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Review article

Use of cyclodextrins in topical formulations: Practical aspects

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Abstract

Cyclodextrins are promising, but using them as an excipients can sometimes be difficult. The aim of this review is to summarize the results of recent studies on the application of parent cyclodextrins (α-, β-, γ-) and some of their derivatives in topical formulations. General properties, current legal status, and toxicological aspects of cyclodextrins are briefly described. The goal of using cyclodextrins to create new formulations with well-known actives, advantages, and limitations in topical formulations is presented, and possible applications in such preparations are also discussed.

Keywords: Cyclodextrins; Topical preparations; Skin penetration; Mucosal delivery; Ocular delivery; Toxicity

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of at least six D-(+)-glucopyranose units linked by α-(1–4) bonds (Fig. 1a) [1]. Natural CDs occur in the form of white crystalline powder, and they form stable hydrates. CDs have quite rigid structures (stabilized by hydrogen bonds between C_2 and C_3 hydroxyl groups) lacking free rotation in α-(1–4) bonds; thus they form torus-like molecules (truncated cone) (Fig. 1b) [1]. CD molecules have a hydrophilic outer surface (all hydroxyl groups in the ring are located in the exterior of torus) and a hydrophobic interior (there are skeletal carbons with hydrogen atoms and oxygen bridges inside the cavity). The non-bonding electron pairs of the oxygen bridges are directed toward the inside cavity, thereby generating high electron density [2,3].

While CDs were first isolated by Villiers in 1891, the main characteristics, preparation, and isolation technique were described by Schardinger [4,5]. In the beginning, only very small amounts of CDs were produced. It was only in the late 1970s when biotechnological development enabled production of purified CDs with high yield that the ''career'' of CDs started.

There are three main natural CDs: α-, β-, and γ-CD composed of six, seven, and eight glucose units, respectively. They differ in ring size and physicochemical properties (Table 1). It is possible to achieve higher homologues, but because of their properties – large cavity dimension, high aqueous solubility, and weak complex formation – they cannot be of practical use. The CDs contain 18 (α-CD), 21 (β-CD), or 24 (γ-CD) hydroxyl groups that can be chemically modified. To improve some physicochemical properties of natural CDs, many types of derivatives have been developed: hydrophilic (methylated, hydroxyalkylated, and branched), hydrophobic (ethylated), ionic (sulphated and phosphated) [2–5]. Derivatisation of parent crystal CDs usually leads to achieving amorphous mixtures of isomers; thus, their aqueous solubility is much higher [6].

The most important attribute of CDs is the ability to create inclusion complexes with a large number of molecules or their portions; however, not all molecules (drugs) can form stable complexes. There are some limitations, like very high aqueous-soluble substances, that generally cannot be included. Recently Martins et al. [7] reported that high soluble drug substances are able to create with CDs

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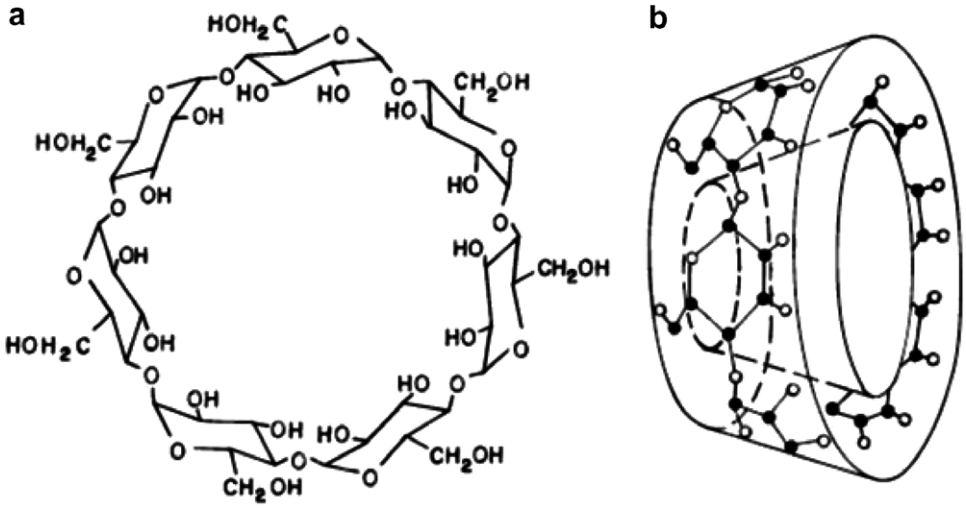

Fig. 1. Structure (a) and torus-like shape (b) of β-CD molecule [1].

rather an association compound in which drug interacts with the hydrophilic outer surface of CD (hydroxyls at position 3).

The size – geometric factor of the molecule is most important because it decides whether the molecule is able to form "stable" inclusion with α-, β-, or γ- CD. If the molecule had adequate properties, it interacts with CD inside cavity without forming covalent bonds; this interaction is "guest/host" type. CD inclusion complex is mainly formed via the substitution of included water by the appropriate "guest" molecule. Release of the enthalpy-rich water molecules from the cavity decreases the energy of the system. A decrease in the energy of the system is due to reduce the contact surface area between the solvent and solute as well as solvent (highly polar water) and imperfectly solvated (hydrophobic) CD cavity. Some other factors, such as hydrogen bonding, changes in surface tension, van der Waals' interactions, and ring strain release, also can have some influence on the complex formation. The complexation is usually a concentration-dependent process and the molar ratio (1:1, 1:2, 2:1, 2:2) can depend on the "guest/host" proportion. It is possible that in solution the molecule interacts with the outer surface of CD and CD complexes agglomerate (self-association) [8–10].

The association/dissociation equilibrium in aqueous solution is one of the most characteristic features of inclusion. Drug release from the CD complex is mainly caused by dissociation due to dilution in fluids. In the case of topical applications, such as ocular, nasal, rectal, or dermal, with minimal or impossible dilution mechanism, the potential mechanism of drug release from CD complex is preferential drug uptake by tissue [11]. As reported by Stella et al. [11] if the drug substances possesses physicochemical properties that allow it to penetrate into or through biological membranes (skin, mucosa, or cornea), then the tissue acts as a sink causing dissociation of the complex. Only a free fraction of drug that is in equilibrium with the complexed fraction may be available for absorption, thus CDs are able to increase bioavailability rather by deliver the drug substance to absorption site and by minimisation of drug hydrophobicity than permeation by itself. The penetration into or permeation through the biological membranes of inclusion complex and CDs are questionable because of their large mass (>1000 Da) and hydrophilicity [3,11–16]. However, there are few works that demonstrate some absorption of CDs by pulmonary, dermal (occluded) or transmucosal route, probably through paracellular pathway [17,18]. There are also suggestions that certain CDs (especially methylated) are able to extract membrane components [3,18]. This problem is wider discussed in Section 2.

2. Toxicological aspects

Natural CDs are regarded as a non-irritant to skin, eyes, and mucosa upon inhalation [2]. However, α-CD may cause some non-corrosive eye irritation. Among CD derivatives, HP-β-CD (HP – hydroxypropylated) is regarded as safe and non-irritating, while methylated CD can cause serious irritations and even corrosion to the eye [3]. Jansen et al. [19] studied the toxicity of solutions containing 12.5% of HP-β-CD and 12.5% or 5% of DM-β-CD (DM – dimethylated) on the corneal epithelium of albino rabbits after single and multiple administrations. They concluded that DM-β-CD is toxic to the cornea in concentrations of 12.5% and 5%, whereas HP-β-CD in concentration of 12.5% is well tolerated. Cytotoxic studies on human corneal epithelia cell line indicated that cytotoxicity of CDs decreased in the following order: α-CD > DM-β-CD > SBE-β-CD (SBE – sulfobutylated) = HP-β-CD > γ-CD [20]. Toxic effect of α-CD and DM-β-CD appeared very quickly (just after 5 min). Cytotoxicity of CD on corneal membranes may be due to its interaction with membranes components, such as cholesterol, phospholipids, and proteins [2,20,21]. However, there is commercially available eye drops drug product (Clorocil®)

Table 1
Physicochemical properties of CDs [2,3]

CD type	Glucose units	Molecular weight	Aqueous solubility [%; w/w]			Cavity diameter [Å]	Cavity volume [Å^3]	Crystal water content [%; w/w]
			20 °C	25 °C	35 °C			
α-CD	6	972	9.0	12.7	20.4	4.7–5.3	174	10.2
β-CD	7	1135	1.64	1.88	2.83	6.0–6.5	262	13.2–14.5
γ-CD	8	1297	18.5	25.6	39.0	7.5–8.3	427	8.13–17.7

containing RM-β-CD (RM – randomly methylated) and chloramphenicol.

For evaluation of the influence of CDs on membrane irritation, damage, or induced cytotoxicity, hemolysis data are useful [3,22]. The haemolytic effect depends on the type (for parent CDs it is in following order: β-CD > α-CD > γ-CD) and concentration (strongly) of CD [3,23], and that of methylated CD is much higher than other CDs [23].

As described by Ohtani et al. [24], CDs extract the lipids from membrane without entry into the membrane. The CDs form a new lipid containing compartment in the aqueous phase that equilibrates freely with the cell surface [24]. α-CD is able to solubilising rather phospholipids than sterols, while β-CD interact mainly witch cholesterol and proteins [18,24,25]. Derivatisation had a big influence on CDs ability to solubilise membrane ingredients, as presented by Motoyama et al. [26]. DM-α-CD markedly release proteins from rabbits red blood cells in opposite to HP-α-CD and α-CD that interact only with phospholipids [26].

Application of CD solution directly onto the mucosa (nasal, buccal or vaginal) is regarded as safe for hydrophilic and natural CDs in a wide range of concentrations, while for methylated CD derivatives, the concentration and the application time should be controlled [21,27,28]. Asai et al. [27] demonstrated that 20% RM-β-CD causes serious damage (cracks) to the rat nasal mucosa and that administration of 10% solution causes irritation and thereafter heavy secretion in paradox protect cell. Even at 5% of RM-β-CD, they noticed some morphological changes in epithelia but only after longer application time. Investigation by Agu et al. [28] on the model of human nasal culture cell demonstrated that changes in ciliary beat frequency caused by 5% DM-β-CD and 5% α-CD are reversible and/or partly reversible, whereas for 10% DM-β-CD changes are irreversible. These results are in good agreement with the studies of Boulmedarat et al. [21] on the toxicity of RM-β-CD on human oral epithelium model cell. There was indicated that 10% RM-β-CD results in toxic and inflammatory effects depending on the exposure time, while 2% or 5% did not induce tissue damage. Romeijn et al. [29] and Boek et al. [30] reported, on the base of studies that were performed *in vitro* using chicken embryo trachea as an tissue model, that methylated β-CD used in nasal formulations (2% or 4% concentration in complexes with estradiol, salmon calcitonin, or dihydroergotamine methansulfonate) had a similar ciliary beat frequency as physiological saline. Test with using cryopreserved human mucosa from the sphenoidal sinus and 2% methylated β-CD solution was also performed, and the ciliary beat frequency was similar for both chicken and human tissues. Also acute histological effects of singly applied 2% DM-β-CD on the epithelium of the nasal cavity *in vitro* were similar to those induced by saline [29]. Methylated β-CD can be regarded as safe below concentrations of 5%; furthermore, *in vivo* conditions – dilution and mucociliary clearance – will provide decrease of CD concentration and irritation in place [21,28]. Calidiol® nasal spray contains estradiol and RM-β-CD and is applied in treatment of osteoporosis in postmenopausal women. It provides very fast drug diffusion to the tissue cells and thus a pulsative therapy. Also HP-β-CD as hydrophilic natural CD is regarded as non-irritant to the mucosa and had no direct influence on mucosa integrity, however it can increases the activity of a lipophilic absorption enhancer. Irie et al. [18] presented that combination of HP-β-CD with 1-[2-(decylthio)ethyl]azacyclopentae-2-one enhanced absorption of CD as well as permeation marker (fluorescein isothiocyanatedextran) via nasal cavity after lag phase. Simultaneous application of CDs with other excipients can potentially influence on their permeation, and not only on permeation of drug substance.

β-CD, applied under occlusion condition onto the skin surface in humans, does not induce irritation or allergenic reaction [2,3]. Skin compatibility with CDs, both natural and a wide range of derivatives, has been summarized recently by Piel at al. [31]. All tested CDs were well tolerated by the *stratum corneum*, with the exception of dimethylated derivatives where changes in corneoxenometry were observed, which can indicate disruption in the lipid bilayers. Overall, natural CDs and their hydrophilic derivatives are not able to permeate skin barrier in significant amounts; thus they are safe for topical applications [3,12,32,33]. As summarized by Loftsson and Masson [12], only 0.02% of the HP-β-CD applied dose was absorbed into mouse skin and only about 0.3% of DM-β-CD dose was absorbed through rat skin. This is in good agreement with the results of Legendre et al. [33] for C^{14}-radiolabeled RM-β-CD, which ones found 0.1–0.2% of this CD in acceptor fluid after 24 h permeation and about 0.7% of CD bounded to the surface of the *stratum corneum*. Even dimethylsulphoxide did not enhance RM-β-CD penetration. Only lipophilic RM-β-CD can interact with membranes more readily but in high concentrations [27,31]. There is also no evidence that CDs, even after prolonged

oral administration, can cause mutagenic or teratogenic effect [2,3,34–36].

Generally, all types of above-discussed CDs can be used in skin and mucosal formulations safely and without risk of irritation; even methylated CDs in low concentrations can be safely applied. Only for aqueous solution/suspension containing high concentration of CDs there is some probability that methylated CD will interact with the *stratum corneum* lipids (cholesterol, triglycerides) and temporarily affect the membrane integrity [27]. As a consequence of low β-CD aqueous solubility (1.85% at room temperature), they cannot affect membrane structure when administrated as solution. For eye preparation γ-CD, HP-β-CD and SBE-β-CD are recommended.

3. Legal status

α-CD (Alfadex) and β-CD (Betadex) have their own monographs in the current European Pharmacopoeia (EP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP). They are classified as an excipients, not as part of the drug substance, although opinions about such classification are divided. The first monograph on Betadex appeared in 1997 in the EP, and β-CD is the most popular and well-investigated CD. Among CD derivatives, only HP-β-CD has a legal status and own pharmacopoeial monographs in current EP and BP, whereas dimethyl-β-CD, 2-hydroxyethyl-β-CD, 2-hydroxypropyl-β-CD, 3-hydroxypropyl-β-CD, and trimethyl-β-CD are regarded as related substances [37]. The problem with derivatives is mainly based on homogeneity of the final product. For example, methylated CDs are commercially available in various qualities, such as dimethyl-β-cyclodextrin with isomeric purity of 50%, 80% or 95% (DIMEB-50, DIMEB-80, and DIMEB-95, respectively) (there is no 100% pure 2,6-dimethyl-β-CD); whereas pharmacopoeial HP-β-CD is characterised by molar substitution in the range 0.4–1.50.

The approval status, toxicological aspects according to accepted application way, and economical aspect of natural CDs do not create limitations for their usage. This situation is still more complicated for CD derivates, with the exception of HP-β-CD, because of higher prices, lack of official monographs, differences in molar substitution number, and purity levels between marked products. The physicochemical properties of CD derivatives, including the ability to form complex with the drug, can be changed by not only the type but also by the number and position of the substituents [38–41]. The degree of substitution is stated as average and does not indicate the substitution position, thus it is not a uniquely characterised derivative, as seen in HP-β-CD [41].

The poor legal status of CDs implicates difficulties for using them as an excipients in the drug products. As stated by European Medicines Agency (EMEA) in the draft of newest guideline concerning excipients, there are four groups of these compounds: excipients described in the EP or in the pharmacopoeia of an EU Member State, excipients described in a third country pharmacopoeia, excipients not described in any pharmacopoeia and novel excipients [42]. Registration requirements for excipients described in pharmacopoeias are well-known, but for non-pharmacopeial substances the requirements are more detailed and expanded, and for novel excipients – even as restrictive in some field as for the drug substances.

4. Application of CDs in topical formulations

4.1. Practical aspects

Practical use of the obtained complex with CDs is more complicated than forming the inclusion. As presented by Szejtli [23], drug formulations with CDs are usually not bioequivalent to their reference products. Even when only better stability is required, the absorption of the drug will be usually affected in the positive or negative way. CDs are able to modify mucosal, dermal, or corneal application both by increasing (supergenerics) [6,43–47] or modifying delivery (retarded or prolonged release) [6,48–50]; hence, no reference products exist for performing a comparative studies. Therefore, for registration purpose, all preclinical studies are necessary, which increase developmental cost for such products, and the reduction of dose does not solve this problem [23].

Additionally, lack of unified valid pharmacopoeial *in vitro* release methods makes difficult to compare a new semisolid formulations with CD addition to those without modification. Dissolution test is of utmost importance in quality control; so for semisolid forms the test should also prove lack of batch-to-batch variations, adequate quality, stability during storage time, and equivalence of the product after quantitative and qualitative changes in the composition [51]. Though in EP performing suitable test is suggested for demonstrating appropriate release of the drug substance, there is no description of such test.

The most popular and FDA-approved method for dissolution/permeation testing from topical preparations is with use of Franz diffusion cell. However, there is no unification of test parameters, the dimensions differ a lot between laboratories and manufacturers. Particularly, two main parameters are different: cross-sectional surface area and volume of receptor compartment [13,33,49,52,53].

4.2. Advantages

4.2.1. Elimination of irritating effect (or toxicity) – when drug substance is irritating to the cornea, mucosa or skin and avoidance of some excipients, such as pH regulators, solubilising agents or organic solvents

Composition of ocular drug products is often a compromise between solubility, pH, viscosity, and stability. Sigurdsson et al. [54] ascertained that CDs are useful for attaining such compromise. The topical availability of dorzolamide from the CD-containing eye drops appeared to be comparable to Trusopt®. In this study, the authors use

RM-β-CD, which is not recommended for ophthalmic application; therefore, the practical aspect of this formulation can be disputable. HP-β-CD enables solubilisation of tropicamid at pH 7.4 phosphate buffer instead of standard 1% tropicamid eye drops buffered at pH 5.0 (irritant to eye) [55].

For ocular or mucosal liquid formulations, it is very important to improve aqueous solubility because it is the only appropriate solvent for safe administration. For nasal drops with disoxaril or eye drops with hydrocortisone, additional solubilising agents or permeation enhancers may have corrosive or toxic effect on the mucosa or cornea [14,56–59].

Bary et al. [56] proved the increased bioavailability for hydrocortisone/HP-β-CD complex in ophthalmic solution compared to drug suspension. In nasal delivery, aqueous solutions are also favoured, and for disoxaril it was only possible thanks to DM-β-CD complexation [14]. Many substances were tested in experimental animals, isolated corneal models, and in pilot study in humans. These studies resulted in a few eye drops products, such as Naclof® or Voltaren® (diclofenac sodium with HP-γ-CD), Indocollyre® or Indocid® (indomethacin with HP-β-CD) and Clorocil® (chloramphenicol with RM-β-CD).

Also for liquid preparations intended for application onto the skin the most biocompatible solvent is water. Montassier et al. [60,61] proposed aqueous-soluble tretinoin/β-CD complexes as an alternative for use 60% ethanol as a solvent. The solubility of drug was the same in both cases. Organic solvents, like ethanol, are corrosive to the skin and their volatility may cause recrystallisation of the drug substance during storage. CDs can be an alternative for them.

It is possible to eliminate some corrosive activity of drug molecules on the tissues by using of CDs. A decrease in the invasive action of celecoxib on the *stratum corneum* by DM-β-CD and HP-β-CD was demonstrated in histological studies [62]. Ventura et al. [62] documented that DM-β-CD alone causes more injuries on the *stratum corneum* than celecoxib/DM-β-CD complex. It is possible because only free components can interact with the *stratum corneum*; the drug substance blocking interior cavity of CD and CD ''holds'' invasive particles inside. HP-β-CD was less effective in elimination of skin toxicity because of lack of complex formation – only external interactions were possible. Anadolu et al. [44] reported that three months of application of hydrogel and moisturising vehicle, both containing retinoic acid/β-CD complex, cause less side-effects than a commercial product without β-CD. The overall percentage of topical side-effects (erythrema, desquamation, xerosis, and irritation) for commercial products was 93%, while for the β-CD-containing formulations it was only 27%.

For reducing skin toxicity of *N*,*N*-diethyl-3-methylbenzamide, two highly hydrophilic CDs, namely HP-β-CD and γ-CD, were used [49]. The reported lower *in vitro* release of the drug substance is probably due to better affinity of the drug to the vehicle.

4.2.2. Improvement of drug substances absorption

In the topical formulations, improved solubility is usually associated with enhanced bioavailability, like for eye drops containing: dorzolamide [54], tropicamide [55], hydrocortisone [56], acetazolamide [63], methazolamide [64], thialidomide [65], ganciclovir [66], disulfiram [67], zinc diethyldithiocarbamate [68], cannabinoids [69], and mycophenolate mofetil [70] or preparation intended for skin application: sericoside [45], piroxicam [52], celeoxib [62], tretinoin [60,61], 4-biphenylylacetic acid [71], hydrocortisone [72], oxybenzone [73], and bupranolol [74]. The increase in bioavailability is mainly due to higher concentration in the site of administration, caused rather by higher aqueous solubility and thus improved availability onto the tissue surface than enhancement activity of CDs by itself. Schoch et al. [75] demonstrated that modified CDs (HP-γ-CD as well as oktakis-γ-CD) significantly increased *in vitro corneal* permeability of diclofenac sodium in comparison to formulation containing Cremophor. Eye permeation enhancement is not a result of tissue damage, rather the effect of interaction CD-drug or CD-tissue (possible an ion channel activity).

The improvement of skin permeation is possible by increasing drug solubility, which improved availability onto the skin surface or by influence on the barrier function of *stratum corneum* (probably only in the case of DM-β-CD) [62].

In the studies of Ventura et al. [62] on percutaneous absorption of celecoxib from 0.01% solution or suspension in presence of HP-β-CD and DM-β-CD, both CDs influenced the *in vitro* drug permeation through human skin by shortening the lag time from 2 h to 35 min for 5% DM-β-CD and to 1 h for the same concentration of HP-β-CD. The cumulative amount of celecoxib permeating through the skin after 24 h was up to 7–8 times greater when either HP-β-CD or DM-β-CD was present in the donor phase in contrast to the uncomplexed drug substance.

4.2.3. Effects on pharmacokinetics

A CD-formulated drug is practically never bioequivalent with the reference product. It can have improved or decreased bioavailability, the drug action can be delayed or accelerated in time. As described earlier, it is possible to reduce lag time for transdermal permeation or achieve faster analgesics and anti-inflammatory effect in eye drops, as for nimesulide-containing formulations [62,76].

Decrease in application frequency is especially useful for elder people. Saari et al. [77] reported that there were no statistically significant differences between three weeks therapy with 0.1% dexamethasone applied three times daily for postcataract inflammation and 0.7% dexamethasone/CD complex applied once daily.

Bilensoy et al. [48] observed that complexation with β-CD reduces the release of clotrimazole from mucoadhesive vaginal gel formulation. The cumulative release after three days was 40% for drug/β-CD complex and 80% for clotrimazole incorporated alone into gel; however, after 24 h

there was no significant difference between both formulations. It is suggested by authors that gel-containing clotrimazole/β-CD can be used as a controlled-release vaginal formulation; in practice, it will be hard to keep any gel system onto vaginal mucosa for more than 4 days. In addition, the initial 20–30% release after 10 h is very high in comparison to only 40% release after 3 days, and the applied experimental model cannot predict the behaviour of the formulation in vaginal fluid.

In transdermal therapeutic system technology, some trials on using CDs to modify release profile have been performed. Davaran et al. [78] used β-CD in nicotine transdermal patch to provide the active in a sufficient, safe, and controlled way. The release is controlled not only by patch membrane but also by dissociation of the inclusion complex, and the complex formation equilibrium can be regulated by CD amount. Pose-Vilarnovo et al. [79] concluded that for hypromelose-based gel system, high CD concentration retards drug release by increasing tortuosity of the diffusion pathway in matrix by "empty" CD cavities.

4.2.4. Improvement in physical-, chemical- and photostability of drug substances

Sometimes it is necessary to prolong drug stability in solution or gel formulations. Kim et al. [80] noticed that rhEGF/HP-β-CD complex formation significantly increased the stability of human epithelial growth factor at 4 °C in comparison to free polypeptide. Stabilising effect of HP-β-CD on chemical and enzymatic degradation of ganciclovir prodrugs in eye drops was observed by Tirucherai et al. [66].

It is usually more beneficial to formulate an aqueous solution formulation for nasal administration instead of a suspension. For new anti-viral compound disoxaril (with very low aqueous solubility of 0.1230 μg/ml), preparing only an acidic pH solution is possible, though it resulted in rapid hydrolysis of the drug. Even at pH 7.0, this drug completely hydrolysed after 36 h at 4 °C. Ventura et al. [14] demonstrated that in the same conditions, disoxaril complexed with DM-β-CD was found intact in amounts of 90% after 15 days. Stability effect decreased when the storage temperature was increased to 37 °C and was 5 times less than in 4 °C. Besides inhibiting hydrolysis, CDs can also eliminate crystallisation during storage of poorly soluble drugs, such as sericoside in extract form. In emulsions containing HP-γ-CD and γ-CD (both o/w and w/o type), no crystallisation was found even after a 6-month storage period at 40 °C, while for sericoside/β-CD complex crystallisation occurred rapidly [45]. It was suggested that it is a result of interaction between (smaller than γ-CD) particles of β-CD and raw vehicle materials.

Photostability effect was observed for sunscreen agents with CDs [50,81,82]. The phenylbenzimidazole sulfonic acid that was complexed with HP-β-CD and next incorporated into cream formulation remains 99% intact during long-term stability studies (6 months at room temperature in dark) in contrast to 81% for uncomplexed agent [82].

4.2.5. Reduction of drug substances metabolism in site of application

Complexation of dexamethasone with β-CD and HP-β-CD protects the drug substance against skin metabolism. Such studies performed by Lopez et al. [46] on the homogenised mouse skin during 2 h resulted in 30% and 65% degradation of dexamethasone for complexed and free drug, respectively. However, this stabilising effect is limited *ex vivo* and *in vivo* by the non-homogenised full skin because CDs are not able to penetrate into viable skin layers.

4.3. Limitations

4.3.1. Low efficiency of complexation

For some molecules, the efficiency of complexation is low and quite large amounts of CDs are necessary to form inclusion complex. It can result in relatively large excess of CDs in the final product, even when the dose of the drug substance is small [83]. For piroxicam and β-CD or RM-β-CD complexes with inclusion stoichiometry 1:1 prepared by freeze-drying method, the content of drug was 22.6 and 19.9%, respectively [52]. This means that for 1% drug formulation, 5% of piroxicam/β-CD complex had to be used, which will result in obtaining a suspension formulation (only 1.85% of β-CD could be dissolved in water at 25 °C). For itraconazole/HP-β-CD-containing vaginal cream, obtaining 1% concentration of drug substance needs addition of 43.6% CDs [84], while for the sunscreen agent – phenylbenzimidazole sulfonic acid – 10-fold molar excess of CDs was necessary to prepare a stable inclusion complex [82]. In most cases, maximal dermal (or transdermal), mucosal or corneal absorption is possible when concentration of CDs is minimized to a small particular range, while the excess of CD amount results in inhibition of absorption [43,56,62,73,74,79,85–87].

Because only the uncomplexed drug can be absorbed thus complex stability constant had a great influence on the drug bioavailability from inclusion complex. Rate constant of complex formation can vary in a wide range: from 10^0 to 10^8 1 m^{-1} s^{-1}. The 0 value of dissociation rate constant means that drug is incapable of forming an inclusion. For complexes with high value of rate constant, like nitrophenol (10^8 1 m^{-1} s^{-1}), the drug diffusion from the complex will be the limiting factor (retarding release). High dissolutions rate constants are important in the case of stability improvement or bitter taste elimination, while in preparations aimed for bioavailability increasement the lower range are necessary. Stability constant most often is between 50 and 200 mol^{-1}, with mean values of 129 and 490 for α- and β-CD, respectively [88].

4.3.2. Large difference in mass between CD and "guest" and relatively low aqueous solubility of natural CDs

Differences in mass between CD and "guest" (1000 and 200–400, respectively) result in small mass loading even when the efficacy of complex formation is excellent; such

effect is a limitation for drugs with high dosage. Thus formulations with small dosage can be produced, such as eye drops containing 1% of indometacin or 1% of diclofenac sodium with presence of 10% and 2% of certain CDs, respectively, while it is impossible to produce topical skin formulations with high drug concentration (10%) in the form of solution or use as an excipient CD with low aqueous solubility, like natural β-CD.

The most popular β-CD is the least soluble among natural CDs. Additionally, CD solubility increases sharply with temperature (Table 1), which can lead to uncontrolled recrystallisation during cooling or rapid changes in storage temperature. Also the CD solubility generally decreases in the presence of organic molecules, such as ethanol or propanol [23].

4.3.3. Interaction with CD inclusion complex

Small lipophilic molecules in aqueous solutions can displace drug "guest" from CD cavity by reducing solubilising or stabilising effect, or creating inclusion with "empty" CDs. The activity of some anti-microbial preservatives can be reduced in aqueous phase by the presence of HP-β-CD [89,90] and β-CD [10,91]. This is important for eye drops where HP-β-CD may have practical application. Probably it was a reason to use thiomersal as a preservative agent for Indocollyre® drug product (0.1% indomethacin and 10% HP-β-CD) [92]. Chan et al. [91] reported that β-CDs in small concentrations (0.25%) are not capable of interacting significantly with four parabens (methyl-, ethyl-, propyl-, and butyl-). However, the degree of interaction was found to increase proportionally with the concentration of β-CD (0.5%, 0.75%, and 1.0%). Methyl-paraben showed a higher extent of interaction than ethyl- and propyl-parabens due to its small size and the steric energy. H NMR studies indicated that methyl group was accommodated inside the hydrophobic cavity while ethyl group was not in the cavity. Ethyl-, propyl- and butyl-parabens showed a regular trend of increase in the extent of interaction – probably due to changes in the orientation of the alkyl chain in the presence of β-CD [91]. This indicates that for new formulations with CDs and water content, appropriate anti-microbial efficacy test had to be examined very carefully. This problem does not occur for γ-CD thanks to the geometric factor – largest dimension of interior of cavity. As an example, Naclof® preparation (containing diclofenac sodium and HP-γ-CD) is preserved with benzalkonium chloride.

Also in semisolid formulations, mainly in aqueous phase, the problem of interaction between some excipients and inclusion complex of the drug can occur. Bilensoy et al. [48] founded that Carbopol 934 interacts with gel formulations containing β-CD and clotrimazole, and causes precipitation during a few hours. The mechanism of this incompatibility stays undefined. It is probably due to the interaction of polymer with an uncomplexed drug – not directly with CD ring. Propylene glycol, commonly used as a co-solvent, can interact also with CD cavity in the presence of the included drug substance. As presented by Doliwa et al. [15] propylene glycol reduced piroxicam/HP-β-CD complex stability about 30 times. This indicates that propylene glycol molecules are able to displace piroxicam from inclusion with HP-β-CD cavity.

Shigeyama et al. [38,93] proved the interaction between β-CD and emulsifying agent – Cremophor 60. In the mixed hydrophilic ointment with purified lanolin (ratio 7:3) fused with α-CD or β-CD the oil droplets were found, whereas no visible structural changes in the presence of DM-β-CD or TM-β-CD were observed. Harada [94] reported that CDs can form inclusion complexes with big molecular particles such as polymers with high selectivity. α-CD formed crystalline compound with poly(ethylene glycol) (PEG) in high yield, whereas β-CD cannot form an inclusion with PEG but it included poly(propylene glycol). The inclusion is formed with PEGs with molecular mass above 200, and most rapidly with 1000.

4.3.4. Changes in rheological properties of semisolid formulations

The rheological changes usually appear as lowering the viscosity of the gel and they depend on CD type [48,95]. Boulmedarat et al. [95] noticed that 5% of methyl-β-CD induced pronounced loss of viscosity in gels based on Carbopol 974P (20 Pa s vs 45 Pa s for the control), whereas γ-CD slightly increased the viscosity compared to the control. They suggested that hydrophobic interactions between polymer chains and CD cavity appeared, thus polymer swelling properties decreased; whereas Jug et al. [52] concluded that the reason of rheological changes in hypromelose gel with β-CD can be a result of possible interaction between the hydrophilic polymer and CD ring (ternary complex formation).

Shigeyama et al. [38] demonstrated that oil droplets coalesced in the cream with a fused mixture of β-CD, resulting in a decrease of viscosity and increase of drug release rate. The rheological changes were a result of interaction of CD with the emulsifying agent and caused ointment base instability after seven days, even in a cold place (4 °C).

4.3.5. Doubts on skin penetration enhancement

One of the biggest hopes for CD application in topical formulations was using them as universal non-irritating penetration enhancer for transdermal and transmucosal application of drug substances. CDs are regarded by some authors as classic enhancers that are able to extract all the major lipid classes and proteins and thus reduce skin barrier function [33,96], while others regarded their action as problematic and rather unproved [97]. Above hypotheses are based on the studies on the animal skin (hairless mice or rats) performed in 1990s by Legendre et al. [33], Vollmer et al. [96] and Bentley et al. [98]. Although Bentley et al. [98] indicated that HP-β-CD caused removal and possible disorganisation of the lipids in the *stratum corneum* and Legendre at al. [33] stated that HP-β-CD exhibited twofold higher activity in removing cholesterol from rat skin than

RM-β-CD, it seems that only methylated CDs applied in high concentrations (10–20%) in aqueous solutions can have influence on the *stratum corneum* [31,74,96]. No effect of possible disruption was seen for β-, HP-β- and γ-CDs [98].

Pretreatment studies realised on the rat skin for bupranolol as drug substance showed no flux increases for 2% and 10% solution of HP-β-CD, while for RM-β-CD flux increased markedly for both concentrations with concentration dependency [74]. Shaker et al. [32] suggested that HP-β-CD and its inclusion complex with corticosterone do not effectively penetrate into or transport through the skin. In hairless mouse skin model, HP-β-CD did not change the barrier function of the *stratum corneum*, nor did it enhance transport of corticosterone. Similar results were obtained during studies with human skin [99–101]. Valjakka-Koskela et al. [99] reported that addition of 10% of HP-β-CD decreases levosimendan flux through human *in vitro* skin from the solution form. Preis et al. [100] reported that incorporation of hydrocortisone/HP-β-CD inclusion complex into gel formulation did not involves changes in the drug permeation through human *ex vivo* skin in comparison to preparation without the CDs. In the same studies incorporation of drug substance/β-CD inclusion complex into formulation resulted in decrease release. Also in the studies by Simeoni et al. [101] with excised human skin, HP-β-CD had no effect on the *stratum corneum* and *epidermal* concentration of sunscreen agent buthyl-methoxydibenzoylmethane in comparison to free molecules applied as solution. Additionally, they found that sulfobutylether-β-CD markedly reduced *epidermal* absorption of such filter without reducing its *stratum corneum* penetration [101]. Some of CDs, like HP-β-CD, β-CD or sulfobutylether-β-CD, may not only had no influence on barrier function but they even, in certain cases, may had protective properties against penetration of drug substances into deeper skin layers [101,102].

Ventura et al. [62] presented opposite statement. They concluded that both HP-β-CD and DM-β-CD enhanced drug flux through human *stratum corneum* and *epidermis* by means of an increase of dissolution rate of the drug as well as a direct action on the *stratum corneum*. The direct impact on the *stratum corneum* is overestimated because during 24 h experiment with 3% solution of CDs, HP-β-CD had no destructive effect and seems that was not able to act as penetration enhancer. Although DM-β-CD in above conditions caused separation of corneocytes layers and had significant influence on barrier function of the skin, its inclusion complex with the drug showed less injurious effect than DM-β-CD alone.

4.3.6. Physicochemical variations

Differences in physicochemical properties of CD derivatives depend on their type, and number and position of substituents. Differences in osmotic pressure for HP- or SBE-derivatives of β- and γ-CDs (commonly used in ophthalmic formulations) were reported [39].

Higuchi and Connors [103] classified inclusion complexes drug substance/CD on the base of CD influence on drug solubility. A-type phase-solubility profiles are obtained when the solubility of the drug substance increase in solution with increasing concentration of CD, while for B-type phase-solubility profiles the inclusion complexes formation are of limited solubility. The solubility of some drug inclusion complexes, especially with β-CD, often gives B-type Higuchi phase-solubility diagrams caused by poor solubility of the ligand, whereas hydrophilic CD derivatives demonstrated A-type.

5. Possible applications of CDs in topical formulations

5.1. Sunscreen creams

CDs are helpful in controlling skin penetration of topically applied sunscreen agents and other chemicals [48,52,73,81,101,104,105]. Sunscreens and some topically applied substances should stay onto skin surface without further cutaneous penetration; thus, lowering their release from the carrier is a beneficial feature. Additionally, sunscreen agents under solar radiation generate a variety of decomposition products that can be harmful to DNA, such as free radicals and active oxygen species, and thus decrease its UV-protection by concentration decrease. As an example, HP-β-CD significantly reduced the release and membrane permeation of oxybenzone without suppressing the UV-absorbing properties of this chemical [52]. Felton et al. [73] demonstrated that skin flux of oxybenzone is related to the CD concentration, maximum flux occurred at 10% HP-β-CD, while 20% CD excess decreased both skin absorption and permeation. By adding excess of CDs, it is possible to form a drug reservoir on the skin surface.

5.2. Liposomes

CDs can be used to improve lipophilic drug entrapment in the aqueous liposomal phase and thus result in a new two-carrier system of drugs-in-CD-in-liposome formulations [106–109]. Maestrelli et al. [53] investigated such system for transdermal delivery of ketoprofen. They achieved improvement in drug entrapment for ketoprofen/HP-β-CD complex in equimolar ratio. Encapsulation efficacy increased with CD concentration; however, high concentration destabilised liposomal membrane. Liposomal formulations resulted in slower and prolonged drug permeation through membrane (about 40% drug permeated after 24 h) in comparison to drug solution (60% after 4 h). The CD interaction with liposome lipid membrane depends on the type of CD, complexed drug substance and lipids [107–109]. Also, some other lipidic systems, such as microspheres, are combined with drug/CD complexes, especially for mucosal delivery [110–113].

5.3. New carrier systems

A new innovative procedure is based on cotton tissue or polymer materials grafted with β-CD to improve medical textiles features, such as anti-bacterial and anti-fungal properties, wound dressings, transdermal therapeutic systems, textiles with sun protection factor, and prolonging skin contact with volatile molecules [105,114–116]. There are also trials to produce high-loading capacity hydrogels consisting of cross-linked polymer networks with covalently bonded CDs [40,117,118].

5.4. CDs as skin penetration co-enhancers

As skin penetration enhancement is difficult by using only CDs and as the influence on drug flux depends not only on CDs and drug substance properties but also on other formulation components, recently CDs have been applied with good efficacy as co-enhancers or in combination with other methods – supersaturation, electroporation, or iontophoresis [16,47,119–122].

6. Conclusions

CDs are certainly a trend in scientific investigations – the number of published works is huge. For topical formulations, there are a lot of limitations besides some advantages, and the number of products is not impressive [123]. High costs of implementation for almost all CDs formulations and problems with quality and legal status of derivatives are also a limiting factor in availability of increased number of products with CDs.

CDs are not able to penetrate biological membranes during topical application in normal conditions, though they are able to change the bioavailability of drugs significantly. Unfortunately, CDs are able to interact with formulation ingredients and cause physicochemical stability problems; selection of proper vehicle seems to be a challenge. Thus it is easier to create solutions or suspensions than formulate complicated semisolids carriers, and this tendency is reflected in the number of products too. In spite of the above scenario, a few quite complex formulations with modified by CDs addition properties appear – liposomes, textiles, hydrogels, microspheres, and emulsions. They are promising forms, but their practical use will be limited to a narrow group of products and are a matter of time.

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