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Dextran-based cyclodextrin polymers: Their solubilizing effect and self-association

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ABSTRACT

Cyclodextrins and their water-soluble derivatives are excellent solubilizers and stabilizers and widely used as enabling pharmaceutical excipients. However, still new cyclodextrin derivatives are being designed and synthesized in an effort to improve their physicochemical properties. In this study physicochemical and self-associating characteristics of dextran-based cyclodextrin polymers, with ether or ester linkage between the β-cyclodextrin and the dextran backbone, were investigated. Methods applied include phase-solubility studies, permeation studies and dynamic light scattering. Results with a model drug, hydrocortisone, showed that the solubilization and binding efficiency slowly declined with increasing molecular weight. The degree of substitution had significant effect on the properties, making the polymers with ether linkage more favorable. Self-association of the drug–polymer complexes was observed, although the instability can hamper their utilization

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides typically consisting of 6–8 α-D-glucopyranose units; they have hydrophilic outer surface and relatively lipophilic central cavity. In the last few decades CDs have received growing attention due to their ability to increase the apparent water-solubility of lipophilic poorly soluble drugs through formation of inclusion complexes (Brewster & Loftsson, 2007; Frömming & Szejtli, 1994; Loftsson & Duchene, 2007). Moreover, CDs are also becoming popular components for fabricating various types of nanoparticles for targeted drug delivery, due to their general complex forming abilities and capability to function as modular building blocks for self-assembled structures (Gref et al., 2006; Zhang & Ma, 2010).

Self-association of natural CDs has been observed and characterized, though information about their water-soluble derivatives is somewhat limited (González-Gaitano et al., 2002; Messner, Kurkov, Jansook, & Loftsson, 2010; Szente, Szejtli, & Kis, 1998). Inclusion complexes of hydrophilic CD derivatives and hydrophobic drugs do also self-associate to form nanoparticles which can be used for drug delivery (Loftsson, Magnúsdóttir, Másson, & Sigurjónsdóttir, 2002; Loftsson, Másson, & Sigurdsson, 2002; Messner, Kurkov, Brewster, Jansook, & Loftsson, 2011a; Messner et al., 2011). However, it is known that in aqueous solutions these nanoparticles are in dynamic equilibrium with non aggregated forms of CD/guest complexes, free CD molecules and free guest molecules, and that they may fall apart upon dilution, filtration, shear mixing and heating. It has been shown that certain polymers can have a stabilizing effect on the self-associated nanoparticles but the stabilization is insufficient for most practical purposes (Messner, Kurkov, Flavià-Piera, Brewster, & Loftsson, 2011b).

CD-based polymers, nanogels and nanosponges have also been synthesized and evaluated as drug delivery systems (Ansari, Torne, Vavia, Trotta, & Cavalli, 2011; Moya-Ortega, Alvarez-Lorenzo, Concheiro, & Loftsson, 2012; van de Manakker, Vermonden, van Nostrum, & Hennink, 2009). Here we design, synthesize and characterize some open-chain dextran-based CD derivatives prepared by "click" chemistry (Nielsen, Wintgens, Amiel, Wimmer, & Larsen, 2010). Dextran is a natural straight chain polymer consisting of α-1,6-glycosidic linkages between glucose molecules with branches attached by α-1,3 linkages. Dextrans with molecular weight (Mw) as high as 70 kDa are commonly used in parenteral solutions as volume expanders as well as in ophthalmic drug formulations and to decrease vascular thrombosis during surgery. Dextrans are modified with alkyne groups, CDs are functionalized with azide groups and the two products linked together by (1,3)-cycloaddition resulting in water-soluble polymers. Their complex forming properties

Abbreviations: CD, cyclodextrin; HC, hydrocortisone; BS, binding sites; SE, solubilization efficiency; BE, binding efficiency.

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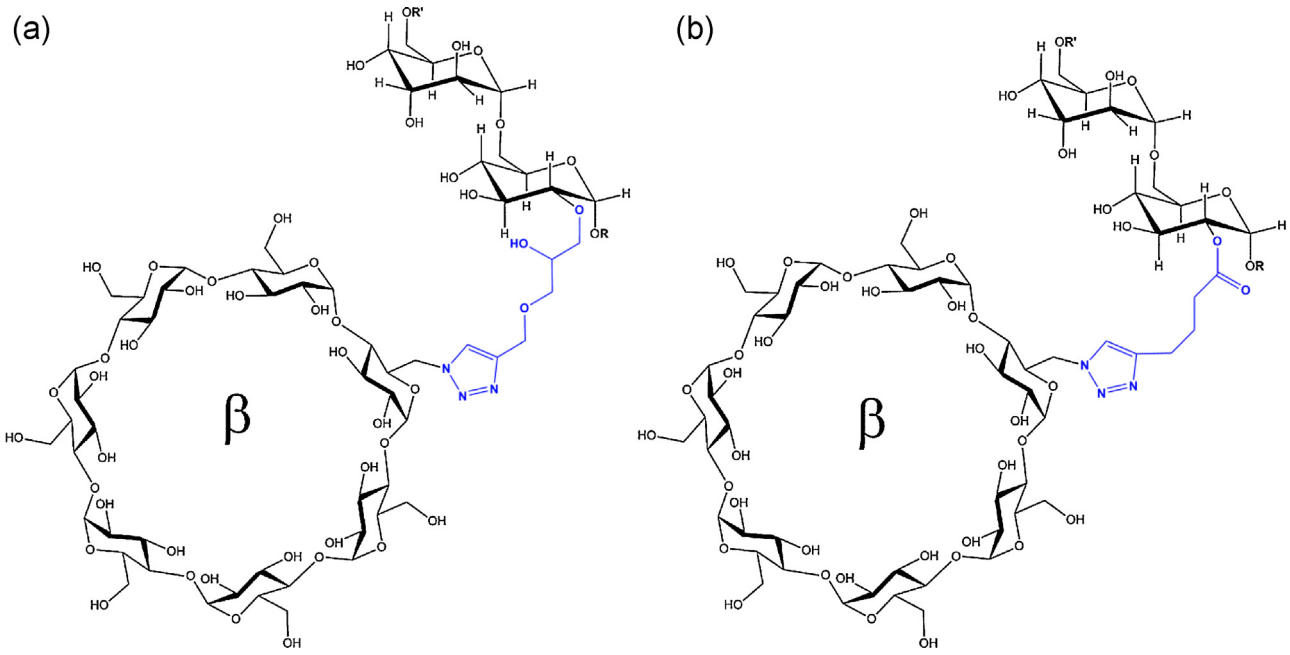

Fig. 1. Chemical structure of a GPβCD (a) and a HXβCD (b) linkage to the base dextran.

and aggregation abilities were examined. These polymers can also form nanoparticles through formation of poly-inclusion complexes with suitable polymers with pendent guest molecules (Layre, Volet, Wintgens, & Amiel, 2009; Nielsen, Steffensen, & Larsen, 2009; Wintgens, Nielsen, Larsen, & Amiel, 2011). The focus of this article is to examine the physicochemical properties (binding, solubilizing) of the CD polymers complexes with a model drug and to assess their ability to self-associate.

2. Materials and methods

2.1. Materials

Hydrocortisone (HC) was purchased from BUFA Chemicals, Netherlands. β-Cyclodextrin (βCD) was purchased from Wacker Chemie AG, Germany. The different dextrans: D6, D10, D20, D25, D40, D70, D110 (Dextran T6, T10, T20, T25, T40, T70, T110, Pharmacosmos A/S, Denmark) with normative molecular weight of 6, 10, 20, 25, 40, 70, 110 kDa were dried overnight in vacuum at 115 °C before use. Main reagents for the synthesis were glycidyl propargyl ether 90% (GPE, Fluka, Germany), 5-hexynoic acid 97% (Aldrich, Germany), 1-adamanatanecarbonyl chloride (Sigma–Aldrich, Germany). Methanol and tetrahydrofuran and was purchased from Sigma–Aldrich. All the solutions and the mobile phase for HPLC measurement were prepared with Milli-Q water (Millipore, Billerica, MA, USA) was used as a mobile phase.

2.2. Synthesis and characterization

The synthesis of the dextran-based cyclodextrin polymers was described earlier (Nielsen et al., 2010). In brief, dextrans were modified with alkynes through either ether- or ester bonds, onto which azide-modified βCD was grafted by a Cu(I)-catalyzed alkyne–azide coupling. The nomenclature of the dextran based CD polymers follows the scheme D-(molecular weight of the base dextran in kDa)-(two letter code for the linkage between the dextran and the CD)-βCD. The HX (from HeXynoic acid) codes an ester joint with 6 carbon atom distance between the dextran and the CD, GP (from glycidyl propargyl ether) codes an ether joint to the dextran. For example, D6HXβCD is a βCD polymer based on Mw 6 kDa dextran, connected at the 6th carbon atom from the CD by an ester bond. Representative chemical structure of a dextran-based CD polymer is shown in Fig. 1.

The CD polymers have been purified by dialysis and characterized by ^{1}H NMR, FT-IR, TGA and ITC. The Mw of the polymers were measured by GPC. DS was calculated from the average Mw of the polymers knowing the Mw of the base dextran and the Mw and number of CD units.

The synthesis of adamantane-grafted hydrophobically modified dextran were prepared (HMD, D40Ada) and characterized according to literature (Layre et al., 2009).

2.3. Quantitative determination of hydrocortisone concentration

Quantitative determination of hydrocortisone was performed on a reversed-phase high performance liquid chromatographic (HPLC) system from Dionex Softron GmbH (Germany) Ultimate 3000 series consisting of a LPG-3400A pump with a built-in degasser, a WPS-3000-TSL autosampler column compartment, a VWD-3100 variable wavelength UV–vis detector and Phenomenex Luna C18 150 mm × 4.60 mm, 5 micron column (Phenomenex, UK) with a matching HPLC KrudKatcher Ultra Column In-Line Filter (Phenomenex, UK). The mobile phase consisted of methanol, water and tetrahydrofuran 70:29:1 (volume ratios). The flow rate was 1.0 ml/min and the retention time was 3.1 min.

2.4. Phase-solubility studies

Saturated solutions of hydrocortisone were prepared in water, as well as, in aqueous CD polymer solutions and the solubility was determined by the heating method (Loftsson & Hreinsdóttir, 2006). Samples were analyzed by HPLC. Phase-solubility profiles were determined according to the method of Higuchi and Connors (Higuchi & Connors, 1965). All the polymers were tested at the same weight concentrations: 0%, 1%, 3%, and 5% (w/v). From the phase-solubility studies the number of drug binding sites (BS) and the solubilization efficiency (SE) were calculated. The number of BS is defined as the number of drug molecules that bind to each polymer

molecule and the solubilization efficiency is defined as milligrams of a given drug that can be solubilized in aqueous solution by one gram of polymer.

2.5. Permeation studies

Drug permeation studies from solutions containing saturated drug/CD polymer complexes (donor phase, 1 ml) were carried out in unjacketed Franz diffusion cells with a diffusion area of 1.77 cm^2 (SES GmbH–Analysesysteme, Germany). The receptor phase (12 ml) consisted of pH 7.4 phosphate buffered saline (PBS) containing 2% (w/v) γCD. The donor and receptor compartments were separated by a single layer semi-permeable cellulose ester membrane (Biotech CE, Spectrum Europe, Breda, NL), with molecular-weight-cutoff (MWCO) of 50, 100, 300 or 1000 kDa, which had been soaked in the receptor phase solution overnight. The MWCO of the membrane was always kept larger than the Mw of the polymer and, thus, single polymer molecules and drug/polymer complexes were able to permeate the membrane. The study was carried out at room temperature under constant stirring rate (300 rpm). Samples were taken at 15, 30, 45, 60, 90 and 120 min, and replaced immediately with equal amount of pure receptor phase. The flux (J) was calculated from the linear part of the permeation profile and the apparent permeation coefficient (P_{app}) was calculated from the following equation:

$$J = \frac{dq}{dt \cdot A} = P_{app} \cdot C_D \quad (1)$$

where dq/dt is the slope (in mg s) of HC permeated through the membrane over time, A is the diffusion area (1.77 cm^2) and C_D is the total drug concentration in the donor phase. The stability of polymer complex aggregates, formed by self-assembly of drug/polymer complexes, was evaluated by the permeation method as described earlier (Messner, Kurkov, Flavià-Piera, et al., 2011). Briefly, the stability of the aggregates was evaluated by measuring the permeation of hydrocortisone/polymer complexes through a cellulose membrane before and immediately after media dilution. The 5% (w/v) samples were diluted to give 1% and 3% CD polymer concentration, the flux of the hydrocortisone was measured and was compared to the flux of the original undiluted samples containing 1% and 3% CD polymer. If the nanoparticles disassembled right after dilution, the drug flux from the two corresponding polymer solutions should be almost identical. If, on the other hand, the aggregates were stable or disassembled somewhat slowly the drug flux from the diluted solutions should be lower than from the equilibrated solutions.

2.6. Particle size distribution

The mean hydrodynamic diameter of the polymers/complexes was determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (from Malvern Instruments) equipped with a He–Ne laser (λ = 633 nm, scattering angle 173°). The samples were measured 11 times for ten seconds at 25 °C, and repeated twice.

3. Theory

Several characteristic features can be obtained from the phase-solubility studies of the CD polymers. Previously, we have defined the number of drug binding sites (BS) on each polymer chain and their solubilization efficiency (SE) (Fülöp, Kurkov, Nielsen, Larsen, & Loftsson, 2012). The BS is represented by the slope of the linear phase-solubility diagram in molar concentrations and it shows how much drug is bound to a given CD polymer in mM per mM. The SE is the slope of the linear phase-solubility diagram where the concentration of the dissolved drug is in mg/ml and the concentration of CD polymer is in g/ml. However, it is also important to explore the drug binding to the individual CD cavities. Here we have to make a couple of assumptions. First the dextran based CD polymers are thought of as single CD cavities linked to a linear polymer chain. Most likely the CD cavities will have different affinities to the lipophilic drug molecules depending on their location within the polymer chain and, consequently, the stability constants, i.e. the stability constants of the drug/CD complexes, for different CD moieties within each polymer chain will be different. However, here we assume that all CD cavities within the polymer will have equal affinity for the drug molecule. Second, it is assumed that in a given complexation medium all polymer molecules contain the same number of drug molecules per polymer molecule. That is all the polymer molecules will bind drug molecules in the same manner and according to the principle of minimum energy.

The total number of the CD cavities in a polymer (n) can be estimated from the Mw of the base dextran, the Mw of the polymer and the Mw of the CD ligand. The number of CD cavities which form drug inclusion complexes at equilibrium (m) can be determined from phase-solubility studies as previously described:

$$m \equiv \mathrm{BS} = \frac{\text{moles of drug bound}}{\text{total moles of CD polymer}} = \frac{d[\mathrm{D}]}{d[\mathrm{CD_{pol}}]} = \text{phase solubility slope} \quad (2)$$

Therefore if all the CD cavities are equivalent toward binding and the drug is solubilized through formation of 1:1 inclusion complexes with a single CD cavity, then the following equilibrium will be attained:

$$m \cdot \mathrm{D} + n \cdot \mathrm{CD_{cav}} \overset{K}{\rightleftarrows} m \cdot \mathrm{D/CD_{cav}} + (n-m) \cdot \mathrm{CD_{cav}} (n > m) \quad (3)$$

where CD_{cav} is an empty CD cavity (i.e. CD moiety) attached to the polymer chain and D/CD_{cav} is a drug-occupied cavity on the CD-polymer.

However, the situation is a bit more complex. The CD polymers can self-assemble to form nanoparticles (or aggregates) that can result in formation of non-inclusion complexes or micellar-type solubilization. Thus, the binding efficiency (BE) is used to characterize drug binding to the polymer instead of some observed K values. Binding efficiency can be calculated from n and m:

$$\mathrm{BE} = \frac{m}{n} \quad (4)$$

4. Results and discussion

All the prepared dextran-based polymers are soluble in water. The investigated polymers belong to 2 groups: in polymers marked with GP the CD moieties are connected to the dextran backbone via ether bonds and in polymers marked with HX the CD moieties are linked to the dextran backbone via ester bonds. The molecular weight of the polymers varies, the shortest polymers contain a 6 kDa dextran backbone and the longest ones contain a 110 kDa dextran backbone. In the following section the physicochemical properties of the polymers are compared as regards to Mw and degree of substitution (DS).

4.1. Phase-solubility

A representative phase-solubility diagram for hydrocortisone in aqueous CD polymer solutions is shown in Fig. 2. Results of the polymer characterization by the phase-solubility technique using hydrocortisone as a model drug are shown in Table 1. The GPβCD polymers displayed in all cases A_L-type phase-solubility profiles, while the HXβCD polymers displayed both A_L- and A_N-type profiles. Because for CD polymers the theory of 1:1 complexes

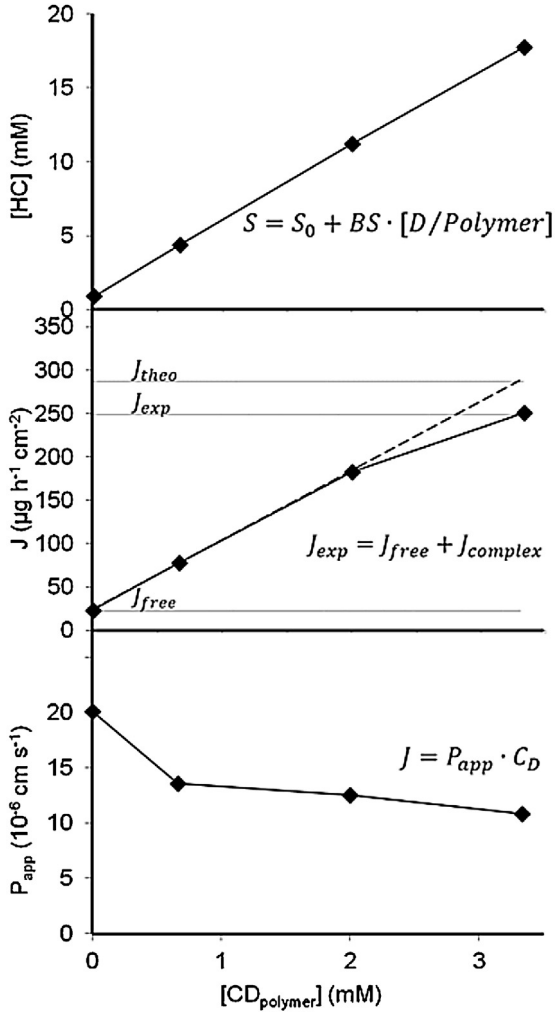

Fig. 2. Phase-solubility (top), and permeation profiles (middle, bottom) for hydrocortisone in aqueous D6 GPβCD 1 solutions. The relevant mathematical equations are also displayed. S, solubility; S_0, intrinsic solubility; BS, number of binding sites; J_{free}, flux of the free drug; J_{theo}, theoretical flux; J_{exp}, experimental flux; $J_{complex}$, flux increasing effect of the complexation; P_{app}, apparent permeability coefficient; C_D, drug concentration in the donor phase; MWCO 50 kDa cellulose ester semipermeable membrane was used.

cannot be used, therefore, instead of the binding coefficient, three physiochemical characteristics were derived from the slope of the phase-solubility curve, introduced in the Theory section: BS, SE and BE.

The Mw of the polymers has an important effect on the observed physicochemical properties (i.e. BS and SE), which is presented in Fig. 3. In the case of GPβCD the polymers were of three different degrees of substitution (DS), where DS is defined as number of βCD moieties per dextran polymer. Fig. 3 shows that if the Mw of the dextran is increased it will contain more CD ligands (BS will increase) at the same DS. The BS increases linearly with the Mw up to the 40 kDa dextran. At 70 kDa and above, the value of BS starts to deviate from linearity, most probably due to increased steric hindrance of the binding sites with increasing Mw of the dextran backbone. In general, SE has the largest value when the Mw is low but then slowly decreases with increasing Mw.

The binding efficiency (BE) has a similar relationship to the Mw as the SE, i.e. has the largest value when the Mw is low (Fig. 4). However, BE does not show monotone decrease with increasing Mw. The BE is between 70% and 80% in most cases.

Table 1
Properties of the dextran-based GPβCD and HXβCD polymers: name, molecular weight (kDa), βCD concentration (w/w %) indicating the number of βCD moieties per weight polymer, degree of substitution, phase-solubility types using hydrocortisone as guest.

Compound	Mw (kDa)	c βCD (w/w %)	DS	Phase-solubility type
D6 GPβCD 1	15	55.1	0.17	A_L
D6 GPβCD 2	19	62.2	0.25	A_L
D6 GPβCD 3	20	64.3	0.27	A_L
D10 GPβCD 1	25	54.6	0.17	A_L
D10 GPβCD 2	30	60.9	0.23	A_L
D10 GPβCD 3	33	63.5	0.26	A_L
D20 GPβCD 1	50	54.6	0.17	A_L
D20 GPβCD 2	54	57.0	0.19	A_L
D20 GPβCD 3	64	62.5	0.25	A_L
D25 GPβCD 1	59	52.6	0.16	A_L
D25 GPβCD 2	78	61.6	0.24	A_L
D25 GPβCD 3	83	63.3	0.26	A_L
D40 GPβCD 1	125	61.8	0.24	A_L
D40 GPβCD 2	118	60.2	0.22	A_L
D40 GPβCD 3	155	67.4	0.33	A_L
D70 GPβCD 1	170	53.4	0.16	A_L
D70 GPβCD 2	194	58.1	0.20	A_L
D70 GPβCD 3	213	61.1	0.23	A_L
D70 GPβCD 4	239	64.3	0.28	A_N
D110 GPβCD 1	272	54.1	0.17	A_L
D110 GPβCD 2	272	54.2	0.17	A_L
D6 HXβCD	19	62.2	0.25	A_L
D10 HXβCD 1	32	61.8	0.25	A_L
D10 HXβCD 2	35	64.4	0.29	A_L
D10 HXβCD 3	38	66.8	0.32	A_L
D20 HXβCD 1	58	59.3	0.22	A_L
D20 HXβCD 2	71	65.1	0.30	A_L
D20 HXβCD 3	77	66.9	0.33	A_L
D25 HXβCD	91	65.7	0.31	A_N
D40 HXβCD	126	61.9	0.25	A_N
D70 HXβCD	297	69.1	0.37	–

The DS (low, mid, high) is marked in Figs. 3 and 4. DS varies between 0.16 and 0.33 amongst the samples; in w/w% of βCD it is between 52 and 67. It has an optimal value, i.e. when DS is low the binding efficiency is better but the CD-moieties are never completely occupied (i.e. the CD polymers are never saturated), i.e. even at high drug concentrations there will always be unoccupied CD cavities (i.e. complexation sites) on the polymers. However, when the DS is high the CD ligands become less accessible probably due to steric hindrance.

The effect of the DS (i.e. the number of βCD moieties per polymer) on BS, SE and BE is presented in Fig. 5 for the two different types of CD polymers with two different base chain lengths. For the HXβCD polymers tested the results show that the DS of the polymers are already above their maximum solubilizing abilities, whilst for the GPβCD polymers the DS values are close to produce optimal solubilization in the polymers. The phase-solubility results show that the GPβCD polymers are perform better than the HXβCD polymers with respect to the calculated physicochemical properties (BS, SE and BE). The DS has significant effect on the accessibility of the CD moieties.

4.2. Permeation profiles

According to Fick's first law of diffusion, if there is no aggregation then the CD polymer concentration–flux diagram would follow the shape of the phase-solubility diagram, in the case of A_L type phase-solubility the flux diagram would be linear as well. In Fig. 2, we can see that there is a deviation from linearity which indicates aggregation. Previously, (Messner, Kurkov, Brewster, et al., 2011) we have shown that at a given CD concentration the expected theoretical flux based on the linear relation (J_{theo}) and

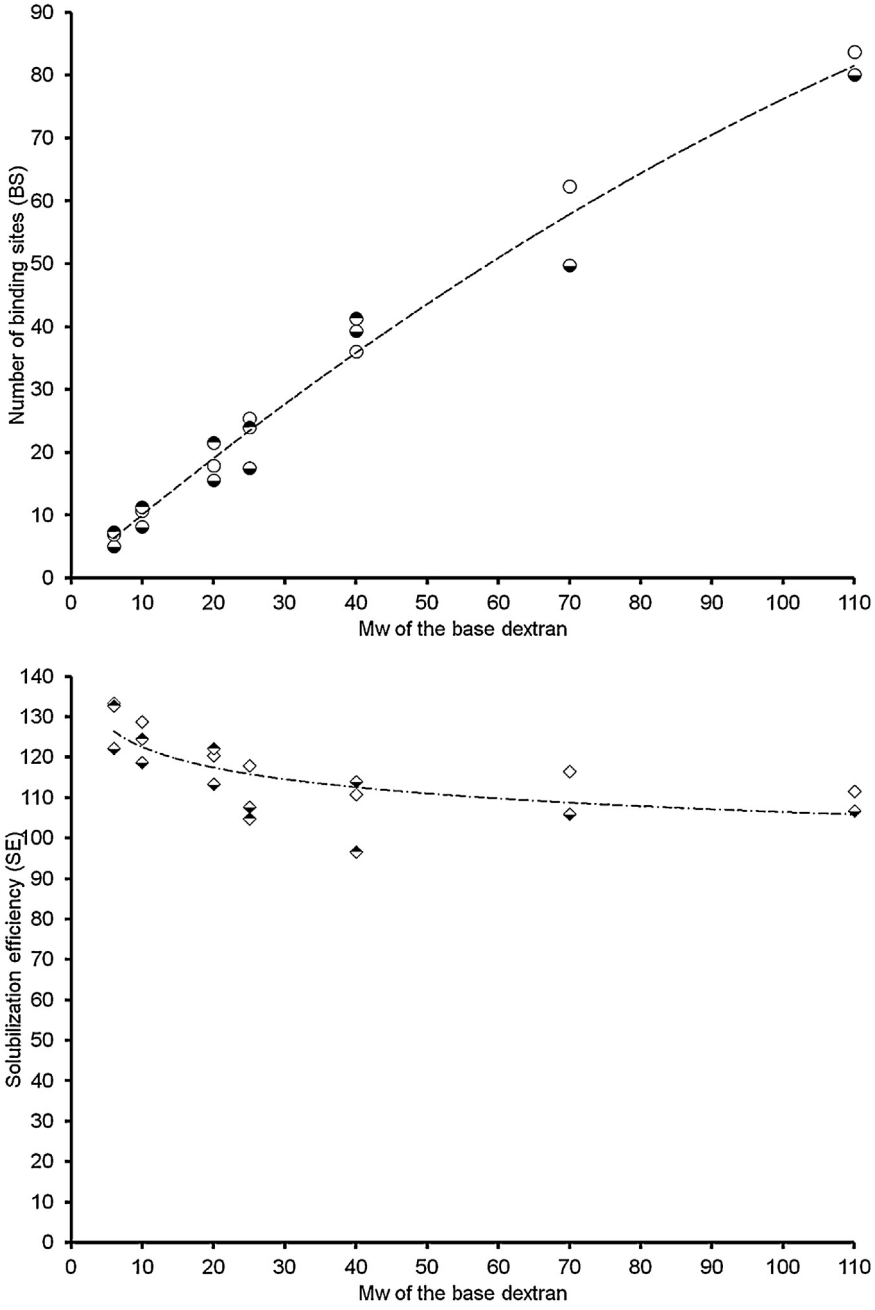

Fig. 3. The number of the binding sites (BS) of the GPβCD polymers of various types of base dextrans (top); ◓ Low DS BS; ○ Mid DS BS; ◒ High DS BS. The solubilization efficiency (SE) of the GPβCD polymers of various types of base dextrans (bottom); ▼ Low DS SE; ◊ Mid DS SE; ▲ High DS SE.

the experimental flux (J_{exp}) can be used to calculate the fraction of aggregation (f_A):

$$f_A = 1 - \frac{J_{exp}}{J_{theo}} \quad (5)$$

Polymers of different molecular weight were studied. Semipermeable cellophane membranes of different MWCO were used to estimate their f_A values from the J_{theo} and the J_{exp} at 5 w/v% CD polymer concentration. However, these membranes cannot be used to compare the tendency of the different polymers to form aggregates, because f_A depends on the MWCO of the membrane and the MWCO of the polymer. For that the MWCO/Mw ratio has to be evaluated together with the f_A values. Our studies have shown that for a CD polymer family with identical chain length but different DS the flux decreases with increasing Mw. In other words, for a given family of polymers with identical backbone chain length the higher the Mw, the lower the MWCO/Mw, the lower the J_{exp} and the higher the f_A. These results are displayed in Fig. 6 showing that the fraction of aggregates is usually between 10% and 30% (and not more than 35%) at 5 w/v% CD polymer concentration.

For the stability tests, samples containing 5% (w/v) of the polymer were diluted to 1% and 3% and the drug flux from those diluted solutions compared to that of 1% and 3% polymer solutions that had been allowed to equilibrate for at least one week. For each polymer, permeation studies were performed on the diluted samples, as well as, on the originally prepared samples of identical concentration (i.e. they contained identical amounts of both the drug and the polymer). This method can be applied to polymers displaying A_L type phase-solubility profiles. If the fluxes after dilution are the same as for the original samples then it means that the aggregates

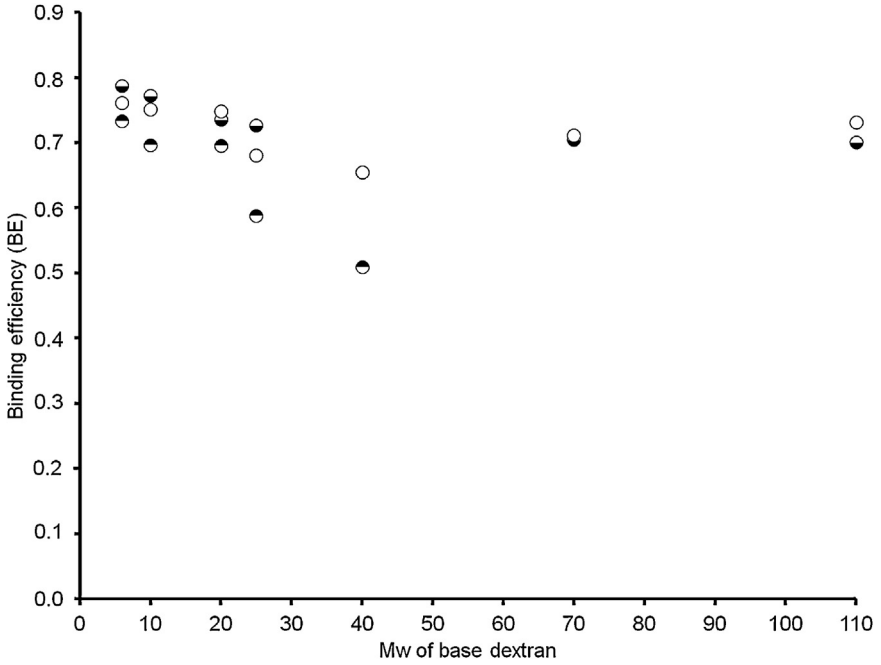

Fig. 4. Binding efficiency (BE) of the GPβCD polymers of various types of base dextrans; ◓ Low DS BE; ○ Mid DS BE; ◒ High DS BE.

are falling apart upon dilution. If the flux is reasonably smaller than before, it means that the aggregates are stable. In some cases 10–20% enhancement was noticed but the results are sometimes inconclusive, the results obtained by comparing the original and diluted samples containing 1% and 3% CD polymer are not comparable. The stability test results show that the aggregates are often disintegrating upon dilution.

4.3. DLS results

Selected CD polymers were investigated by Dynamic Light Scattering. In general, the results show two size populations, the first one is related to the free polymer and the other one can be associated with the aggregation. First, the 1% aqueous solutions of the dextran based CD polymers were measured, then

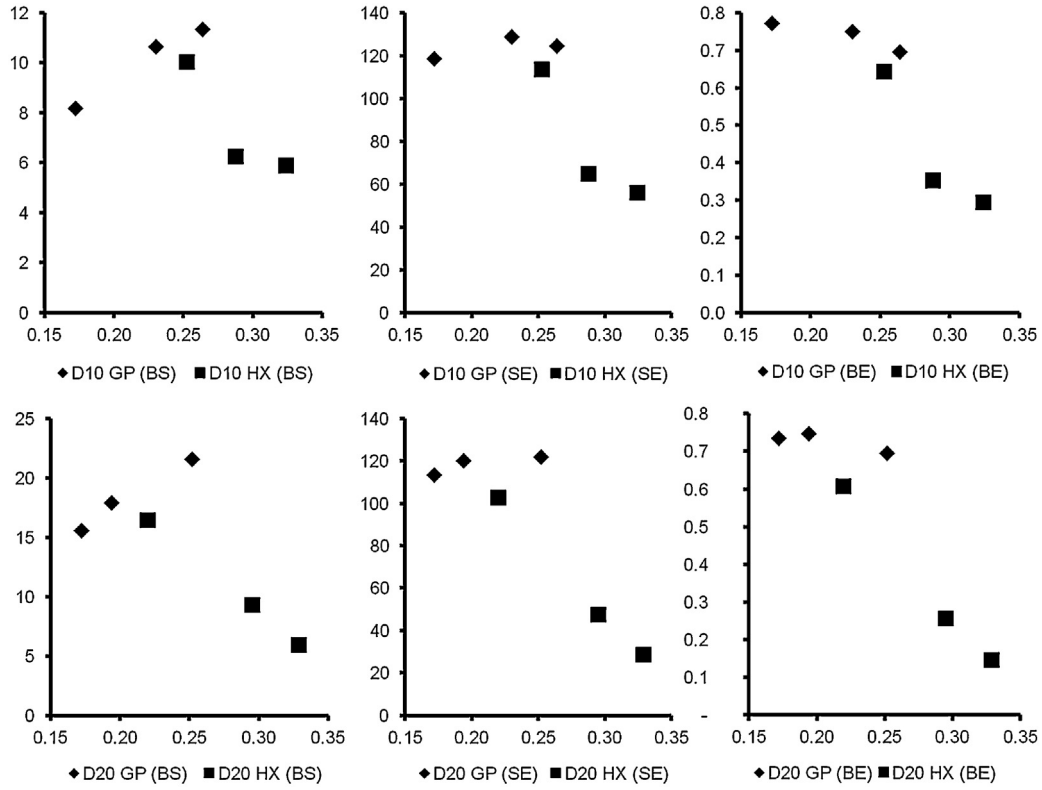

Fig. 5. The effect of DS (*x* axis; no units) on the physicochemical properties in the case of the CD polymers: D10 GPβCD and D10 HXβCD (top), and D20 GPβCD and D20 HXβCD (bottom); the properties from left to right: BS (no units), SE (mg hydrocortisone/g polymer), BE (no units).

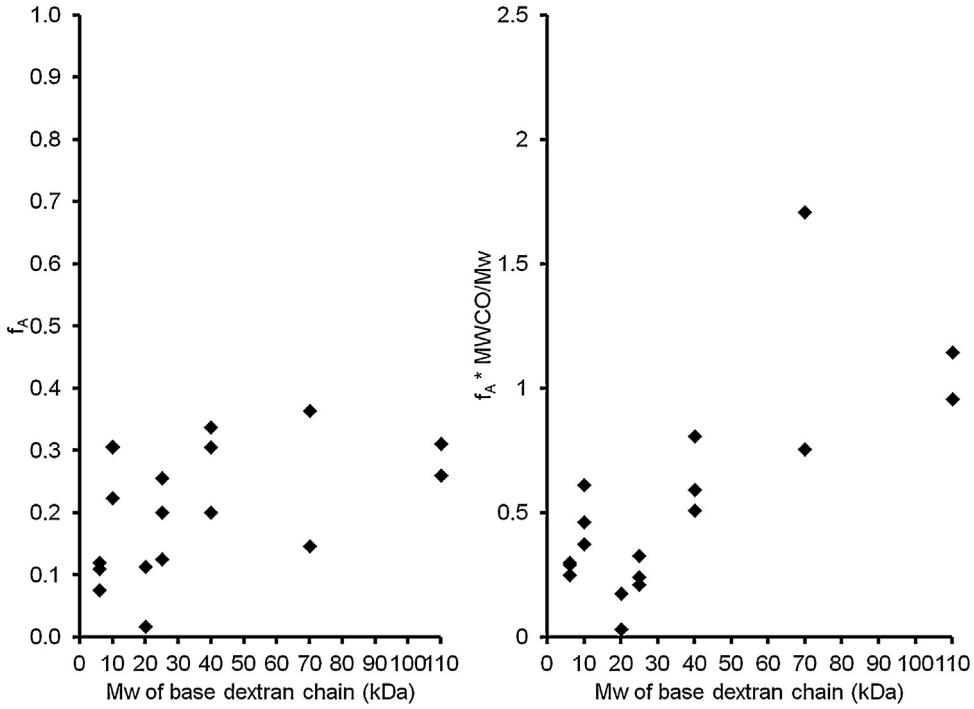

Fig. 6. Fraction of aggregates (f_A) and f_A*MWCO/Mw for the GPβCD polymers. f_A values were calculated from J_{theo} and J_{exp} at 5 w/v% CD polymer concentration.

they were saturated with hydrocortisone and finally about 0.1% of a hydrophobically modified dextran (HMD) (D40Ada) (Wintgens et al., 2011) was added to the CD polymer/drug complexes to induce formation of nanoparticles where inclusion complex formation between the βCD cavities and the adamantane ligands stabilized the nanoparticles. The results are presented in Table 2. As expected the size of the first population increases with increasing polymer Mw. The second population was observed in almost every case with a size that was determined to be between 350 and 700 nm, indicating formation of nano-sized aggregates.

In case of GPβCD the size of the first population (d_1) increases upon addition of hydrocortisone, but it decreases in the case of HXβCD. This decrease can possibly be due to increased lipophilicity and tighter package of the hydrocortisone-containing polymer chains, which also could explain the weaker solubilizing effect of the HXβCD polymers. The sizes of the second populations (d_2) are increasing in the case of GPβCD polymers after the addition of hydrocortisone. For the HXβCD polymers clear conclusions cannot be made because the appearance of populations with sizes above micron were disturbing the measurements, in the case of polymer with the smallest Mw the size of the aggregate increased.

Most of the polymer precipitates upon addition of 0.1% (w/v) of the hydrophobically modified polymer due to the formation of macroscopic particles, except in the case of D6 GPβCD, which forms a cloudy solution of nanoparticles of similar size as the aggregates, but the size of the first population did decrease, due to the inclusion complexation between the CD polymer and HMD.

The DLS peaks are quite wide for these CD polymers; the polydispersity index is usually around 0.3. This could be due to the non-spherical nature of the polymer molecules, their slight difference in molecular structure and conformation, and due to the fact that the aggregates are dynamic systems constantly being formed and disassembled in aqueous solutions.

Table 2
DLS results of some dextran-based CD polymers with similar DS; the two size populations of the aqueous polymer solutions, the complexes with hydrocortisone and after adding hydrophobically modified dextran (D40Ada); d_1 and d_2 are the diameters of the first and second size population (n.a.: size not available, when the size of the second population was higher than a micron).

Polymer	Aqueous solution		Complex with HC		After adding HMD	
	d_1 (nm)	d_2 (nm)	d_1 (nm)	d_2 (nm)	d_1 (nm)	d_2 (nm)
D6 GPβCD 2	6.9	534	8.7	616	13.8	699
D25 GPβCD 3	14.4	709	16.9	748		
D40 GPβCD 1	17.3	360	18.5	n.a.		
D70 GPβCD 4	24.5	464	21.7	513		
D6 HXβCD	7.1	207	8.0	659		
D25 HXβCD	15.5	n.a.	12.9	570		
D40 HXβCD	18.2	786	17.2	n.a.		
D70 HXβCD	26.8	n.a.	14.6	709		

5. Conclusion

Dextran-based CD polymers synthesized by linking the βCD molecules to the dextran backbone with either ether linkage (GPβCD) or ester linkage (HXβCD) were studied. In aqueous solutions the polymers formed complex with hydrocortisone, and the physicochemical properties of the complexes and their aggregation were investigated.

Both the permeation and DLS techniques showed that the examined dextran-based CD polymers self-assemble to form aggregates. The fraction of aggregation is usually between 10% and 30%, but their stability is limited, the aggregates disintegrating upon dilution with the medium.

From the phase-solubility properties the number of the binding sites (BS), the solubilization efficiency (SE), and the binding efficiency (BE) were calculated. BS increases linearly with increasing Mw of the dextran polymer up to about 40 kDa, but then it displays a negative deviation from linearity. Optimal SE and BE were obtained for low Mw polymers and then the SE and BE values decreased slowly with increasing polymer chain length. All physicochemical properties are highly dependent on the degree of substitution (DS), i.e. the number of βCD moieties on the polymer chain. The DS has an

optimum below which the SE and BE of the polymers are enhanced with increasing DS and above which the accessibility of the βCD moieties becomes hampered due to steric hindrance of the binding sites. In comparison of the two types of polymers, the GPβCD polymers were more effective in solubilizing hydrocortisone.

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