Thiolated cyclodextrins (CDs) are obtained by covalent attachment of thiol moieties on the oligomeric backbone of different types of CDs. First, thiolated CDs were introduced in the field of drug delivery as mucoadhesive oligomers forming disulfide bonds with cysteine-rich substructures of mucus glycoproteins providing a prolonged mucosal residence time. In the following, their in-situ gelling and permeation enhancing properties were shown. Furthermore, inclusion complexes formed with thiolated CDs can enhance drug solubility and provide a sustained release. These in combination unique properties make thiolated CDs a useful tool for different drug delivery systems and regenerative medicine. Within this review article, an overview of the synthesis of thiolated CDs and their key properties is provided. Moreover, the potential of thiolated CDs for different applications in drug delivery is shown.
1. Introduction

Thiolated polymers as excipients for drug delivery systems were first introduced in the late 1990s [1]. Due to the covalent attachment of thiol moieties on polymers, various additional functions are introduced. Polymers without thiols are simply like proteins without cysteine. The capability of thiolated polymers to form disulfide bonds not just within their polymer chains through an oxidative cross-linking but also with endogenous proteins such as mucins, keratins, and cysteine-rich receptor proteins opens the door for virtually unlimited medicinal applications. Due to the formation of disulfide bonds with cysteine-rich subdomains of mucus glycoproteins they were shown to provide comparatively much higher mucoadhesive properties [2]. Furthermore, features like in-situ gelling [3], efflux pump-inhibiting and permeation enhancing properties [4] were demonstrated. These unique properties of thiolated polymers resulted already in numerous clinical trials and product developments [5]. As many thiolated polymers have a polysaccharide backbone such as chitosan [6–10], hyaluronic acid [11–15], alginate [16–20] or starch [21–25], whose performance is mostly independent from the polymer chain length, it could have been that thiolation of CDs will show the same potential. Nevertheless, it took a decade from the discovery of thiolated polymers until first thiolated CDs were used in drug delivery [26] and these early-stage pharmaceutical applications focused more on the use of the high binding affinity of thiols to gold nanoparticles [27,28] than on the interactions of thiolated CDs with endogenous proteins [29].

Just in the last decade, the potential of thiolated CDs on the interphase between drug delivery systems and biological substrates was discovered and features such as mucoadhesive, in-situ gelling and permeation enhancing properties were shown. These studies confirmed on the one hand that by thiolation of CDs the same properties as known for polymers can be introduced. On the other hand, these studies showed also that thiolated CDs exhibit a greater potential than thiolated polymers for numerous applications because of their comparatively small size and lipophilic core. Owing to their small size, they can penetrate the mucus gel layer with a mesh size of (10–200 nm)³ to a comparatively higher extent getting more tightly anchored in deeper mucus regions close to the absorption membrane and interacting more intensively with cysteine-rich membrane-bound proteins. In the case of ocular delivery, they are even small enough drug carriers to remain invisible on the cornea [30]. Furthermore, by the incorporation of hydrophobic drugs in the lipophilic core of thiolated CDs, their solubility can be strongly improved, a protective effect can be provided, an unpleasant taste can be masked and drug release can be controlled.

Within this review article, an overview of the synthesis of thiolated CDs and key features including mucoadhesive, in-situ gelling, inclusion complex forming, solubility improving and permeation enhancing properties are discussed. Moreover, the potential of thiolated CDs for different applications in drug delivery systems is highlighted in detail.

2. Synthetic pathways for thiolated CDs

CDs are cyclic oligosaccharides derived from starch-containing six (α-CD), seven (β-CD), or eight (γ-CD) glucopyranose units linked by α-(1–4) bonds [31]. They exhibit two types of –OH groups at three different positions: primary –OH groups at the C-6 position and secondary –OH groups at C-2 and C-3 position. These –OH groups are located on the edges providing a hydrophilic exterior, whereas C-H units at the inside create a hydrophobic interior [32,33]. The hydrophobic cavity of CDs can host various molecules making them a useful tool for various industries such as the food, cosmetic or pharmaceutical industry [34,35,36].

All modifications of CDs take place at the hydroxyl groups because of their nucleophilicity [37]. As the –OH group at C-6 position is most nucleophilic [38,39], it is easily attacked by electrophilic reagents. In comparison secondary –OH groups at C-2 and C-3 position are less nucleophilic and reactive. In addition, hydrogen bonding between them lowers their accessibility for reactants. As the hydroxyl group at position C-2 is most acidic, it is also an interesting target for derivatization whereas position C-3 is least accessible and requires alkylation or sulfonylation prior to derivatization [40].

In general, thiolated CDs can be formed by substitution of hydroxyl groups with thiols groups (I), by the attachment of thiol-bearing ligands (II) and by a ring-opening of the glucopyranose units followed by the conjugation of sulphydryl ligands (III). The reactivity of thiol groups on CDs is determined by their pKa. Thiol groups easily lose their proton (H⁺) forming thiolate anions (–S⁻). The lower the pKa value of –SH groups is, the higher is the concentration of thiolate anions at physiological pH as shown in Fig. 1. The pKa value of thiolated CDs can be determined by UV spectrophotometry [41,42].

The rate of thiol oxidation and thiol/disulfide exchange reaction is inversely dependent on the pKa of the attacking –SH ligand, as the thiolate anion is more nucleophilic than the thiol [43]. Therefore, the decrease in pKa of –SH groups results in an overall rate enhancement until the thiolate anion becomes the dominant species and then drops in nucleophilicity resulting in slower kinetics [44]. Alkyl thiols have a pKa in the range of 8–10 [5,45,46]. The pKa of hydroxyl to sulphydryl conversions such as thiolated HP-β-CD, per-6-thiolated α-CD and tetradeca-thiolated β-CD, however, was found to be in a narrow range between 7.8 and 8.2 providing limited flexibility on that parameter [47,41,48]. On the contrary, the covalent attachment of sulphydryl ligands exhibiting a broad range of pKa values according to their chemical structure opens the door to a huge variety of thiolated CDs from very low to very high reactivity such as S-protected γ-CD [42] or pre-activated α-CD [49]. Furthermore, additional functionalities such as charges can be introduced via ligands.

Sulphydryl groups are sensitive towards oxidation and can be easily oxidized at pH greater than 5 [11] as shown in Fig. 2. Oxidation of –SH groups during storage or application, however, can deteriorate the interactions between thiolated CDs and endogenous thiols limiting their efficiency. Moreover, due to a small difference in electronegativity between hydrogen and sulfur atom of thiol groups, a non-polar covalent bond is formed between

![Relative reactivity](image-url)
thiolated via groups but here all the primary –OH groups on C-6 of CD were first modified using regioselective solvents. Halogenation of polysaccharides takes place at higher temperatures leading to an insufficient degree of substitution (DS) but reduces also the reaction time from over 20 h to a few minutes, it seems to be advantageous [48]. Moreover, microwave irradiation has great potential in green chemistry [59] because the hydrogen bonding between them makes them rigid and less flexible as compared to C-6 position primary –OH groups.

Thiolated β-CD, for instance, was almost stable towards oxidation at pH 5, whereas a significant decline in thiol groups was observed at pH 6 and 7.2 [51]. Similarly, thiolated HP-β-CD was progressively oxidized at pH 6.0 and 7.2 with just 60% and 40% remaining –SH groups after 3 h, respectively [41].

2.1. Hydroxyl to sulhydryl conversions

CDs can be thiolated by direct conversion of hydroxyl groups to sulhydryl groups. Hydroxyl groups of CDs are first substituted by halogens and then transformed into thiol groups under inert condition using regioselective solvents. Halogenation of polysaccharides is a temperature-dependent reaction as degradation of polysaccharides takes place at higher temperatures leading to an insufficient degree of substitution (DS) and low recoveries of the products [52]. In order to achieve a higher degree of halogenation and at the same time to avoid degradation of CDs, the reaction was initially proceeded at 90 °C for 1 h and afterwards continued at 70 °C [51]. This pathway offers the possibility to generate thiolated CDs with a high DS [51,42].

Manta et al. explored a three-step method for the synthesis of thiolated CDs. This synthetic pathway involves epoxy activation of β-CD to generate oxirane groups by the addition of epichlorohydrin or 1,4-butanediol diglycidyl ether (DGE) in alkaline medium, then conversion into thiosulfate groups by using sodium thiosulfate and finally reduction to –SH groups with thiopropyl-agarose [53]. Peralta-Altier et al. also used the same method with the only difference of epoxy activation of β-CD with DGE for the synthesis of thiolated β-CD [54]. Moreover, a two-step method was explored to modify β-CD. First, to activate –OH groups on β-CD, sodium hydride (NaH) was added followed by the addition of allyl bromide to the reaction mixture to generate β-CD-allyl ether. In the next step, thiolated β-CD was produced via the addition of thioacetic acid by photo-conjugation [55]. These synthetic pathways for thiolated CDs are illustrated in Fig. 3.

2.1.1. Per-6-thiolated CDs

In all above mentioned thiolated synthetic pathways, CDs were thiolated via substitution of one or two –OH groups with –SH groups but here all the primary –OH groups on C-6 of CD were first substituted with halogen (iodine) and then replaced by –SH groups without opening the ring of CD [28,56].

In this method per-6-modification of all primary –OH groups of CD was done by a selective reaction [57]. As compared to previously described thiolated CDs synthesis methods, in this synthetic pathway along with triphenylphosphine (Ph₃P), anhydrous N,N-dimethylformamide (DMF) was used for halogenation at C-6 position to generate phosphonium salt to activate all primary –OH groups towards nucleophilic displacement under inert environment [58]. Anhydrous DMF was used as a solvent in order to improve the stability and solubility of intermediate reagents [37]. Moreover, in this method comparatively less reactive reagents attack more selectively the C-6-position –OH groups as more reactive reagents will not only react with –OH groups at C-6 positions but also with –OH groups at C-2 and C-3 positions [37].

2.1.2. Tetradeca-thiolated CDs

Tetradeca-thiolated CDs are also called per-2,6-thiolated CDs and these thiolated CDs were synthesized using microwave irradiation. In comparison to per-6-thiolated CDs, in this method –OH groups at C-2 position were also substituted by –SH groups along with C-6 position primary –OH groups. As primary –OH groups at C-6 position are easily attacked by an electrophilic reagent as compared to secondary –OH groups at C-2 and C-3 position because the hydrogen bonding between them makes them rigid and less flexible as compared to C-6 position primary –OH groups. Moreover, due to the presence of two –OH groups at the secondary side, the steric hindrance for substitution of secondary –OH at C-2 and C-3 position is larger than that at the primary side. In detail, β-CD was treated with thiourea in acidic conditions for the protonation of –OH groups at primary and secondary positions. The protonated –OH groups have high reactivity in microwave condition and reaction proceeds via isothiouronium ion intermediate that after hydrolysis provides thiol groups. A yield of 85% was obtained with this method.

As this method does not only significantly increases the degree of substitution (DS) but reduces also the reaction time from over 20 h to a few minutes, it seems to be advantageous [48]. Moreover, microwave irradiation has great potential in green chemistry [59] with high practical yield [60].

2.2. Covalent attachment of sulhydryl ligands

Stickland et al. synthesized thiolated β-CD by introducing a tosylate group to β-CD backbone by replacing primary –OH group followed by nucleophilic substitution of this tosylate group using sodium azide to produce monoazide intermediate, that was reduced to amine. Finally, 6-O-amino-β-CD was coupled with the N-hydroxysuccinimide (NHS) ester of lipic acid dissolved in anhydrous DMF to give thiolated β-CD (6-O-lipoyl-β-CD) [61]. The same procedure was followed by Henke et al. but diithiopropionic acid was coupled via an amide linkage to β-CD amine to obtain thiolated β-CD derivatives [62]. Nucleophilic substitution of tosylate group with 1,10-decanedithiols or triethylene dithioglycol also leads to the formation of thiolated CDs [63]. A two-step synthesis method was introduced by Shih et al. to modify β-CD with allyl-lether. First, NaH was added to activate hydroxyl groups on β-CD followed by the addition of allyl bromide in excess to generate β-CD-allyl ether [64]. Finally, β-CD-AE was photo conjugated with thioacetic acid via radical-mediated thiol-allyl-lether addition to obtain thiol-substituted β-CD [55].

2.3. Ring-opened thiolated CDs

Periodate cleavage of vicinal diols followed by reductive amination is another useful technique for the thiolation of CDs. Hydroxyl
groups on neighboring carbon atoms, so-called vicinal diols, are oxidized for the generation of aldehydes by using sodium periodate (NaIO₄). Periodate acts by selectively oxidizing the vicinal hydroxyl groups at carbon atoms 2 and 3 to aldehydes thereby breaking the C–C bond. The degree of oxidation depends on the applied concentration of NaIO₄ [64,65]. In the second step, imines or so-called Schiff’s bases are formed between the aldehydes and sulfhydryl ligands bearing a primary amine group such as

**Fig. 3.** Synthetic pathways of different thiolated CDs. [A] Hydroxyl to sulfhydryl conversions and [B] covalent attachment of sulfhydryl ligands.
cysteamine. As these imine substructures are unstable under acidic conditions, they are subsequently reduced with NaCNBH₃ to secondary amines as illustrated in Fig. 3. In this way, cationic substructures in the form of secondary anions are introduced in CDs [29]. The amount of thiol groups immobilized on the backbone of different thiolated CDs is given in Table 1. There are several other studies published describing various esterification methods for CDs, although none of them had the purpose to attach thiol groups [66,67 68,69].

2.4. S-protected thiolated CDs

As thiol groups are highly susceptible to oxidation and oxidize at pH ≥ 5, thiolated CDs were S-protected in order to improve their stability. The high reactivity of thiols is disadvantageous as they are already inactivated on the way to their target site by the formation of disulfides with other endogenous thiol substructures such as mucins or keratins. Thioamides like 2-mercaptopyridine or 2-mercaptonicotinic acid (2-MNA) can convert to thiol and are more reactive than simple alkyl thiols [7,84]. Thiolated CDs were S-protected by the introduction of a protective group, like mercaptonicotinamide, by a disulfide bond. Upon contact with the mucus, a thiol/disulfide exchange occurs between the cysteine moieties of the mucin proteins and the thiomer, whereby mercaptonicotinamide is a harmless leaving group and the backbone of the polymer is covalently attached to the mucus [85].

Ring-opened S-protected thiolated α-CD was obtained via the covalent attachment of L-cysteine-2-mercaptonicotinic acid conjugate (Cys-MNA) ligand to oxidized α-CD [70]. In this method, the degree of S-protection was dependent on the degree of oxidation of α-CD and oxidation of α-CD was gradually increased with increasing concentration of NaO₄ to generate carbonyl groups in dextrose molecule of α-CD via the oxidation of vicinal diols. To minimize non-specific oxidation of α-CD the reaction was performed at 30 °C for 2 h [86]. The same method of S-protection was also followed by Asim et al. for mucoadhesive S-protected thiolated cyclodextrin-iodine complexes as shown in Fig. 4.

These S-protected thiolated CDs, however, were synthesized under harsh conditions via an oxidative opening of the glucose rings and cytotoxic effects were observed due to the cationic nature [71]. To overcome these shortcomings S-protected thiolated CDs were developed by reductive amination without ring-opening via formation of disulfide bonds between thiol groups of thiolated γ-CD and the aromatic ligand 2-mercaptonicotinic acid (2-MNA) [42]. As these S-protected thiolated CDs are more reactive than unprotected thiolated CDs, they are also referred as pre-activated thiolated CDs. S-protected thiolated CDs with the amount of 2-MNA attached are shown in Table 1.

The covalent attachment of different S-protection groups to thiolated CDs, however, results in changes in their chemical structure due to conjugation and subsequently alters the pKa values. Therefore, this S-protection of thiolated CDs does not only provide stability towards oxidation but also increases the reactivity of the thiols. As the π-system of the pyridine has an electron-withdrawing effect, free thiol moieties are released after mercaptopyridine group cleavage showing their high reactivity.

Table 1

| Thiolated CDs | Method of Synthesis | –SH groups (µmol/g) | –S– (µmol/g) | MNA (µmol/g) | Refs. |
|---------------|---------------------|---------------------|--------------|--------------|-------|
| α-CD          | Mono-6-thiolation   | 1163 ± 96           | 160 ± 97     | ———          | [51]  |
| α-CD          | Mono-6-thiolation   | 1426 ± 66           | ———          | 4285 ± 439   | [70]  |
| α-CD          | Di-6-thiolation     | 1144 ± 154          | ———          | 3804 ± 302   | [72]  |
| α-CD          | Per-6-thiolation    | 4244 ± 402          | 786 ± 139    | ———          | [47,73]|
| β-CD          | Mono-6-thiolation   | 1784 ± 201          | 223 ± 108    | ———          | [29]  |
| β-CD          | Di-6-thiolation     | 1564 ± 171          | ———          | ———          | [76]  |
| β-CD          | Per-6-thiolation    | 7-SHC/CD            | ———          | ———          | [28,77]|
| β-CD          | Tetradeca-thiolation| 8144 ± 317          | 1786 ± 118   | ———          | [48]  |
| β-CD          | Mono-6-thiolation   | 1-SHC/CD            | ———          | ———          | [53,78]|
| β-CD          | Mono-6-thiolation   | 1-SHC/CD            | ———          | ———          | [54,79]|
| β-CD          | Mono-6-thiolation   | 0.362 mM            | ———          | ———          | [55]  |
| β-CD          | Mono-6-thiolation   | 1-SHC/CD            | ———          | ———          | [61,80]|
| β-CD          | Mono-6-thiolation   | 1-SHC/CD            | ———          | ———          | [62]  |
| β-CD          | Mono-6-thiolation   | 1-SHC/CD            | ———          | ———          | [63]  |
| β-CD          | Heptakis-6-thiolation| 7-SHC/CD            | ———          | ———          | [81,82]|
| β-CD          | Per-6-thiolation    | 98% thiols          | ———          | ———          | [83]  |
| HP-β-CD       | Mono-6-thiolation   | 975 ± 42            | 149 ± 37     | ———          | [41]  |
| γ-CD          | Di-6-thiolation     | 1385 ± 84           | 180 ± 48     | 1153 ± 41    | [42]  |
3. Safety of thiolated CDs

As CDs are widely used in the pharmaceutical field, data of numerous toxicological studies are available [87,88,89]. Orally administered CDs were shown to be non-toxic due to lack of absorption from the gastrointestinal tract [36,90,88]. Exposure to HP-β-CD and sulfolubet ether β-cyclodextrin (SBE β-CD) solutions had only negligible effects on Caco-2 cell monolayer integrity [91]. Similarly, Mitra et al. effectively used CDs as nasal absorption promoters without any toxic effects [92]. Arimori and Uekama showed the iv pharmacokinetics in rabbits from α-CD and γ-CD aqueous solutions containing prednisolone as compared to an aqueous solution of its phosphate ester prodrug reflecting identical pharmacokinetics [93]. Moreover, Masuda et al. proved a protective effect of CDs showing that α-CD is able to protect against the irritating effect of flurbiprofen after ocular administration in rabbits and humans [94]. In case of thiolated CDs, however, there are comparatively less safety data available so far.

As oligosaccharides CDs are prone to degradation by various enzymes such as cyclomaltodextrinase, α-amylase or glucoamylase. Of relevance for the degradation of CDs are salivary and pancreatic α-amylases [95]. The degradation of CDs is based on a multiple attack reaction, whereas the first reaction is the ring-opening, that is also the rate-determining step [96]. In general, α-CD is hardly degraded by α-amylases and the degradation of β-CD is rather slow in comparison to γ-CD [97]. This is due to the higher flexibility of the γ-CD ring, making it more accessible for the hydrolysis by α-amylases. Thiolated γ-CDs were shown to be degraded by α-amylase at a rate comparable to unmodified γ-CD [42].

Cytotoxic effects of all thiolated and S-protected CDs on Caco-2 cells were investigated by various toxicological assays performed including resazurin assay [98], lactate dehydrogenase (LDH) assay [99], hemolysis assay [100] and MTT assay [101]. All thiolated and S-protected thiolated CDs were found non-toxic except first-generation thiolated cationic β-CD [29] that showed a pronounced toxic effect increasing over time. The comparatively high toxicity of cationic thiolated β-CD can be explained by the positive charge of secondary amino groups that destabilizes cell membrane integrity [102]. The mercaptocitroninamide or 2-mercaptocticonic acid (2-MNA) are mostly used for S-protection due to safety reasons as mercaptocitronin acid and mercaptocitroninamide are safer than mercaptopyridine [85,103]. As proof of concept, in-vivo studies also confirmed the safety of per-6-thiolated CDs [47]. Moreover, Gimenez et al. showed via an in-vitro MTT cytotoxicity assay on a supramolecular system composed by violaine complexed by thiolated β-CD-Au-NP on V79 and HL60 cell lines that such systems are non-toxic [101,104,105]. In another study with thiolated CD, it was proven that cetirizine incorporated in thiolated CD shows minimal conjunctival and corneal irritation without inflammatory effects being observed during 72 h of study [72]. These results provide evidence that the protective property of CDs toward the irritating effect of drugs is not significantly affected by their thiolation.

4. Key features of thiolated CDs

Key features of thiolated CDs are based on mucoadhesive properties by the formation of disulfide bonds with cysteine-rich subdomains of endogenous proteins, cross-linking properties, inclusion complex formation, solubility enhancement, permeation enhancement, controlled drug release, and metal-binding properties. An overview of the versatile properties of thiolated CDs is provided in Fig. 5.

4.1. Mucoadhesion

The phenomenon of mucoadhesion is believed to be based on associative interactions between mucoadhesive materials and the mucus gel layer [85]. The complex structure of mucus offers many opportunities for adhesive interactions either through non-specific Van der Waals forces or specific interactions such as ionic interactions between complementary chemical structures [106]. In the case of polymers, mucoadhesion can also be achieved by an interpenetration process of the polymer chains in the mucus gel layer followed by chain entanglements. As in the case of CDs, however, neither sufficiently strong adhesive interactions nor chain entanglements are feasible, they are not at all mucoadhesive. Hence, thiolation as illustrated in Fig. 6 is key to introduce mucoadhesive properties and there are already numerous studies available providing evidence for that. Improvement in mucoadhesive properties of thiolated α-CDs is based on the formation of disulfide bonds between thiolated CDs and cysteine-rich subdomains of mucus glycoproteins [50]. For instance, thiolated β-CDs showed prolonged mucoadhesion on buccal mucosa. After 3 h, more than 50% of the β-CD-Cs remained attached to the buccal mucosa and showed a 34.5-fold improvement in mucoadhesion [29]. Similarly, in-vitro mucoadhesion studies on porcine vaginal mucosa revealed that thiolated β-CDs derivatives display significantly higher mucoadhesive properties than the corresponding unmodified β-CD. According to these results, immobilization of thiol groups on different thiolated β-CDs derivatives showed 5.8-, 15.5-, and 17.1-fold improved mucoadhesion on the porcine vaginal mucosa compared to unmodified β-CD [76]. Moreover, ocular mucosa has also been investigated for the mucoadhesive properties of thiolated α-CDs that showed up to 32-fold improved mucoadhesion on porcine ocular mucosa compared to unmodified α-CD [72]. In contrast to thiolated polymers, thiolated CDs are invisible on the ocular surface [72] and sprayable for nasal application [107]. Furthermore, Denora et al. showed that thiolated HP-β-CD is able to remain longer in contact with the esophageal mucosal layer as compared to unmodified HP-β-CD [41]. It was proven for thiolated polymers that the higher the amount of –SH groups immobilized on the polymer backbone is, the higher are their mucoadhesive properties [108]. Therefore, a highly thiolated CD namely tetradeca-thiolated β-CD was accessed for comparison reason and mucoadhesive studies on different porcine mucosae were performed [48]. Tetradeca-thiolated β-CD showed higher mucoadhesive properties on all types of mucosae compared to previously tested less thiolated CDs [42,71,51,72,76,29,70,41]. For instance, only 46% of non-ionic thiolated β-CD remained on the intestinal mucosa, whereas almost 80% of tetradeca-thiolated β-CD remained on the same mucosa. Similarly, only 30% of thiolated β-CD remained on the vaginal mucosa, whereas 55% of tetradeca-thiolated β-CD remained on the vaginal mucosa. This study strongly supports the theory that a higher degree of thiolation improves mucoadhesion [109].

The mucoadhesive properties of thiolated CDs can be even further improved via S-protection as reported in numerous review articles on thiomers [85,110–114]. In addition to disulfide bond formation of S-protected thiolated CDs with cysteine-rich subdomains in mucin, these thiolated oligomers are also believed to react with glycoproteins in many different ways.

Mucoadhesive properties of S-protected thiolated α-CDs on the urinary bladder mucosa were evaluated using a fluorescence marker. The study revealed that S-protected thiolated α-CDs display significantly higher adhesion than the corresponding thiolated and unmodified α-CDs. Almost 33.8% thiolated α-CD was retained on the bladder mucosa, whereas more than 70% S-protected thiolated CD remained on the same mucosa. S-protected thiolated
CDs also showed improved mucoadhesive properties on porcine intestinal mucosa as compared to thiolated CDs [70]. More details about the mucosal residence time of S-protected thiolated CDs on different mucosae is provided in Table 2.

An extended residence time on intestinal mucosa leads to improved patient compliance as the frequency of dosing can be reduced and an increased concentration of the drug at the target site can be attained. Due to the strong mucoadhesive properties of per-6-thiolated CD, for instance, a comparatively high furosemide concentration should be maintained in plasma for 8 h after its oral administration [47]. Moreover, thiolated CDs turned out to be beneficial for the treatment of Eosinophilic Esophagitis.
Thiolated CDs interact with cysteine-rich subdomains found in mucin to form new disulfide bonds [48] leading to an increase in viscosity of thiolated CDs/mucin blends. In the presence of per-6-thiolated α-CD, the dynamic viscosity of mucus was increased 5.8-fold [47]. Moreover, in the presence of tetradeca-thiolated β-CDs 13.5-fold improvement in viscosity of mucus was observed due to a much higher degree of thiolation [48]. These results confirm the hypothesis that an increase in viscosity correlates with the amount of sulfhydryl moieties on the oligo- or polymeric backbone [125]. In-situ gelling properties of highly thiolated CDs are illustrated in Fig. 7. As a high increase in viscosity, however, can hinder the penetration of thiolated CDs into deeper mucus regions, an even higher degree of thiolation might not be of interest anymore.

Apart from mucus glycoproteins, thiolated CDs can of course cross-link with various other types of polymers forming hydrogels. In-situ gelling hydrogels are applied in-vivo by direct injection [126,127]. Generally, in-situ gelling hydrogels are not suitable for hydrophobic drugs due to limited solubility and the lack of interactions between the hydrophobic drug and the hydrophilic polymeric backbone of the hydrogel [128]. Therefore, thiolated CDs are used for in-situ hydrogel formation due to their hydrophobic cavity for drug entrapment and thiol groups working as cross-linkers for hydrogels. Peng et al. developed an in-situ gelling hydrogel system comprising thiolated β-CD and maleimide functionalyzed dextran that is able to bind and release hydrophobic drugs such as retinoic acid in a sustained manner [129]. Moreover, Arslan et al. also fabricated well-defined responsive hydrogels using thiol-ene conjugation of thiolated CDs as cross-linkers and thiol-reactive telechelic polymers [130].

Arslan et al. conjugated per-6-thiolated CD and telechelic maleimide functionalized linear poly(ethylene glycols) (PEG) obtaining well-defined hydrogels [83]. Gel formation appeared through multiple Michael reaction between thiols and maleimide groups. The synthesis protocol employs the utilization of maleimide

4.2. Cross-linking properties

The mucosal residence time can also be improved by increasing the viscosity of formulations due to cross-linking properties [112]. The application of high viscous gels, however, results in an insufficient distribution of the formulation over the target mucosa. Therefore, in-situ gelling properties are beneficial [117], as formulations exhibiting such properties can be applied in low viscous form spreading over the target mucosa still providing a prolonged residence time due to gelation on the site of application via inter- and intra-molecular disulfide bond formation. The sol-to-gel transition of thiomers is dependent on various parameters such as pH [118–120], temperature [121,122] and electrolytes concentration [123,124]. At physiological pH, sufficiently high amounts of negative thiolate anions (–S\(^{-}\)) are essential for oxidation and cross-linking. Thiolated CDs display such in-situ gelling properties due to the oxidation of –SH groups, which results in the formation of inter- and intra-molecular disulfide bonds. In contrast to thiolated polymers exhibiting high in-situ gelling properties just by disulfide bond formation within their polymeric chains, thiolated CDs show pronounced in-situ gelling properties only in reaction with polymers such as mucus glycoproteins.

(EoE) due to an extended time of contact between the administered drug and the oesophageal mucosa [41].

Similarly, formulations can be developed for vaginal applications as a liquid or semi-solid dosage forms. Moreover, aqueous formulations consisting of thiolated CDs might be helpful in reducing the blurred vision, itching and local irritation often caused by ophthalmic emulsions, ointments, and gels [72,115,116]. Significantly improved mucoadhesive properties of thiolated CDs will reduce the need of additional excipients in ophthalmic formulations typically used for increasing viscosity of the formulation.

Table 2

| Types of thiolated CDs | Intestinal mucosa | Buccal mucosa | Vaginal mucosa | Ocular mucosa | Bladder mucosa | Oesophageal mucosa | Ref. |
|------------------------|------------------|--------------|----------------|--------------|---------------|-------------------|-----|
| Thiolated-γ-CD         | 50.9-fold        | ——           | ——             | ——           | ——            | ——               | [42]|
| S-protected thiolated α-CD | ——           | ——           | ——             | ——           | ——            | ——               | [70]|
| S-protected thiolated α-CD | 38-fold        | ——           | ——             | ——           | ——            | 25-fold           | [71]|
| Thiolated β-CD         | 46.4-fold        | ——           | ——             | ——           | ——            | ——               | [31]|
| Thiolated β-CD         | 49.1-fold        | 34.5-fold    | ——             | ——           | ——            | ——               | [29]|
| Tetradeca-thiolated β-CD | 78.6-fold      | 60.3-fold    | 49.3-fold      | ——           | ——            | ——               | [48]|
| Thiolated β-CD         | ——              | ——           | ——             | ——           | ——            | 11.9-fold         | [41]|
| S-protected thiolated α-CD | ——           | ——           | ——             | ——           | ——            | ——               | [76]|

In-situ gellation of tetradeca-thiolated CD with mucus.

In-situ gelling properties of highly thiolated CDs are illustrated in Fig. 7. As a high increase in viscosity, however, can hinder the penetration of thiolated CDs into deeper mucus regions, an even higher degree of thiolation might not be of interest anymore.

Fig. 7. In-situ gelation of tetradeca-thiolated CD with mucus.
end-functionalized PEGs as the matrix and per-6-thiolated CD as a cross-linker, whereby reaction between the –SH groups and maleimide produces rapid gelation. Chemically homogenous hydrogels were generated through specific cross-linking chemistry compared to traditional gels obtained through random cross-linking of polymer chains.

Moreover, because of their unique polymerization properties, star polymers have received increasing interest [131–133]. Star polymers are branched polymers having many linear chains attached to the central core [134]. Modification of CDs has offered opportunities to develop CD-based star polymers via thiol-ene reaction, a versatile approach different from polymerization techniques [135–138]. Zhang et al. synthesized CD-based star polymers via thiol-ene addition of per-6-thiolated-β-CD with different (meth)acrylic monomers and vinyl terminated polymers. In this study, the precursors were conjugated with thiolated β-CD via thiol-ene click reaction and then ring-opening polymerization (ROP) was investigated as an initiator to obtain a thiol-ene product [139]. In another study, thiolated β-CD was cross-linked with allyl groups of bifunctional linear PEG via radical-induced thiol-ene click chemistry as displayed in Fig. 8 [140]. Depending on the polymer chain length, well-defined hydrogel networks were formed by this gelling process.

In-situ gelling thiomers show also potential in the field of tissue regeneration as highlighted in numerous studies [141–149]. Thiolated CDs were therefore also investigated for tissue engineering. Shih et al. designed a thiol-ene photopolymerized hydrogel using thiolated β-CD and PEG encapsulating an anti-inflammatory and anticancer hydrophobic molecule curcumin [55]. This thiolated β-CD cross-linked PEG-based hydrogel demonstrated higher drug loading efficiency as compared with pure PEG-based hydrogels. Moreover, encapsulation of pancreatic MIN6 β-cells into these hydrogels showed enhanced cell proliferation and insulin secretion as compared to hydrogels cross-linked by dithiothreitol (DTT). These thiolated-CDs immobilized hydrogels do not only permit cell encapsulation but may also allow creating hydrogels with dynamically tunable properties in the presence of cells.

4.3. Inclusion complex formation and solubility enhancement

CDs consist of several glucose units forming a truncated cone. This cone can be pharmacologically used for the encapsulation of drugs. The formation of the host–guest complex is favored by hydrophobic interactions, electrostatic interactions, Van der Waals contributions, hydrogen bonding, the release of conformational strain and charge-transfer interactions [146]. Based on the cavity size, appropriate drugs can be incorporated into CDs. In general, smaller drugs containing an aliphatic chain can be encapsulated in α-CD, whereas β-CD can incorporate aromatic and heterocyclic structures. The γ-CD being the biggest CD can host macromolecules and steroids [147]. To which extent the ring-opening of thiolated CDs as described above enlarges the cone structure so that drugs of even higher molecular mass can be incorporated, has so far not been evaluated. The incorporation of drugs in CDs can be used to improve various pharmaceutically relevant properties. The most prominent advantage is certainly the solubility enhancement of poorly soluble drugs by incorporating them into the hydrophobic cavity of CDs resulting in water-soluble complexes [148]. The improved solubility leads in many cases to an improved bioavailability [149].

The limiting factor for the potential use of β-CD in the pharmaceutical field is its insufficient aqueous solubility due to self-aggregation by H-bonds [150]. The substitution of H-bond donor and acceptor substructures by other functional groups around the CD ring, however, is assumed to improve its aqueous solubility. This theory is at least true for β-CD [151,152]. Ijaz et al. proved this theory by thiolation of β-CD increasing with this excipient solubility of miconazole. As a class II drug according to the Biopharmaceutical Classification System (BCS), miconazole shows low water solubility, but high intestinal permeability. Its solubility was increased up to 150-fold due to inclusion complex formation with thiolated β-CD, whereas in the presence of unmodified β-CD an only 2-fold increase was observed. In another study, a 257-fold improvement in the solubility of miconazole nitrate was observed [51]. The solubility of the hydrophobic anti-inflammatory steroid prednisolone was strongly improved by 6-S-glycosyl-6-thio derivatives of β-CD compared with unmodified β-CD. Thiolated CD derivatives with α-anomeric configuration showed an enhanced solubility in water compared to the corresponding β-anomers of β-CD reflecting the low solubilizing properties of these derivatives towards prednisolone [153]. Kim et al. showed that cysteinyl β-CD-baicealin inclusion complex has improved solubilizing efficiency and antioxidant activity of baicealin than the corresponding complex formed with unmodified β-CD [154]. Other inclusion complexes of thiolated CDs with acyclovir for vaginal application [76], trimethoprim for intravesical use [70] and cetirizine for oral delivery [72] showed enhanced drug solubility. Denora et al. demonstrated that water solubility of budsone (BUD) in the presence of thiolated HP-β-CD increases about 85 times due to the thiol residues on the CD backbone that might influence the lipophilic character of the cavity [41]. Furthermore, thiolated CDs were investigated for solubility improvement of BCS Class IV drugs. Per-6-thiolated α-CD showed significant enhancement in solubility of furosemide (FUR) [47]. The interactions of this BCS Class IV drug with thiolated CD displayed typical A_L type solubility curves, indicating a soluble binary complexes

Fig. 8. Schematic illustration of hydrogel formation via radical thiol-ene click reaction. Adapted from reference [140].
formation at a stoichiometric ratio of 1:1. Improved solubility of poorly soluble drugs with thiolated CDs is advantageous in situations where the drug is poorly soluble and rapidly washed off by body fluids. Recently, Zhang et al. synthesized β-CD polymers (β-CDPs) by conjugation of thiolated β-CD and maleimide-functionalized hydroxypropyl-β-CD (HPCD-AMI) via thiol-maleimide click chemistry forming inclusion complexes of these polymers with curcumin. Phase solubility studies showed that after the complexation of curcumin with β-CDs, solubility increases up to 3780 times in comparison to the β-CD complex [155]. The ability of thiolated CDs to form complexes with different drug molecules is summarized in Table 3.

Another important aspect is the stability improvement of incorporated drugs. Numerous studies prove evidence that photo-stability, chemical and thermal stability of drugs can be strongly improved [156,157,158].

4.4. Permeation enhancement

According to Clausen et al., the mechanism being responsible for the permeation enhancing effect of thiomers is based on the inhibition of protein tyrosine phosphatase (PTP) being involved in the opening of tight junctions (TJs) [167]. Occludin, a primary transmembrane protein that builds up TJs is dephosphorylated by this enzyme. Opening of TJs occurs by phosphorylation of occludin mediated by protein tyrosine kinase, whereas dephosphorylation of these groups results in a closing of TJs [168]. PTP inhibition leads therefore to TJs opening with improved paracellular drug transport [169]. The active center of PTP bearing a cysteine substructure is inhibited by reduced glutathione (GSH) via disulfide bond formation that is in the presence of thiolated CDs formed in high amounts by converting oxidized glutathione (GSSG) to GSH [167]. The prolonged residence time of thiolated CDs on the mucosa due to their mucoadhesive properties [42] can shift the balance between GSSG and GSH towards GSH.

More recently, Zhang et al. showed that even further mechanisms are involved in the TJs opening effect of thiomers [170]. When getting into contact with highly expressed cysteine-rich membrane receptors, thiolated polymers tend to interact with receptors like epidermal growth factor receptor (EGFR) or insulin-like growth factor receptor (IGFR) due to high affinity between their thiol groups and cysteine-rich receptors. This results in the activation of protein tyrosine kinases Src by phosphorylation. Then these phosphorylated Src regulates the claudin-4 proteins, resulting in the opening of TJs. Additionally, some other groups like amino groups on thiomers might control TJs function via interaction with integrin receptor making thiolated CDs bearing amino substructures such as ring-opened thiolated CDs to an interesting tool for permeation enhancement. Per-6-thiolated CDs were evaluated for their permeation enhancing properties. The transport of furosemide (FUR) across Caco-2 cells was 6.87-fold improved due to the addition of per-6-thiolated α-CD in comparison to the corresponding unmodified α-CD serving as a control. Moreover, in the presence of per-6-thiolated α-CD, the permeation of FUR was 6.55-fold improved in comparison to control on freshly excised small intestine. In rats, the relative bioavailability of 19.8% was achieved for per-6-thiolated α-CD-FUR complex as compared to 4% in case of the FUR control formulation as shown in Fig. 9.

The integrity of TJs is measured by its trans-epithelial electrical resistance (TEER) value [171] and the improved permeation across the Caco-2 cell monolayer of thiolated CDs was associated with a decrease in TEER suggesting a reversible TJs opening for paracellular transport. On the contrary, in the case of latrunculin A and N-ethylmaleimide irreversible TJs opening occurred decreasing the TEER irreversibly [172,173]. The permeation enhancement of per-6-thiolated α-CD was found to be superior over polyacrylic acid-cysteine conjugates [174] and polysulfonate thiomers [175].

In contrast to other permeation enhancers, systemic toxic side effects of thiolated CDs can be excluded as they are simply too big to be absorbed from the mucosal membrane. In addition, they are biodegradable [42]. Moreover, as the permeation enhancing effect of thiolated CDs is based on a completely different mechanism than that of all other permeation enhancers such as medium-chain fatty acids, the combination of thiolated CDs with other permeation enhancers should result in an even additive effect.

4.5. Controlled drug release

Thiolated CDs are used as drug carriers in drug delivery systems, as rate and time of drug release can be controlled with these excipients by different ways as described in the following.

4.5.1. Controlled release from thiolated CDs

Due to drug-thiolated CDs complex formation, the local therapeutic effect of encapsulated drugs is prolonged by the controlled release of drugs from drug-thiolated CDs complex. For instance, Asim et al. developed an S-protected thiolated cyclodextrin-iodine complex that showed prolonged intestinal mucosal

### Table 3

Inclusion complex formation of thiolated CDs with different drugs.

| Thiolated CD | Drug            | CD:Drug | Analytical methods | Inclusion constant Kc (M⁻¹) | Solubility improvement | Refs. |
|-------------|-----------------|---------|--------------------|-----------------------------|------------------------|-------|
| α-CD        | Trimethoprim    | 1:1     | FT-IR             | —                           | 2-fold                 | [70]  |
| α-CD        | Cetirizine      | 1:1     | —                 | —                           | —                      | [72]  |
| α-CD        | Furosemide      | 1:1     | HPLC              | 1090                        | 85-fold                | [47]  |
| α-CD        | Iodine          | 1:1     | HPLC              | 5.37 × 10⁴                  | —                      | [71]  |
| α-CD        | Bisphenol       | —       | Spectroscopy      | 1.3 × 10⁴                   | —                      | [159] |
| α-CD        | Dimethyl phthalate| —    | Spectrophoto-metrically | 8.5 × 10²                 | —                      | [160] |
| β-CD        | Miconazole      | 1:1     | HPLC              | —                           | 150-fold               | [29]  |
| β-CD        | Miconazole      | 1:1     | HPLC              | 190                         | 257-fold               | [51]  |
| β-CD        | Acyclowin       | 1:1     | HPLC              | —                           | —                      | [76]  |
| β-CD        | β-Lapachone     | 1:1     | —                 | 1.23 × 10³                  | —                      | [26]  |
| β-CD        | Curcumin        | 1:1     | UV–visible spectroscopy | 5.40 × 10⁴                  | 3780-fold              | [155] |
| β-CD        | Ferrocene       | 1:1     | UV–visible spectroscopy | 2.37 × 10⁴                  | —                      | [161] |
| β-CD        | Azobenzene      | 1:1     | UV–visible spectroscopy | —                           | —                      | [162] |
| β-CD        | Coumarin        | 1:1     | Fluorescence anisotropy | —                           | —                      | [163] |
| β-CD        | Ferrocene       | 1:1     | Chrono coulometry | 7.6 × 10⁴                  | —                      | [82]  |
| β-CD        | Benzimidazole   | 1:1     | Spectroscopy      | —                           | —                      | [164] |
| β-CD        | Baicalein       | —       | UV/Vis spectroscopy | —                           | 100-fold               | [154] |
| β-CD        | Prednisolone    | 1:1     | X-ray powder diffraction | —                           | —                      | [165] |
| β-CD        | Coumarin dye    | 1:1     | Spectroscopy      | —                           | —                      | [156] |
| HP-β-CD     | Budesonide      | 1:1     | HPLC              | 2013                        | —                      | [41]  |
residence and sustained release of the antimicrobial agent. Approximately 44% of the antimicrobial agent was released from the S-protected thiolated α-CD-iodine complex in 3 h, whereas in the same time period 95% iodine was released from the unmodified α-CD-iodine complex. Moreover, this complex exhibited pronounced antimicrobial activity against Staphylococcus aureus and Escherichia coli [71]. Ijaz et al. showed that encapsulation of cetirizine into thiolated CD resulted in significantly reduced local ocular mucosal irritation and prolonged drug residence time on the ocular mucosal surface [72]. Similarly, thiolated poly (aspartic acid)-CD complex loaded with prednisolone exhibited a prolonged drug release profile [165]. Zhang et al. synthesized thiolated CD-functionalized mesoporous silica nanoparticles (MSNPs) loaded with doxorubicin. These MSNPs complexed with thiolated CDs showed sustained release of doxorubicin triggered by acidic pH for superficial bladder cancer therapy [176]. Similarly, a pH-responsive controlled release of doxorubicin was achieved in the acidic environment in tumor cells using the supramolecular assembly of per-6-thiolated CD complexed with drug [161]. Moreover, Zhang’s group showed a sustained release profile of curcumin from β-CD polymers-curcumin inclusion complexes that were synthesized by thiolated β-CD and maleimide-functionalized HP-β-CD conjugation. It was observed that the release of curcumin from HP-β-CD-curcumin complex was around 20%, whereas curcumin released from β-CD polymers-curcumin inclusion complexes was up to 80% [155]. Overall, thiolated CDs maintained equilibrium effectively between excellent adherence and controlled drug release within the system. In another study, Ceña’s team proposed docetaxel-loaded self-assembled nanocarriers comprising thiolated β-CD/calixarene giant surfactants. These nanocarriers exhibited a cytotoxic effect on prostate cancer and glioblastoma cells by inclusion complex formation with well-establish sustained drug release [177]. Moreover, Sciortino’s group showed controlled docetaxel release lasting about 60 days after an initial burst of about 20% from thiolated CDs nanoparticles [178].

4.5.2. Controlled release from hydrogels
Thiolated CD-based hydrogels have potential as drug carriers. Peng et al. designed hydrogel-based drug carriers using per-6-thiolated CD, poly(ethylene glycol), and dextran [179]. Modified dextrans were cross-linked to form a hydrogel using either per-6-thio-β-CD or a combination of mono-6-thio-β-CD and di-thiolated poly(ethylene glycol). A sustained release of the model hydrophobic drug trans-retinoic acid was observed from these thiolated CDs based hydrogels in-vitro and in-vivo, as CDs act as a binding site for retinoic acid. Moreover, Shih et al. prepared thiolated cyclodextrin functionalized hydrogels that showed higher drug loading efficiency and prolonged delivery of curcumin in-vitro [55]. In another study, a light-responsive hydrogel system of thiolated β-CD functionalized dextran and azobenzene functionalized dextran was designed for the light-controlled release of proteins. On UV light exposure, trans-azobenzene was isomerized to cis configurations resulting in the dissociation of inclusion complex formed with thiolated CD leading to the conversion of hydrogel into a sol. This light-responsive gel-to-sol transition was utilized for the controlled release of the entrapped model protein green fluorescent protein [180].

4.5.3. Targeted drug release
Another pharmaceutical application of CDs is targeting to cell receptors for drug delivery. In this concept, thio-PEG5000-bearing CDs were synthesized to demonstrate their binding...
**Fig. 10.** Schematic illustration of supramolecular cross-linked nanogels by host–guest interaction between dextran grafted benzimidazole (Dex-g-BM) and thiol-β-cyclodextrin. Reproduced after permission from reference [164].

**Fig. 11.** Schematic illustration of AuNP carriers with β-lapachone, using: i) β-CD-SH and mPEG-SH for AuNP-1 (RhoCD-SH and mPEG-SH for RhoCD-AuNP-1), ii) β-CD-SH, mPEG-SH and NHS-PEG-SH for AuNP-1.5 (RhoCD, mPEG-SH, NHS-PEG-SH for RhoCD-AuNP-1.5) and iii) anti-EGFR. Reproduced from ref. [26].
behavior towards saccharide-specific cell wall lectins [181]. These thiolated CDs enhance targeted cell binding activities [182,183] and have a wide application in targeted drug delivery [184,185].

Chen’s group proposed polyrotaxane-based glycopolymers with thiolated CDs exhibiting their specific binding toward lectin that confirm enhanced affinity and selectivity toward a specific target receptor [183]. Gabius et al. confirmed the thiol substituted CD-based glycoclusters as inhibitors of lectin-gene-transfected tumor cells as targets [185]. In another work, an oligosaccharidyl-thio-β-CD was synthesized as a potent drug-targeting vector [186]. Similarly, pH and reduction sensitive supramolecular nanogels were introduced that were cross-linked by a host–guest complex between thiolated-β-CD and dextran grafted benzimidazole. These nanogels were pH-sensitive under acidic conditions (pH < 6) and reduction sensitive to a biologically relevant stimulus. They showed enhanced drug release at low pH or high glutathione (GSH) concentrations as illustrated in Fig. 10 [164].

The star polymers are also investigated for the delivery of chemotherapeutic drugs and microRNAs (miRNAs). For instance, Yang et al. reported about cell-penetrating poly(disulfide) (CPD) star polymers consisting of a thiolated β-CD core and CPD arms. This system showed rapid cellular uptake and intracellular release of miRNAs due to the rapid degradation of the polymer under reductive cytosolic environment providing a comparatively high transfection efficiency [187].

Cationic CDs have been developed as a new class of synthetic DNA non-viral vectors for transfection as viral vectors have immune reaction risk [184]. CDs neutralize the charges on the phosphate DNA backbone and stimulate nanoparticulate complex for cell delivery [188]. Amphiphilic thiolated β-CDs were studied for condensation of plasmid DNA and cell transfection and thiolated CDs were found more effective than unmodified CDs with 20,000-fold increased transfection efficiency [189]. In another study, it was shown that plasmid DNA can be readily released from the CD complexes when exposed to negatively charged vesicles and that HepG2 cells can be transfected with targeted CD complexes containing mRNA [190].

4.6. Metal-binding properties

Thiolated polymers are generally recognized for their strong metal ion binding characteristics [191]. Most proteolytic enzymes require cofactors such as divalent cations to maintain their structural integrity. The –SH group has a high affinity for metals. Thiolates bind tightly with heavy metals such as mercury (Hg) [192,193] and arsenic (As) [194]. The term mercapto is derived from the Latin word mercurium captāns as thiols are famous to capture mercury. Thiolated CDs were shown to efficiently bind covalently with heavy metals such as gold (Au), silver (Ag), palladium (Pd) and platinum (Pt).

Thiolated CDs-functionalized gold nanoparticles (Au-NPs) are being used in drug delivery systems. Park et al. reported a sophisticated system for the anti-cancer drug β-lapachone complexed with thiol-functionalized Au-NPs [26]. On the surface of the Au-NPs three different ligands were attached: polyethylene glycol (PEG) as an antifouling agent, anti-epidermal growth factor receptor (anti-EGFR) antibodies as targeting moiety and thiolated β-CDs as a drug carrier as exhibited in Fig. 11. The release of cytotoxic drugs from Au-NPs is highly influenced by the glutathione concentration in the cell. Glutathione-mediated release of the anticancer drug from Au-NPs was demonstrated by A549 cells (high glutathione concentration) and MCF-7 cells (low glutathione concentration). After entry of NPs into cells, β-CD-β-lapachone inclusion complexes were released by reduction of S–Au bond displaying high glutathione concentrations to tumor cells.

Aykaç et al. showed the formation of Au-NPs decorated with lactose and β-CD. These NPs were capable to be recognized by different cell receptors to deliver the anticancer drug methotrexate (MTX) as shown in Fig. 12 [195]. Shi et al. synthesized Au-NPs for drug delivery application with surface-bound per-6-thiolated-β-CD, which were complexed with adamantane-appended Pt(IV) that is a pro-drug of cisplatin. When human neuroblastoma (SK-N-SH) cells were treated with drug-loaded vehicles, within 24 h after exposure clusters of Au-NPs were observed in the nuclear regions of living cells. Moreover,
thiolated CDs coated Au-NPs delivery vehicle is shown cytotoxic to SK-N-SH neuroblastoma cells but less cytotoxic than cisplatin, suggesting that the pro-drug Pt(IV) complex may not be reduced to cytotoxic Pt(II) species in the extracellular environment [196].

5. Concluding remarks

Sulphydryl groups (–SH) are the most important bridging structure in nature and are essential for numerous physiological processes within the body. The chemical modification of well-established oligomers by immobilization of sulphydryl ligands on CDs backbone leads to thiolated CDs. These thiolated CDs exhibit many new physicochemical properties and substantial improvements in different characteristics. Thiolated CDs having free thiol groups are easily oxidized at higher pH but S-protected thiolated CDs protect thiol groups against oxidation at higher pH values. The thiol groups on CDs backbone stimulate disulfide bonds formation between the oligomers and cysteine subunit of mucus glycoproteins to prolong the mucosal residence time at the site of action. Improved mucoadhesion was achieved on different mucosae with thiolated CDs that could be a promising auxiliary agent for delivery systems providing prolonged drug residence time. Similarly, S-protected thiolated CDs showed significantly improved mucoadhesion on intestinal mucosa owing to enhanced patient compliance. Highly thiolated CDs exhibited substantial improvement in viscosity of mucus as increase in viscosity was directly proportional to degree of thiolation. These in-situ gelling properties are based on the oxidation of thiol groups resulting in inter- and intra-molecular disulfide bond formation. Moreover, the incorporation of drugs in thiolated CDs can be used to improve various pharmaceutically relevant properties. The most prominent advantage is the solubility enhancement of poorly soluble drugs by incorporating them into the hydrophobic cavity of thiolated CDs resulting in water-soluble complexes. On the one hand, the inclusion complex formation enhances the solubility of poorly soluble drugs providing a sustained release and on the other hand the mucohesive nature of thiolated CDs can guarantee a prolonged residence time on mucosal membranes. Furthermore, thiolated CDs showed ability of permeation enhancement of BCS Class IV drugs via TJ openings. All these unique and multifunctional properties make thiolated CDs a powerful, promising and versatile tool for different drug delivery systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] A. Bernkop-Schnürch, V. Schwarz, S. Steiningr, Pharmaceut. Res. 16 (1999) 876–881.
[2] A. Bernkop-Schnürch, A. Weithaler, K. Albrecht, K. Greimel, C.C. Thurner, H. Friedl, A. Bernkop-Schnürch, Carbohydr. Polym. 9 (2012) 1331–1341.
[3] C. Leichner, M. Jelkmann, Bernkop-Schnürch, Adv.Drug Deliv. Rev. (2019).
[4] S. Dünnhaupt, J. Barthelmess, D. Rahmat, K. Leithner, C.C. Thurner, H. Friedl, A. Bernkop-Schnürch, Carbohydr. Polym. 9 (2012) 765–772.
[5] C. Menzel, J. Silbernagel, F. Laffleur, C. Leichner, M. Jelkmann, C.W. Huck, S. Hussain, A. Bernkop-Schnürch, Int. J. Pharmaceut. 503 (2016) 199–206.
[6] E. Esquivel, M. Almadn, J. Valdez, Int. J. Polym. Sci. 2015 (2015).
[7] A. Bernkop-Schnürch, D. Guggi, Y. Pinter, J. Control. Release 94 (2004) 177–186.
[8] X. Zhu, M. Su, S. Tang, L. Wang, X. Liang, F. Meng, Y. Hong, Z. Xu, Mol.Vision 18 (2012) 1973.
[9] I.P. de Sousa, W. Suchaoin, O. Zupanić, C. Leichner, A. Bernkop-Schnürch, Carbohydr. Polym. 152 (2016) 632–638.
[10] K. Kafejduski, R.K. Jett, F. Foger, H. Hoyer, M. Welre, M. Hoffer, A. Bernkop- Schnürch, Int. J. Pharmaceut. 343 (2007) 48–58.
[11] X. Li, G. Yu, K. Jin, Z. Yin, Die Pharmazie-An Int. J. Pharmaceutical Sciences 67 (2012) 224–228.
[12] J. Fu, X. Shi, Y. Jin, Drug Delivery Sci. Technol. 30 (2015) 74–81.
[13] J. Nowak, F. Laffleur, Bernkop-Schnürch, Int. J. Pharmaceut. 478 (2015) 383–389.
[14] S. Hauptstein, S. Dezorzi, P. Prüfert, B. Matuszczak, A. Bernkop-Schnürch, Carbohydr. Polym. 124 (2015) 1–7.
[15] A. Jindal, M. Wasnik, H.A. Nair, Indian J. Pharmaceut. Sci. 72 (2010) 766.
[16] G. Xu, L. Cheng, Q. Zhang, Y. Sun, C. Chen, H. Xu, Y. Chai, M. Lang, J. Biomater. Appl. 31 (2016) 721–729.
[17] A.A. Kassem, D.A.E. Issa, G.S. Kotry, R.M. Farid, Drug Dev. Ind. Pharm. 41 (2017) 120–131.
[18] T.H. Kim, J.-C. Kim, Int. J. Polym. Mater. Polymer Biomater. (2019) 1–10.
[19] M. Jelkmann, S. Bonengel, C. Menzel, S. Markovic, A. Bernkop-Schnürch, Int. J. Pharmaceutics 546 (2019) 117477.
[20] C. Saikia, M.K. Das, A. Ramteke, T.K. Maji, Int. J. Polym. Mater. Polymer Biomater. 66 (2017) 349–358.
[21] K. Chauhan, S. Hussain, A. Bernkop-Schnürch, Int. J. Pharmaceutics 666 (2019) 5762–5772.
[22] S. Das, M.K. Das, Int. J. Appl. Pharm. 11 (2019) 53–62.
[23] C. Saikia, A. Hussain, A. Ramteke, H.K. Sharma, T.K. Maji, Starch-Stärke 66 (2014) 760–771.
[24] C. Park, H. Youn, H. Kim, T. Noh, Y.H. Kook, E.T. Oh, H.J. Park, C. Kim, J. Mater. Chem. B 9 (2019) 2318–2319.
[25] S. Tamagaki, T. Masuda, Memoirs of the Faculty of Engineering, Osaka City University, 1995, pp. 93–99.
[26] M.T. Rojas, R. Koeniger, J.F. Stoddart, J. Am. Chem. Soc. 117 (1995) 11820–11825.
[27] C. Leichner, M. Jelkmann, A. Bernkop-Schnürch, Carbohydr. Polym. 123 (2015) 187–195.
[28] T. Loftsson, in, Google Patents, 2006.
[29] R. Challa, A. Ahuja, J. Ali, R. Khar, Aaps Pharmaceutic 6 (2005) E329–E357.
[30] E.M. Del Valle, Process Biochem. 39 (2004) 1033–1046.
[31] J. Szegli, Chemical Rev. 98 (1998) 1743–1754.
[32] G. Aicrav, C. Gonzalez-Barreiro, J.C. Mejuto, R. Anal. Ose. J. Chim. Fundam. 23 (2009) 1631–1640.
[33] H.J. Buschmann, J. Schollmeier, J. Cosm. Sci. 53 (2002) 185–192.
[34] T. Loftsson, M.E. Brewster, J. Pharm. Pharmacol. 62 (2010) 1607–1621.
[35] A.R. Khan, P. Forog, K.J. Stine, V.T. D’Souza, Chem. Rev. 98 (1998) 1997–1996.
[36] W. Saeinger, M. Noltemeyer, P. Manor, B. Hingerty, B. Klar, Bioorg. Chem. 5 (1977) 187–195.
[37] A. Hybl, R.E. Rundle, D.E. Williams, J. Colloid Interf. Sci. 531 (2018) 261–268.
[38] M. Hussain Asim et al. / Coordination Chemistry Reviews 420 (2020) 213433
