved in 1982 in Japan and subsequently in a number of other countries, including Germany[29,30].

A third example of a prostaglandin marketed as a CD complex is limaprost alphfadex (Opalmon/ Prorenal; Ono)[29,30,36,37]. This prostaglandin analogue was developed for the treatment of vascular disease and was shown to have improved antiplatelet aggregation and vasodilation activity relative to PGE_1. Importantly, the compound was orally available and showed a good separation between its therapeutic action and unwanted side effects (mainly oxytocin-like effects). The limoprost–α-CD complex was found to be safe and effective in the treatment of Buerger's disease and was approved in 1988.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a mainstay for the treatment of pain[38]. The major drawbacks of these otherwise useful compounds include upper gastrointestinal irritation and bleeding[39]. Piroxicam (Feldene; Pfizer) is an illustrative example. The drug is useful in the treatment of osteoarthritis and rheumatoid arthritis, as well as gout, acute musculoskeletal disorders and dysmenorrhea[40–43]. It has a relatively long pharmacokinetic half-life, meaning that it can be taken once a day, in contrast to many other NSAIDs. The parent drug is also poorly water soluble (~30 μg per ml), poorly WETTABLE (that is, with a contact angle of 70°) and highly crystalline (with a melting point of 202 °C and a ΔH_{melt} of 106 J per g)[44–47]. This imparts to the compound poor dissolution properties, as well as dissolution-limited oral pharmacokinetics.

CDs were applied to this compound in an effort to improve several properties, including safety and drug dissolution rate. These improved characteristics reduced gastrointestinal irritation, and allowed more rapid drug absorption and a more rapid onset of the analgesic effect. Complexation studies indicated that a molar ratio of 2.5 per 1 was optimal for the drug–CD combination

and, given the low piroxicam dose (20 mg), this did not add excessive bulk to the formulation[48]. The apparent solubility of piroxicam in the β-CD formulation was increased fivefold relative to the uncomplexed drug substance. This enhanced solubility was associated with an increased dissolution rate and higher plasma levels at early time in humans, which, in turn, directly correlated with an increased absorption rate[48,49]. There was no change in terminal half-life or total area under the curve when piroxicam or the piroxicam–β-CD complex were compared. With regard to efficacy, piroxicam–β-CD has been demonstrated to have a faster onset of action in a number of clinical trials[48,50,51].

Although differences in the tolerability of piroxicam and its β-CD complex require the analysis of epidemiological data, acute studies might provide some useful insight. Several studies that have assessed the endoscopic appearance of the stomach of volunteers taking either piroxicam or the piroxicam–β-CD complex have revealed significantly better outcomes in the case of administration of the complexed drug[48,52,53]. Similarly, radio-adhesive substrates demonstrated fewer gastric lesions with the piroxicam–β-CD complex relative to piroxicam[48,54]. Several branded piroxicam–β-CD products are available, including Brexin (Chiesi) and Cicladol (Chiesi).

Applications of randomly methylated β-CD. Randomly methylated β-CD (RMβCD) (FIG. 5) provides good biocompatibility and useful complexing efficiencies, and is beginning to be used in marketed products throughout the world. An eye drop preparation of the antibiotic chloramphenicol has been developed by Oftalder and marketed as Clorocil in Portugal[55]. The recently introduced nasal product Aerodil, which contains RMβCD, is a complex between the indicated CD and β-oestradiol[56–60]. A number of potential advantages are apparent for such an oestradiol delivery system. Although the safety of hormone-replacement therapy has been the subject of recent debate[61,62], its efficacy in reducing menopausal symptomatology is well established. Traditional dosing strategies include oral administration of conjugated equine oestrogens, oestradiol esters or micronized oestradiol, as well as the use of transdermal patches to deliver this sex hormone[63–65]. Although both routes have a number of limitations, both provide relatively constant blood levels of drug, in contrast to endogenous oestrogen release, which tends be more pulsatile.

The RMβCD-based nasal product avoids a number of issues related to oral or transdermal administration[56]. Nasal administration results in direct systemic uptake, and so the first-pass effect is reduced or eliminated. In addition, the nasal route is convenient, non-invasive and provides for consistency of drug absorption. Drug uptake is rapid, and peak plasma concentrations are achieved within 10–30 min of drug dosing. Drug levels also dissipate rapidly and return close to baseline within 2 hours[56]. This pattern is more akin to physiological oestrogen secretion than that associated with oral or transdermal approaches. Another feature of this route is that, unlike oral dosing of oestrogens that generate a high oestrone/oestradiol ratio, nasal delivery gives rise to a more physiological ratio of the two hormones[56–60].

A number of clinical trials have confirmed these product design principles. Studd *et al.* studied 420 postmenopausal women and found that the nasal product based on oestradiol and RMβCD was effective in ameliorating menopausal symptoms as early as four weeks after initiation of therapy in a dose-dependent manner, and continued to improve the post-menopausal symptoms even after 12 weeks[56]. The minimum effective oestradiol dose was 200–400 μg per day. These doses were similar in efficacy to 2 mg of oestrogen administered orally, although the reduced systemic exposure results in a potentially improved safety profile. Inter- and intrasubject variability was less than that demonstrated in the oral oestrogen group.

Applications of hydroxypropylated β-CD. Two hydroxypropylated CDs (HPβCDs) have been approved in various world markets (United States and Europe)[25,27,66–69]. HPβCD (FIG. 5) is available in registered oral, intravenous, buccal, rectal and ophthalmic products, whereas HPγCD is available in an eye drop formulation that contains the anti-inflammatory agent diclofenac sodium[70]. Of the HPβCD products, the oral and intravenous solutions of itraconazole (Sporanox; Janssen) have the most widespread use[71].

Itraconazole is a triazole-type drug that exerts its effect by inhibiting fungal cytochrome P450 and inhibiting the biosynthesis of ergosterol, an essential component of the fungal membrane. The compound is noteworthy in that it was the first approved orally bioavailable agent with significant clinical activity against both *Candida spp.* and *Aspergillus spp.*, the two most common human fungal pathogens[72–75]. Formulation development for this drug was complicated by its challenging set of physico-chemical properties, which include a pK_a of 4, a LOG P >5 and an aqueous solubility at neutral pH estimated at 1 ng per ml[71,76]. The production of solid oral dosage forms was eventually made possible by using solid solution technology in which the drug and a polymeric carrier (hydroxypropyl methylcellulose (HPMC)) were sprayed on inert sugar spheres to form a thin film. As the film dissolves in the stomach, the molecularly dissolved drug is released at supersaturated levels. The co-dissolving HPMC inhibits

Box 2 | Pharmaceutical applications of CDs

Cyclodextrins (CDs) can be used to achieve the following:

- **Enhance solubility**
- **Enhance bioavailability**
- **Enhance stability**
- **Convert liquids and oils to free-flowing powders**
- **Reduce evaporation and stabilize flavours**
- **Reduce odours and tastes**
- **Reduce haemolysis**
- **Prevent admixture incompatibilities**

LOG P
The logarithm of the partition coefficient of a substance in octanol–water.

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