Expert Opinion

Recent advances and future directions in amphiphilic cyclodextrin nanoparticles

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Cyclodextrins are known to be promising excipients in the pharmaceutical industry, with their ability to include hydrophobic guest molecules masking the physicochemical properties of the guest, such as poor water solubility, stability problems and undesired side effects. These enabling excipients, which are produced on a large scale and incorporated into various marketed products worldwide, are now modified to render amphiphilic properties that enable them to be used to prepare nanoparticles. Amphiphilic cyclodextrins have the ability to form nanoparticles without the presence of a surfactant by different preparation techniques that are discussed in this review. Classification and physicochemical properties of these interesting molecules as well as the efficacy and safety of nanoparticles prepared from different amphiphilic cyclodextrins are discussed in light of the current literature work with *in vitro* and *in vivo* findings. Cyclodextrin nanoparticles of different nature effectively carry drugs or molecules with bioavailability problems arising from poor aqueous solubility, stability under physiological conditions or side effects associated with the molecule itself or excipients used in the formulation of these problems drugs. In conclusion, amphiphilic cyclodextrins emerge as promising alternatives for tumor drug delivery and passive and active targeting with non-toxic, non-hemolytic properties as injectable, nanosized carriers.

Keywords: amphiphilic cyclodextrin, encapsulation, nanoparticle, preparation, synthesis

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1. Introduction

Cyclodextrins (CD) are unique molecules with 'pseudo-amphiphilic' structure providing a wide range of applications in the pharmaceutical field owing to their complex forming capability, which allows cyclodextrins to include hydrophobic molecules in their hydrophobic cavity and to mask the physicochemical properties of the guest molecule. Cyclodextrins are cyclic oligosaccharides obtained by the enzymatic degradation of starch. Major natural cyclodextrins are crystalline, homogeneous, non-hygroscopic substances that have a torus-like macro-ring shape built up from glucopyranose units, as shown in **Figure 1** [1-3]. Cyclodextrins are named depending on the number of glucopyranose units. Major and industrially produced cyclodextrins are named as follows; α-cyclodextrin possessing six units, β-cyclodextrin possessing seven units and γ-cyclodextrin possessing eight units.

Natural cyclodextrins have been reported to form total or partial inclusion complexes with several drugs to improve aqueous solubility, stability under physiological or ambient conditions, reduce or mask completely the side effects associated with the included drug and to increase compatibility of the drug with other drugs in the formulation or excipients while improving patient compliance by masking the taste or odor of the active ingredient [4-8].

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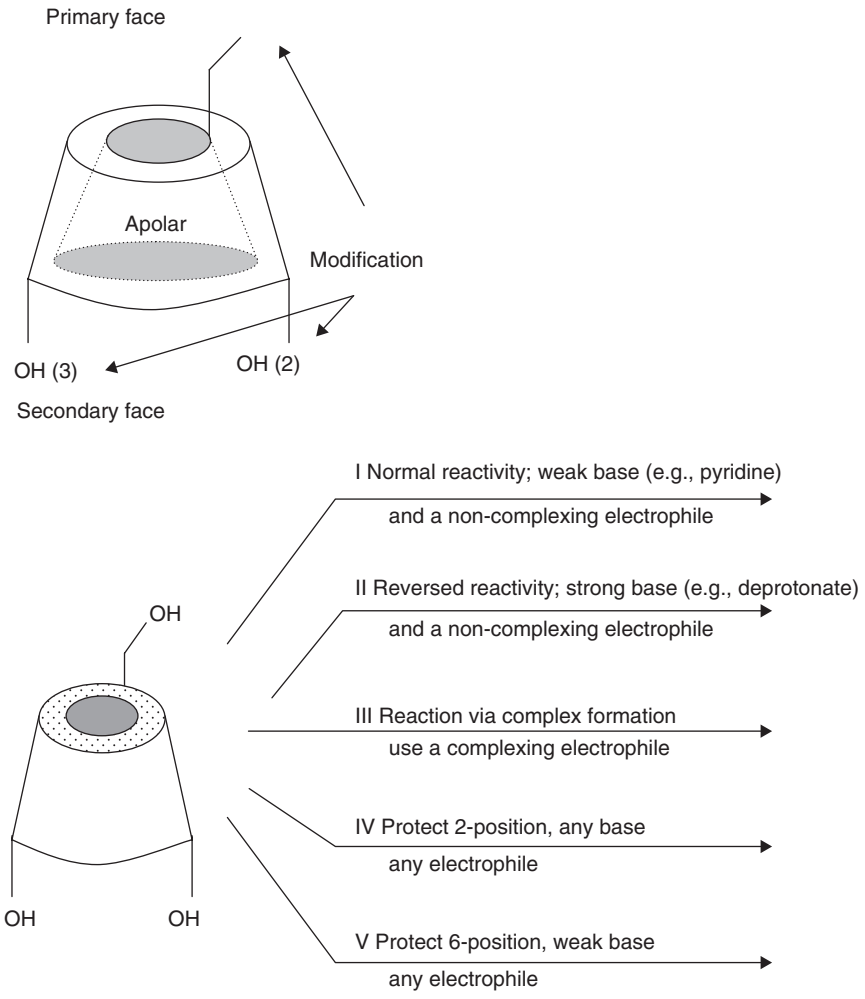

Figure 1. Schematic representation of natural cyclodextrin structure and possible modification sites and strategies.

Contrary to the advantageous nature of cyclodextrins for molecular inclusion, their surface characteristics make it more difficult for the very hydrophilic cyclodextrin molecule to interact with lipophilic biological membranes. For this reason, natural cyclodextrins have been chemically modified in order to achieve one or more of the following goals: to alter their water solubility, reduce the nephrotoxicity and hemolysis encountered on intravenous (i.v.) administration, ameliorate the interaction with biological membranes and provide controlled drug release profiles. Modification of natural cyclodextrins has been the aim of many research groups, to improve safety while maintaining the ability to form inclusion complexes with various substrates. Some groups have also focused on improving the interaction between the drugs and the cyclodextrins, whereas others have attempted to prepare materials that can be chemically defined more precisely.

Two of the natural cyclodextrins are known to be parenterally unsafe owing to nephrotoxic effects [9]. The exact mechanism of the nephrotoxicity of α- and β-cyclodextrin is unknown, but is believed to be related to either cyclodextrin uptake by kidney tubule cells resulting in disruption of intracellular function, or the extraction of lipid membrane components by the cyclodextrins. The latter hypothesis is suggested to be of validity because a linear correlation has been demonstrated between the ability of some cyclodextrins to disrupt cellular membranes and kidney nephrotoxicity [2,6]. The ability of cyclodextrins to cause hemolysis to erythrocytes and membrane irritation seems also to correlate with their ability to extract lipid membrane components such as cholesterol and phospholipids [10,11].

Parallel to the increasing interest and successful regulatory and commercial outcome of nanoparticulate pharmaceutical products, cyclodextrins have also been under investigation for incorporation into nanoparticulate drug delivery systems for several purposes along with polymers. Nanoparticles are of pharmaceutical interest because of their active and passive targeting properties and their ability to deliver poorly soluble drugs in particular without requiring potentially toxic co-solvents and drugs with stability problems to target tissues or cells. Nanoparticles are also considered to be more stable

than liposomal delivery systems under physiological conditions. However, a major drawback is associated with the drug loading capacity of polymeric nanoparticles. Classical emulsion polymerization procedures result in very low drug loading capacities. This results in the administration of excessive quantities of polymeric material, which in the case of relatively more toxic polymers may impair the safety of the drug delivery system [12-14].

2. Amphiphilic cyclodextrins

Amphiphilic cyclodextrins have been synthesized to solve problems of natural cyclodextrins that limit their pharmaceutical applications. The main reasons for the synthesis of amphiphilic cyclodextrins can be classified as follows.

1. Enhancement of interaction of cyclodextrins with biological membranes through a relative external hydrophobicity.
2. Improvement of interaction of cyclodextrins with hydrophobic drugs by creating a second zone of attraction for hydrophobic drugs other than the cyclodextrin cavity, which is the long aliphatic chains in this case.
3. Allowing self-assembly of cyclodextrins, resulting in the spontaneous formation of nanosize carriers in the form of nanospheres or nanocapsules depending on preparation technique.

The major advantage of amphiphilic cyclodextrins is their self-alignment or packaging properties at interfaces, which is sufficient to form nanoparticles spontaneously without the presence of a surfactant along with their demonstrated ability of forming inclusion complexes with various drugs in their cavity and within the long aliphatic chains [15-18].

Amphiphilic cyclodextrins are generally classified according to their surface charge as follows: non-ionic amphiphilic cyclodextrins; cationic amphiphilic cyclodextrins; and anionic amphiphilic cyclodextrins.

2.1 Non-ionic amphiphilic cyclodextrins

Non-ionic amphiphilic cyclodextrins are obtained by grafting aliphatic chains of different length onto the primary and/or secondary face of the CD main glucopyranose unit seen in **Figure 1**. Different derivatives depicted in **Figure 2** are named after their structure in the literature.

1. *Lollipop cyclodextrins* [19] are obtained by the grafting of only one aliphatic chain to 6-amino-β-cyclodextrin.
2. *Cup-and-ball cyclodextrins* are synthesized by the introduction of a voluminous group such as the *tert* butyl group, which is linked to the end of the aliphatic chain in order to prevent self-inclusion of the pendant group [20,21].
3. *Medusa-like cyclodextrins* are obtained by grafting aliphatic chains with length between C10 to C16 to all the primary hydroxyls of the cyclodextrin molecule [22-24].
4. *Skirt-shaped cyclodextrins* consist of α-, β- and γ-cyclodextrins per-modified with aliphatic esters (C2 to C14) on the secondary face [25-28].
5. *Bouquet-shaped cyclodextrins* result from the grafting of 14 polymethylene chains to 3-monomethylated β-cyclodextrin, meaning 7 chains on each side of the cyclodextrin ring molecule [29]. Per(2,6-di-*O*-alkyl) cyclodextrins where the alkyl chain may be propyl, butyl, pentyl, 3-methylbutyl or dodecyl also take part in the bouquet family [4].
6. *Cholesteryl cyclodextrins* have been introduced recently as more complicated derivatives [30]. They were designed assuming that cyclodextrin is the hydrophilic head group and cholesterol is the hydrophobic part.

Interfacial properties of non-ionic amphiphilic CDs at air–water or oil–water interfaces have been demonstrated by different groups [31-33]. Aliphatic chain length, chain structure (linear or branched) and bond type (ester, ether, amide, etc.) of the aliphatic chain affect interfacial properties of amphiphilic cyclodextrins. Self-alignment of the amphiphilic cyclodextrin molecule at the air–water interface was demonstrated to be aliphatic chains perpendicular to and CD ring parallel to the film [32]. As far as the inclusion-forming properties of these new derivatives are concerned, it was suggested that leaving the wider side of the cavity –secondary face – unsubstituted may facilitate entrance of the drug into the cavity of the amphiphilic cyclodextrin [6,18,34,35].

2.2 Cationic amphiphilic cyclodextrins

Recently, cationic amphiphilic cyclodextrins were synthesized and characterized carrying an amino group as the ionic group. A 'stealth' cationic amphiphilic cyclodextrin because of the oligoethylene glycol group it carries, which is heptakis (2-ω-amino-*O*-oligo-(ethyleneoxide)-6-hexylthio)-β-CD, was reported for intracellular drug delivery [36]. Structural properties of cationic amphiphilic cyclodextrins were believed to arise from the balance between hydrophobic tails (thioalkyl chains) and the hydrophilic moieties (ethylene glycol oligomers). Ethylene glycol chains are believed to increase the colloidal stability of nanoaggregates of cationic amphiphilic cyclodextrins. Amphiphilic alkylamino α- and β-cyclodextrins have also been reported regarding their synthesis, characterization and film-forming properties with water-soluble molecules [37].

A series of polyamino-β-cyclodextrins have been reported [38] with complete substitution by amine groups at the 6 position shown in **Figure 1**. Neutral cyclodextrins have been reported to interact with nucleic acids and nucleotides and enhance transfection efficiency *in vivo*. Cationic cyclodextrins, on the other hand, have shown even greater ability to bind nucleotides and enhance delivery by viral vectors. The main advantage of polycationic cyclodextrins and their nanoparticles is their enhanced ability to interact with nucleic acids combined with their self-organizational properties [38].

2.3 Anionic amphiphilic cyclodextrins

Anionic amphiphilic cyclodextrins carry a sulfate group that renders anionic property to their structure. An efficient synthetic route to obtain acyl-sulfated β-cyclodextrins has

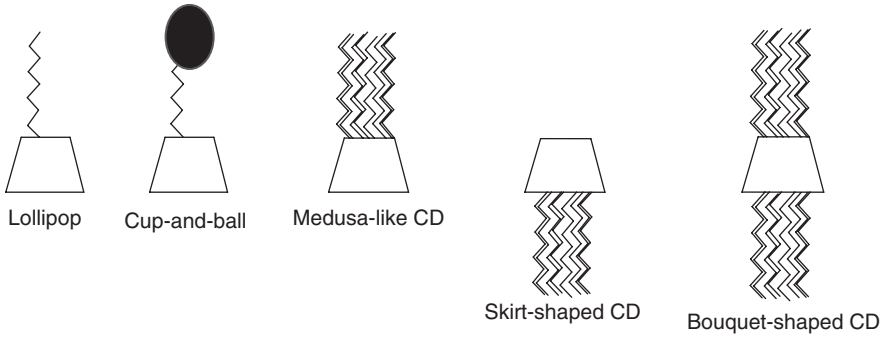

Figure 2. Some non-ionic amphiphilic cyclodextrins reported in the literature.

been introduced in which the upper rim is functionalized with sulfates and the lower rim with fatty acid esters. These derivatives are able to form supramolecular aggregates in aqueous media [39].

Sulfated amphiphilic α-, β- and γ-cyclodextrins have been shown to form 1:1 inclusion complexes with antiviral drug, acyclovir. This was explained by the fact that non-covalent interactions between acyclovir and non-sulfated amphiphilic cyclodextrins (non-ionic amphiphilic CDs) took place both in the cavity of the cyclodextrin and inside the hydrophobic zone generated by alkanoyl chains. However, in the case of sulfated anionic amphiphilic cyclodextrins, the interactions appear to take place only in the hydrophobic region of the alkanoyl chains [40].

Fluorine-containing anionic β-cyclodextrins functionalized at the 6-position by trifluoromethylthio groups have also been reported [41]. These molecules show amphiphilic behavior at the air–water interface and are believed to be good candidates for a new class of amphiphilic carriers. The synthesis of new amphiphilic perfluorohexyl- and perfluorooctyl-thio-β-cyclodextrins and their alkyl analogue, nonanethio-β-cyclodextrin, has been described [42]. The ability of these products to form nanoparticles was also investigated by particle size measurement by photon correlation spectroscopy (PCS) and imaging techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) following freeze-fracture.

Fluorophilic cyclodextrin derivatives with a net negative charge of -70 mV have been obtained as a result of combinations of cyclodextrins and a linear perfluorocarbon [43]. These derivatives may also be considered to belong to the non-ionic class; however, owing to their high negative charge, they have been included in the anionic amphiphilic cyclodextrins. 2,3-di-*O*-decafluorooctanoyl-γ-cyclodextrin was obtained with a protection–deprotection-based synthetic method and characterized further by sophisticated techniques, including thin-layer chromatography (TLC), Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), elemental analysis and time-of-flight mass spectrometry (TOF-MS).

3. Amphiphilic cyclodextrin nanoparticles

Amphiphilic cyclodextrins form nanoparticles spontaneously. Nanoparticles can be nanospheres or nanocapsules, depending on preparation method. For manufacturing nanoparticles, three different techniques have been reported in the literature. Among these techniques, the nanoprecipitation technique is generally preferred because it is a simple technique resulting in unimodal distribution with good reproducibility. The main preparation techniques for amphiphilic cyclodextrin nanoparticles can be listed as follows.

1. *Nanoprecipitation technique* [44-47]. This preparation technique is characterized by homogeneous size distribution, low polydispersity index, and easy and rapid production, involving mild solvents such ethanol or acetone. Forms both nanospheres and nanocapsules.
2. *Emulsion/solvent evaporation technique* [48]. Multistep technique that involves working with potentially more toxic solvents such methylene chloride, heterogeneous size distribution. Forms nanospheres.
3. *Detergent removal technique* [49]. Reconstitution of nanospheres from mixed micelles of detergent and amphiphilic CD by dilution or dialysis of *n*-octylglucoside. Mild conditions and avoidance of organic solvents are the main advantages. Forms nanospheres.

Nanocapsules involve an oil phase (generally Miglyols or benzyl benzoate) that is not present in nanospheres. Particle size distribution of nanocapsules is mostly affected by the size of the oil droplet formed during the preparation along with molar concentration and nature of amphiphilic cyclodextrin. This causes the nanocapsules to be generally larger than nanospheres. On the other hand, nanospheres are not significantly affected by amphiphilic cyclodextrin concentration and can be formed with very high concentrations of amphiphilic cyclodextrins. The modification site of the cyclodextrin (primary or secondary face) is influential for nanosphere size because modification on the secondary face results in a larger surface area. The presence and the concentration of a surfactant such as Pluronic F68 do not affect the particle size of nanospheres

Table 1. Major parameters affecting physicohemical properties of non-ionic amphiphilic cyclodextrin nanoparticles.

Formulation parameter	Nanoparticle property affected by parameter
Amphiphilic cyclodextrin nature	
Substituted face	Particle size, drug loading
Alkyl chain length	Particle size, physical stability, molar cyclodextrin concentration required to form nanoparticles
Chain nature (linear or branched)	Particle size, polydispersity, physical stability
Encapsulated drug property	
Aqueous solublity	Drug loading, drug release
Molecular mass	Drug loading, drug release
$k_{1:1}$ association constant	Drug loading, drug release
Partition coefficient	Drug loading, drug release
Manufacturing process	
Preparation technique (nanoprecipitation, emulsion/solvent evaporation, detergent removal)	Particle size, drug loading, drug release
Loading technique	Drug loading, drug release

and nanocapsules [50-52]. **Table 1** presents the principal parameters affecting *in vitro* characteristics of nanoparticles, such as particle size, physical stability, drug loading and release profiles.

Nanospheres and nanocapsules of amphiphilic cyclodextrins were imaged with different microscopical techniques, such as Cryo-TEM, atomic force microscopy (AFM) and scanning tunnelling microscopy (STM). SEM imaging, on the other hand, results in shrinkage or disruption of the nanoparticles due to electron bombardment. **Figure 3** presents TEM photomicrographs after freeze-fracture for 6-*O*-CAPRO-β-CD nanospheres and nanocapsules, respectively [33,36]. **Figure 4** represents AFM photographs of amphiphilic cyclodextrin nanoparticles loaded with the anticancer drug paclitaxel [53].

Drug loading into nanospheres and nanocapsules is governed by the loading technique used, as can be seen in **Table 1**. Amphiphilic cyclodextrin nanoparticles can be loaded with the following techniques modified in order to achieve higher encapsulation for poorly soluble drugs that can be complexed to amphiphilic cyclodextrins before nanoparticle preparation [12,54].

1. *Conventional loading:* Drug solution is added to organic phase during preparation with the nanoprecipitation technique.
2. *Pre-loading:* Nanoparticles are prepared directly from pre-formed drug:amphiphilic cyclodextrin complexes.
3. *High-loading:* Nanoparticles are prepared directly from pre-formed drug:amphiphilic cyclodextrin complexes and loaded further by the addition of excess drug solution in the organic phase.

The high-loading technique results in a two- to threefold increase in drug entrapment for hydrophobic drugs. Other factors influencing drug loading to amphiphilic cyclodextrin nanospheres are related to drug physicochemical properties, such as drug:CD association constant $k_{1:1}$ representing the affinity of the drug to the CD cavity, oil/water partition coefficient, and aqueous solubility, as seen in **Table 1**. Affinity of the drug to the CD cavity is correlated with drug loading capacity. Lipophilic drugs interact with both the CD cavity and the long aliphatic chains situated on either the primary or the secondary face.

Drug release properties of amphiphilic cyclodextrin nanospheres are affected by various parameters, including drug lipophilicity, drug:CD association constant and loading technique, with release profiles varying from 2 to 96 h depending on the above parameters. Nanocapsules, on the other hand, exert rather different drug release profiles that are mostly dependent on lipophilicity and aqueous solubility of the drug. Lipophilicity of the drug is inversely correlated with the rate of release [55,56]. Nevertheless, preparing nanoparticles directly from pre-formed inclusion complexes helped to reduce the initial burst effect observed in general for nanospheres due to their very large surface area.

Nanoparticles were designed primarily for targeting colloidal carriers of nanosize to tumor tissues by means of the leaky vasculature in tumor regions, which is known as the enhanced permeation and retention (EPR) effect. Since then nanoparticulate drug carriers have been used mainly for cancer therapy through passive and active targeting to cancer cells. Thus, amphiphilic cyclodextrin nanoparticles were also focused mainly on cancer therapy in the literature. Nanoparticles prepared from non-ionic amphiphilic cyclodextrins have been loaded with different anticancer agents with bioavailability problems, such as tamoxifen citrate, paclitaxel and camptothecin. Tamoxifen, an antiestrogen drug used for the first-line and adjuvant therapy for metastatic breast cancer as long-term chemotherapy has been incorporated into amphiphilic cyclodextrin nanoparticles prepared using the amphiphilic cyclodextrin, β-CDC6, in order to reduce the severe side effects associated with the non-selective dose-dependent cytotoxicity of tamoxifen during long-term chemotherapy. Tamoxifen citrate-loaded nanospheres and nanocapsules with ~ 65% entrapment efficiency liberated the drug with a controlled release profile up to 6 h with the previously described high-loading technique used as the loading method [57]. Anticancer efficacy of tamoxifen citrate-loaded nanospheres and nanocapsules was demonstrated to be equivalent to tamoxifen citrate solution in ethanol against MCF-7 human breast cancer cells. Transcription efficiency of the tamoxifen citrate nanocapsules and nanospheres was evaluated against MELN cells in the presence of 17-β-estradiol (E2) for the inhibition of E2-mediated luciferase

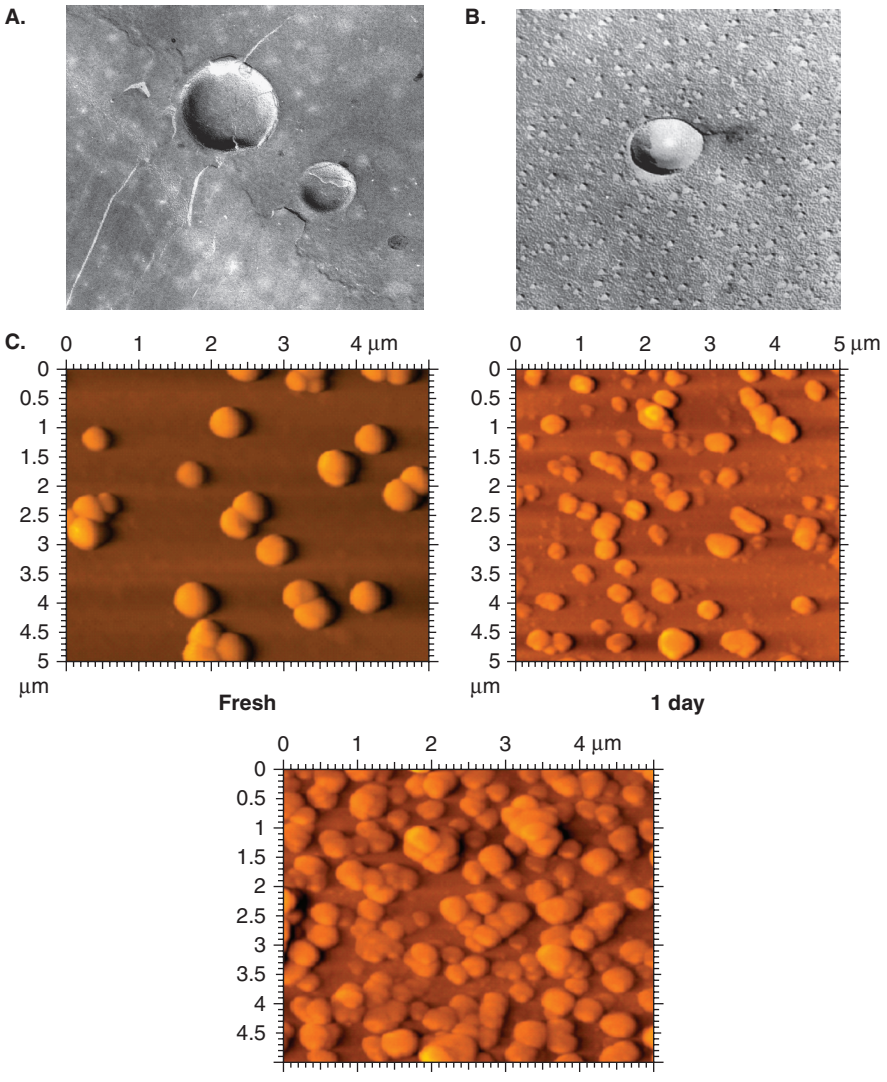

Figure 3. Cryo-transmission electron microscopy images of 6-*o*-CAPRO-β-CD nanospheres (A) and nanocapsules (B). C. Atomic force microscopy photomicrographs of paclitaxel-loaded amphiphilic β-cyclodextrin nanospheres stored as aqueous dispersion [51].
A. Reproduced with permission from [12]. **C.** Reproduced with permission from [53].

gene expression and demonstrated concentration-dependent transcription efficiency [58].

Paclitaxel is a potent anticancer agent with bioavailability problems arising from its poor aqueous solubility, tendency to precipitate on dilution in aqueous media and solubilizers used in its commercially available injectable formulations. This drug has been loaded into nanoparticles prepared from amphiphilic β-cyclodextrin modified on the primary face with 6C aliphatic esters, 6-*O*-CAPRO-β-CD. Paclitaxel-loaded 6-*O*-CAPRO-β-CD nanospheres and nanocapsules were characterized with a diameter of ~ 150 nm for nanospheres and 500 nm for nanocapsules with high entrapment efficiencies. Blank nanoparticles were physically stable when stored as aqueous dispersion for 12 months. The *in vitro* release of paclitaxel from nanoparticles was completed in 24 h [59]. Amphiphilic β-cyclodextrin nanoparticles were evaluated for safety in comparison with the commercial vehicle Cremophor® EL (BASF, France), which is associated with severe side effects, in terms of hemolysis and cytotoxicity. 6-*O*-CAPRO-β-CD nanospheres were reported to be significantly less hemolytic than paclitaxel solution in the Cremophor vehicle on human red blood cells. Cytotoxic effects of blank nanoparticles were evaluated comparatively against L929 mouse fibroblast cells and a significant difference in cytotoxicity of up to 100-fold reduction was observed for amphiphilic cyclodextrin nanoparticles, as seen in **Figure 4**.

Parallel to the blank vehicles, paclitaxel-loaded nanoparticles were also evaluated for their safety and efficacy. First of all, paclitaxel-loaded 6-*O*-CAPRO-β-CD nanospheres and nanocapsules were evaluated for their physical stability in a

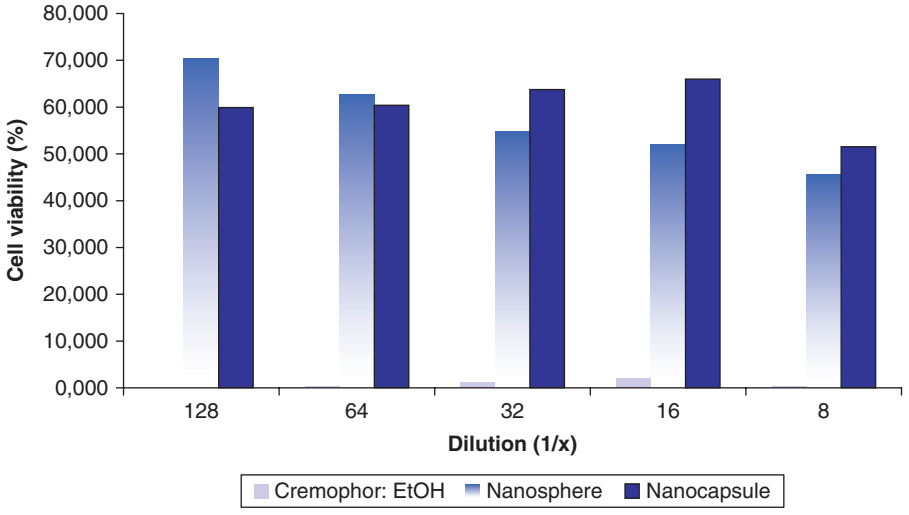

Figure 4. Cell viability of L929 cell line on treatment with different dilutions of commercial vehicle, blank amphiphilic cyclodextrin nanoparticles and blank amphiphilic cyclodextrin nanocapsules determined with MTT assay [51].
Reproduced with permission from [53].

Table 2. Drug-loading parameters for camptothecin in PLGA, PCL, β-CDC6 and 6-O-CAPRO-β-CD nanoparticles.

Formulation	Entrapped drug quantity (μg/ml)	Associated drug %
β-CDC6	28	9
6-*O*-CAPRO	48	13
PLGA	13	2.6
PCL	7	1.4

1-month period in aqueous dispersion form with repeated particle size and zeta-potential measurements and AFM imaging to evaluate recrystallization in aqueous medium. Paclitaxel-loaded amphiphilic cyclodextrin nanoparticles were found to be physically stable for a period of 1 month, whereas recrystallization occurs within minutes when diluted for i.v. infusion [53]. Nanoparticles significantly reduced the hemolytic properties caused by commercial vehicle, as observed from hemolysis assay data and SEM photomicrographs of Cremophor- or nanoparticle-treated erythrocytes. Paclitaxel-loaded amphiphilic nanoparticles were demonstrated to show similar anticancer efficacy against MCF-7 cells when compared with paclitaxel solution in the Cremophor vehicle [53].

Another potent anticancer drug, camptothecin, which is reported to be clinically inactive because of its stability problem under physiological conditions, which causes the drug to be converted from its active lactone form to its inactive carboxylate form, was formulated using two different amphiphilic β-cyclodextrins, β-CDC6 and 6-*O*-CAPRO-β-CD. Cyclodextrin nanoparticles have succeeded in maintaining camptothecin in its active lactone form with considerable loading values and release profiles prolonged up to 96 h [60]. This study is significant because amphiphilic cyclodextrin nanoparticles have been compared with polymeric nanoparticles (poly(lactic-*co*-glycolic acid) [PLGA] and PCL) for the first time in terms of particle size, drug loading and drug release properties. It was found that for camptothecin, cyclodextrin nanoparticles were significantly more efficient for drug loading, which was four- to sixfold higher than PLGA or PCL nanoparticles' loading values, as seen in **Table 2**. *In vitro* release profiles of polymeric and cyclodextrin nanoparticles seen in **Figure 5** are also significant in the sense that amphiphilic cyclodextrin nanoparticles displayed prolonged release profiles for camptothecin, with β-CDC6 having a release period of up to 6 days and 6-*O*-CAPRO-β-CD releasing the drug within a 14-day period. On the other hand, both polymeric nanoparticles PLGA and PCL released the drug completely within 48 h [60]. *In vivo* antitumor efficacy of amphiphilic cyclodextrin nanoparticles in comparison with PLGA or PCL nanoparticles in rat glioma model was also evaluated and 6-*O*-CAPRO-β-CD in particular displayed a significantly higher survival rate in tumor-induced rats [61].

Biodistribution of non-ionic amphiphilic cyclodextrin (β-CDC6) nanoparticles on i.v. injection was evaluated using radiolabeled β-CDC6 nanoparticles in a mice model [62]. Blood kinetics and organ distribution of ^{125}I-labeled β-CDC6 nanoparticles were determined for a period of 10 min – 6 days and nanoparticle accumulation was observed mainly in reticuloendothelial system (RES) organs, showing a biodistribution profile in the order liver > spleen > heart > lungs > kidneys > stomach > skin > brain. No particular sign of toxicity was observed in animals that received β-CDC6 nanoparticle injection [62].

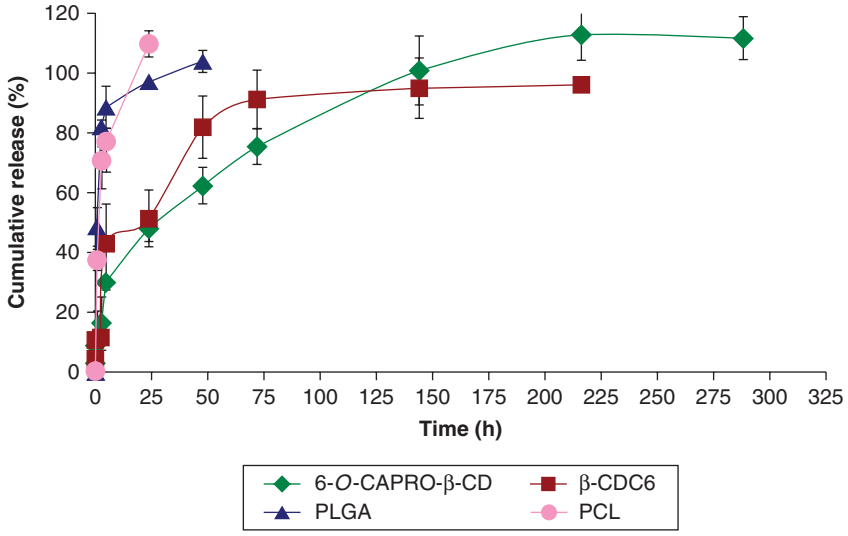

Figure 5. *In vitro* release of camptothecin from amphiphilic cyclodextrin and polymeric nanoparticles [57].
Reprinted with permission from [60].

Recently, Quaglia *et al.* [63] grafted oligoethyleneoxide side chains to non-ionic amphiphilic cyclodextrins modified with 16C aliphatic chains linked with thiol bonds. Docetaxel, an anticancer drug, was loaded to nanoparticles prepared from this new derivative using the emulsion-solvent evaporation method, achieving a very slow release profile of 8 weeks for docetaxel. A non-hemolytic property of oligoethylene oxide possessing non-ionic amphiphilic cyclodextrins was also demonstrated towards red blood cells. When HEp-2 cells were exposed to free docetaxel and docetaxel bound to non-ionic amphiphilic cyclodextrin nanoparticles, significantly higher cell damage and cell death were observed for nanoparticle-associated docetaxel. It was also reported that to prevent rapid clearance of nanoparticles from the bloodstream, biomimetic properties induced by oligoethylene oxide groups are essential to facilitate targeting of nanoparticles to tumor tissues. It was also believed that sustained release properties of these cyclodextrin nanoparticles contributes to the cytotoxic efficiency once the nanoparticle is internalized by the cell or forms a gradient around the tumor cell releasing anticancer drug for a prolonged period.

Cationic nanoparticles of heptakis(2-ω-amino-*O*-oligo (ethylene oxide)-hexylthio-β-CD have encapsulated anionic porphyrins by entangling porphyrin within aliphatic chains aligning both faces of the cationic amphiphilic cyclodextrin molecule. These nanoparticles have been demonstrated to preserve the photodynamic properties of the entrapped photoactive agent. Photodynamic efficacy of the carrier/sensitizer nanoparticles was proven by *in vitro* studies on tumor HeLa cervical carcinoma cells showing significant cell death on illumination with visible light [64].

Sulfated and non-sulfated amphiphilic cyclodextrin nanoparticles were evaluated for their physicochemical properties. These molecules were acylated on the secondary face and sulfated on the primary face. It was observed that sulfated amphiphilic cyclodextrins with hydrophilic–lipophilic balance (HLB) values > 8 were able to form spontaneous nanoaggregates in water. Sulfatation was reported to lead to stabilization of nanoparticle size and was believed to confer a biological activity to these interesting molecules [65].

Perfluoro-β-cyclodextrins were used to prepare nanocapsules with a single step nanoprecipitation technique. Highly fluorinated materials have multiple properties such as repellence to water and oil, unique dielectric, rheological and optical properties, as well as their exceptional chemical and biological inertness. The fluorinated chains, owing to their strong hydrophobic and fluorophilic character, enhanced particle size, stability, prolonged intravascular persistence and increased drug encapsulation capability. Thus, fluorophilic cyclodextrin nanoparticles were believed to be a suitable carrier for oxygen solubilization and delivery. Oxygen delivery of perfluorinated amphiphilic cyclodextrin nanocapsules was compared with water and showed a prolonged delivery of oxygen. Fluorophilic nanocapsules were believed to give better results than fluorocarbon emulsions as oxygen carriers because of the higher number of particles in the colloidal solution, which permits a greater rate of dissolved oxygen [43].

Perfluoroalkyl-α-cyclodextrin nanoparticles were prepared with a nanoprecipitation method without using a surface-active agent. It was found that fluorinated amphiphilic cyclodextrin nanoparticles had an average diameter of 100 nm and were stable for > 9 months as suspensions. Acyclovir was encapsulated into these nanoparticles using the high-loading technique with an encapsulation efficiency of 40%, and burst effect was delayed up to 3 h [66].

Amphiphilic β-CD nanocapsules prepared from amphiphiphilic cyclodextrins modified on the secondary face

were loaded with non-steroidal anti-inflammatory drug indomethacin for *in vivo* evaluation of ulcerogenic effects of indomethacin and the impact of nanoparticle encapsulation on the unwanted side effects of the bound drug on oral administration. An *in vivo* rat model was used to evaluate the nanocapsules. Gastrointestinal mucosa of the rat was significantly protected from the ulcerogenic effects of the active ingredient indomethacin when compared with administration of indomethacin in free form [67].

Most of the amphiphilic cyclodextrin nanoparticles were designed and developed for injection. Thus, they need to be applied to the patient in sterile form. Sterilization is a major challenge in new drug delivery systems and needs to be optimized in order not to disrupt the stability and integrity of the carrier system and the drug that is encapsulated. Three different sterilization techniques, autoclaving, filtration and gamma sterilization, were evaluated for β-CDC6 nanoparticles loaded with the model drug tamoxifen [68]. Membrane filtration was not suitable for injectable amphiphilic cyclodextrin nanoparticles because nanoparticle sizes were too close to the filter pore size of 0.22 μm. Autoclaving did not affect the nanoparticle yield but caused a significant increase in particle size and aggregates. Gamma irradiation with a dose of 25 kGy was demonstrated to be a suitable sterilization technique because no significant change was observed for mean diameter and zeta-potential of the nanoparticles and for drug entrapment efficiency and *in vitro* release profiles of tamoxifen.

Cytotoxicity of nanocapsules was investigated against L929 mouse fibroblast cells and human polymorphonuclear PMNC cells with MTT assay [50]. Non-surfactant nanoparticles and nanoparticles manufactured with increasing concentration of surfactant (Pluronic F68) were evaluated in terms of cytotoxicity to various cell lines. Non-surfactant β-CDC6 nanocapsules were less cytotoxic than nanocapsules containing surfactants. Cytotoxicity of the nanoparticles mostly arises from surfactant presence and was concentration dependent [50]. Nanospheres of β-CDC6 prepared without surfactant and with Pluronic F68 of varying concentrations between 0.1 and 1% were found to be slightly less cytotoxic than nanocapsules to both cell lines. Cytotoxicity increased with increasing concentration of surfactant and the most suitable percentage for surfactant if required was found to be 0.1% in this study [50].

4. Expert opinion

Amphiphilic cyclodextrins are emerging as promising derivatives to obtain safe and effective nanoparticles with high loading capacities for hydrophobic drugs, stabilizing properties for drugs or molecules that are labile to external conditions such as heat, light, hydrolysis, and so on, and to mask the undesired side effects of drugs on oral or intravenous administration. The presence of several different derivatives with a wide range of physicochemical and biological properties is another major asset of modified cyclodextrins that allows researchers to synthesize and use derivatives to achieve certain properties.

Various derivatives that can be possible candidates for obtaining amphiphilic cyclodextrins have been reported. For example, other than the derivatives that have already been mentioned in this review, carboxymethylethyl-β-cyclodextrin (CME-β-CD) is an ionizable cyclodextrin that can be used to develop an enteric carrier system because it possesses a pH-dependent solubility range in water. It is soluble only at low pH but is freely soluble in neutral or alkaline pH regions [69,70]. Acylated cyclodextrins are known for the delayed release properties on oral administration [71,72]. Sulfated β-cyclodextrins are reported to have interesting chemical and biological properties because of their angiogenic and antiviral properties [7,72,73]. Large-scale production and commercialization as excipients is valid for certain cyclodextrins, such as hydroxypropyl-β and γ-cyclodextrins [74-76] and sulfobutylether-β-cyclodextrins [77,78], through major companies such as Wacker, Roquette, CyDex, Cyclolab, and so on. Cyclodextrin polymers, on the other hand, are able to form hydrogels, beads, and wound-care material that enables cyclodextrins to be effective as drug delivery systems of different nature [79,80]. Recently, beads made from α-cyclodextrins have been reported that are promising for cosmetic application because they can be applied easily to the skin with instant melting and good occlusive properties when applied topically, and are promising for oral administration of lipophilic drugs with bioavailability problems [81,82].

Different strategies are also available for preparing nanoparticles that contain cyclodextrins as excipients. Other than direct preparation from amphiphilic cyclodextrins, researchers have focused on the following three strategies.

1. Preparation of nanoparticles from polymers in the presence of cyclodextrins as stabilizers [83,84].
2. Preparing nanoparticles from polymers incorporating or conjugated to natural or modified cyclodextrins [85-90].
3. Loading polymeric nanoparticles with drug:cyclodextrin complexes to improve drug loading and modify drug release [91-95].

Combination or conjugation of polymers with cyclodextrins allows nucleic acid, gene, DNA or small interfering ribonucleic acid (siRNA) delivery and is a promising alternative for protein delivery. For example, transferrin-containing cyclodextrin polymers have been reported for siRNA delivery and a tadpole-shaped polymer synthesized via coupling PLA onto an aminoalkylated-β-cyclodextrin (CDenPLA) has been reported to encapsulate and stabilize model protein bovine serum albumin. Nucleic acid delivery, on the other hand, was realized with β-cyclodextrin-modified polyethyleneimine (CD-PEI) nanoparticles [91].

In summary, cyclodextrin-based nanoparticles can be considered as alternatives or promising delivery systems for proteins and for tumor-targeted delivery. Functionalization of amphiphilic cyclodextrins stands out as an important

challenge for the future in this field. Researchers have already obtained targeted cyclodextrins by modification of natural cyclodextrins with polyethylene glycol (PEG) groups and folate-conjugation to target the abundant folate receptors on tumor cell surfaces [96,97]. Amphiphilic cyclodextrin nanoparticles that do not carry functional groups for active targeting benefit from the EPR effect, also known as passive targeting, thanks to their favorable size, surface charge and stability properties. Functionalization of amphiphilic cyclodextrins with active targeting moieties along with hydrophilic polymers such as PEG or polyethylene oxide (PEO) may allow the targeting of amphiphlic cyclodextrin nanoparticles, specifically tumor cells, avoiding non-selective cytotoxicity and dose-dependent side effects of anticancer drugs.

The existence of various cyclodextrin manufacturers and research groups working on cyclodextrins worldwide provides a strong platform for cyclodextrin research and development. There is an increasing number of studies in the world literature on nanoparticles for therapeutic and/or diagnostic applications and consequently regulatory approval, such as for Abraxane, which is albumin nanoparticle-bound paclitaxel approved by the FDA in 2005 for the treatment of metastatic breast cancer. These developments clear the way for cyclodextrin nanoparticles to be commercialized and used in major diseases such as cancer.

On the other hand, research on amphiphilic cyclodextrins and nanoparticles prepared from these molecules still lacks some important points that should be elucidated. Primarily, amphiphilic cyclodextrin nanoparticles reported in the literature fall within the range 100 – 500 nm regarding particle size. In this case, they will not be circulating for long in the blood before they are recognized by macrophages and taken up to RES organs. Therefore, research should focus on methods to prolong the circulation time for amphiphilic cyclodextrin nanoparticles either by reducing the particle size to < 100 nm or by the introduction of hydrophilic groups such as PEG or PEO to the nanoparticle structure either by chemical grafting or by coating to prevent opsonization after injection. Reducing the size drastically may also result in toxicity issues and accumulation of nanoparticle material in lungs or kidneys, and thus chemical modification of amphiphilic cyclodextrins to alter their surface charge or render a protein-repellant effect is believed to be more promising.

The pharmacokinetic profile of amphiphilic cyclodextrins on injection or after oral administration is another issue that needs to be clarified further. Only one work exists for β-CDC6 nanoparticles injected into rats that gives biodistribution data, but this field needs to be clarified in terms of plasma drug and cyclodextrin concentrations in different *in vivo* models. Biphasic release profiles obtained with amphiphilic cyclodextrin nanoparticles can then be of interest for the dosing of certain drugs that are in chronic or long-term use, especially in cancer patients.

Declaration of interest

The authors state no conflicts of interest and have received no payment in the preparation of this manuscript.

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