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ORIGINAL ARTICLE

Drug loading in cyclodextrin polymers: dexamethasone model drug

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Abstract In pharmaceutical formulations cyclodextrins (CDs) are used to improve the aqueous solubility, stability, dissolution rate, bioavailability and/or local tolerance of drugs. Moreover, water-soluble polymers can be used to stabilize drug/CD complexes through formation ternary complexes. Alternative approach is to use CD-polymers, which can both enhance the aqueous solubility of a drug and result in sustained drug release. The aim of this work was to compare the solubilizing effects of ternary drug/CD/polymer complexes with two novel high molecular weight CD-polymers, i.e. poly(ethylene glycol) based γ-cyclodextrin (γCD) polymer (*PEG/γCD*) and epichlorohydrin-γ-cyclodextrin polymer (*EPI/γCD*) using dexamethasone (Dex) as a model drug, as well as the drug loading capacity of those selected CD-polymers. Hydroxypropyl methylcellulose and carboxymethylcellulose sodium salt were shown to have negligible effect on the solubilizing efficacy of γCD while hexadimethrine bromide increases the solubilization efficacy. The stability of the polymers was tested and it was adequate for the experimental conditions used. The solubilization efficacy of both CD-polymers was higher than that of the parent γCD and these γCD based polymers are able to load greater amount of Dex than the parent γCD.

Keywords Water-soluble polymers · Cyclodextrin-polymers · Dexamethasone · Solubility · Drug loading

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Introduction

Cyclodextrins (CDs) are cylindrical oligosaccharides with a lipophilic central cavity and hydrophilic outer surface. In aqueous solutions, CDs are able to form inclusion complexes with many drugs by taking up some lipophilic part of the drug molecules into cavity [1]. When used in pharmaceutical formulations, they can improve the aqueous solubility, stability, dissolution rate, bioavailability and/or local tolerance of drugs [2, 3]. Addition of small amounts of water soluble polymers can enhance cyclodextrin complexation, solubilization efficiency and bioavailability of drugs [4, 5]. The polymers stabilize drug/CD complexes through ternary complexes formation. Furthermore, ternary complexes containing matrix-forming polymers, such as hydroxypropyl methylcellulose (HPMC), can result in sustained drug release [6].

Corticosteroids, such as dexamethasone (Dex), are generally lipophilic and possess poor aqueous solubility. The intrinsic solubility of Dex is, for example, only about 80 μg/ml in both pure water and aqueous eye drop vehicle [7]. The solubility of Dex can be enhanced through formation of ternary CD-polymer complexes [8, 9]. Alternative approach is to use CD-polymers, which can both enhance the aqueous solubility of the drug and give sustained drug release. The CD-polymers consist of mixture of isomers that, like some other CD derivatives, offer the

advantages of amorphous solid state drug complexes resulting in enhanced bioavailability after oral drug delivery [10, 11]. The CD-polymers can form nanoparticles or gels in aqueous media that can be explored as drug delivery systems [12]. Here we describe two novel CD-polymers and their initial evaluation as drug delivery systems.

Experimental section

Materials

The parent γ-cyclodextrin (γCD) was purchased from Wacker Chemie (Burkhausen, Germany) and was dried at 115 °C in vacuum overnight prior to usage. Hydroxypropyl methylcellulose 4000 cP (HPMC), carboxymethylcellulose sodium salt 250 kDa (NaCMC) and hexadimethrine bromide (purity ≥ 94% by titration) (HDMBr) were purchased from Sigma (St. Luis, USA). Following materials were used for synthesis of the CD-polymers: 1-chloro-2,3-epoxy propane and sodium hydroxide purchased from Acros Organics (Halluin, France), deuterium oxide 100% (Euriso-Top, Gif-sur-Yvette, France), *N,N*-dimethylformamide, (99.8%, $H_2O \leq 0.01\%$, Fluka, Germany), pyridine (anhydrous, 99.8%, Aldrich, Steinheim, Germany) and 4-(dimethylamino)pyridine (99%, Aldrich, Steinheim, Germany), all substances were used as received. PEG600-diacylchloride was prepared by a previously described method [12]. Dexamethasone (Dex) was purchased from Fagron (Amsterdam, Netherlands). All organic solvents for HPLC were purchased from Sigma (St. Luis, USA). MilliQ water (Millipore, USA) was used for HPLC analysis and for preparation of all solutions.

Synthesis

Poly(ethylene glycol) based γ-cyclodextrin polymers (*PEG/γCD*) was prepared by previously described method [12]. All glassware was dried at 130 °C for 2 h and cooled in a desiccator prior to use. γCD (3.0 g, 2.31 mmol) was dissolved in 100 mL *N,N*-dimethylformamide under nitrogen. PEG600-diacylchloride (4.05 g, 6.36 mmol) was dissolved in 5 mL DMF and added to the γCD solution. Pyridine (10.27 mL, 127.2 mmol) and 4-(dimethylamino) pyridine (10 mg, 0.08 mmol) were added and the temperature elevated to 80 °C. The solution was stirred under nitrogen for 24 h. Then the solvent was removed in vacuo and the remaining solid was dissolved in 100 mL MilliQ water. The pH was adjusted to approximately 7 by addition of saturated sodium bicarbonate solution. The CD-polymer was dialyzed against MilliQ water for 4 days and lyophilized yielding 3.3 g of white solid.

Epichlorohydrin-γ-cyclodextrin polymers (*EPI/γCD*) was prepared by previously described method [13]. Briefly, 10 g of γCD were dissolved in 20 mL of aqueous 0.1 M sodium hydroxide solution under constant stirring. The mixture was left overnight at 30 °C. Then epichlorohydrin (4.2 mL) was rapidly added to the solution under vigorous stirring. Five hours later the polycondensation was stopped by addition of 20 mL of acetone. The mixture was poured into a beaker and acetone removed by decantation. Water (100 mL) was added and pH of the aqueous solution adjusted to 10.5 by addition of concentrated aqueous hydrochloric acid solution. The mixture was stirred over night at 50 °C. After cooling to room temperature, the solution was neutralized and diafiltrated (molecular weight cut-off (MWCO) 10,000 Da) under pressure (2 bars). The obtained solution was lyophilized yielding 4.5 g of EPI/γCD [14].

^{1}H NMR analyses were conducted in deuterated water with a Brucker DRX400 spectrometer (5 mm TXI (H/C/N) xyz-gradient probe) with a delay time (d1) set for 30 s. Size exclusion chromatography was made in MilliQ water with 0.1 M NaN_3 at a TSK-gel type SW 4000–3000 column detected by a Wyatt miniDawn light scattering detector and a Wyatt Optilab Rex. refractive index detector. Dialysis was made with cellophane membranes MWCO 6–8 kDa, 25.5 mm diameter, 5 mL/cm (Sprectra/Por, Spectrum Europe., Netherlands). CD-polymers were lyophilized with a Heto CT 60e.

Stability testing

PEG/γCD and EPI/γCD solutions in pure water, aqueous 0.1 N HCl solution, and aqueous 0.01 N NaOH solution were prepared (2 mL) and autoclaved (Astell MXN 472, UK) three consecutive times, each time at 121 °C for 20 min. After cooling to room temperature the samples were filtrated through a cellulose membrane filter 0.45 μm (Spartan 13, Whatman, Germany). Quantitative determination of γCD was performed by HPLC.

Dexamethasone solubility

The effect of HPMC, NaCMC and HDMBr (a neutral, anionic and cationic polymer, respectively) on the γCD solubilization of Dex was investigated. In addition the solubilising effects of EPI/γCD and PEG/γCD were investigated. Excess amount of Dex was added to 1% (w/v) γCD solutions containing 0.25% (w/v) HPMC, NaCMC or HDMBr. In case of the CD-polymers, the complexation medium was aqueous solution consisting of either EPI/γCD or PEG/γCD at concentrations equivalent to 1% (w/v) γCD based on their γCD content. The solubility of Dex was determined by the heating method [15]. Briefly, excess

amount of Dex was added to the solution and the aqueous drug suspension formed autoclaved (Astell MXN 472, UK) at 121 °C for 20 min. After cooling to room temperature small amount of Dex was added to promote drug precipitation and the aqueous suspensions allowed to equilibrate under constant agitation for 7 days. Then the suspensions were filtrated through a cellulose membrane filter 0.45 μm (Spartan 13, Whatman, Germany). The suspensions prepared with the CD-polymers were centrifuged in order to prevent the retention of the polymers in the filter and samples were analyzed by HPLC.

Viscosity determinations

The viscosities of γCD (1% (w/v)), PEG/γCD (1.26% (w/v)) and EPI/γCD (2% (w/v)) solutions were determined using Brookfield digital viscometer (DV-I+, Mega, USA) connected to water bath (Polystat, USA) operated at 25 ± 2 °C and 37 ± 2 °C. 1.26% (w/v) PEG/γCD and 2% (w/v) EPI/γCD corresponded to 1% γCD based on chemical analysis of the polymers. Samples were allowed to equilibrate for 24 h prior to the viscosity determinations. All measurements were done in triplicate.

Drug loading and release studies

Unjacketed 12 mL flat ground joint Franz diffusion cells (SES Analysesysteme, Germany) were used to determine drug loading and drug release from the polymers. Donor phases consisted of aqueous γCD (1% w/v), PEG/γCD (1.26% w/v) and EPI/γCD (2% w/v) solutions prepared in phosphate-buffered saline (PBS buffer) containing 0.1, 0.2, 0.4 or 1% (w/v) Dex. The solutions were autoclaved (Astell MXN 472, UK) at 121 °C for 20 min and allowed to equilibrate at room temperature (22–23 °C) under constant agitation (KS-15 control, Edmund Bühler GmbH, Germany) for 16 h. The donor phase (1.5 mL) was added to Franz diffusion cell containing aqueous 2% (w/v) γCD solution as a receptor phase. The two phases were separated by a semipermeable cellophane membrane (single layer of Sprectra/Por membrane, Spectrum Europe., Netherlands) with a MWCO 12–14 kDa. Prior to usage the cellophane membrane was soaked in the receptor phase for 5 h. Samples were taken from the receptor phase at 15, 30, 45, 60, 90, 120, 180, 360, 240 and 480 min and analyzed by HPLC.

Drug loading (DL) was calculated from following Eq. 1:

$$DL = \frac{P}{J_{\max}} \tag{1}$$

with the use of the maximum flux (J_{max}), obtained at the highest Dex concentration and the permeability coefficient (P), which is obtained from the three lowest pairs of variants (Dex concentration([dex]) and flux (J)) by using Eq. 2:

$$P = \frac{\sum_{n=1}^{3}\left([dex]_n - \overline{[dex]_3}\right)(J_n - \overline{J_3})\right)}{\sum_{n=1}^{3}\left([dex]_n - \overline{[dex]_3}^2\right)} \tag{2}$$

where

$$\overline{[dex]_3} = \frac{\sum_{n=1}^{3}[dex]_n}{3} \tag{3}$$

and

$$\overline{J_3} = \frac{\sum_{n=1}^{3} J_n}{3} \tag{4}$$

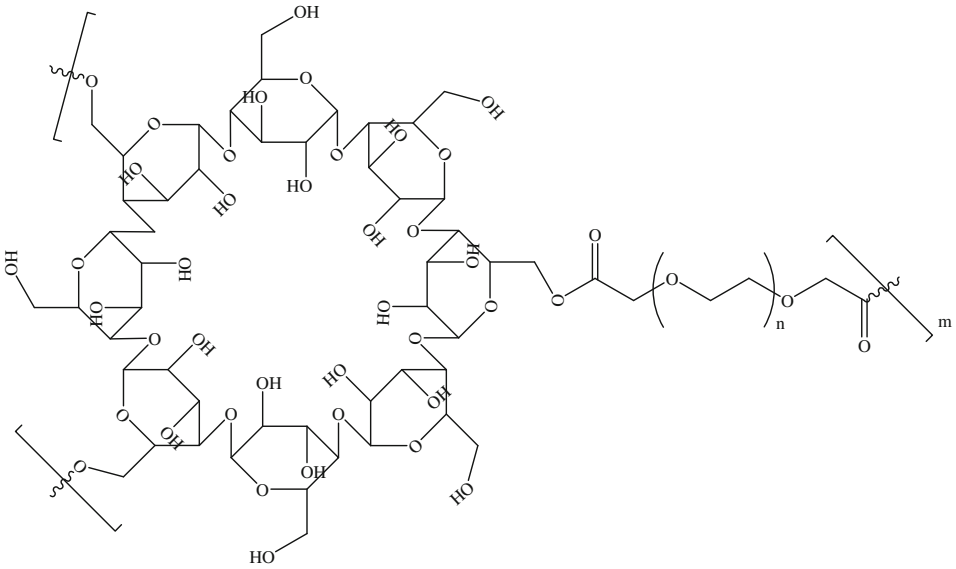
Quantitative analysis

Both, γCD and Dex were analyzed by Dionex Ultimate 3000 (Dionex, Germany) HPLC system (injection volume 20 μL). γCD was separated by a 10 μ Luna NH2 100A (250 × 4.6 mm) column (Phenomenex, UK) using 67% (v/v) CH_3CN in pure water as a mobile phase (1.0 mL/min) and differential refractive index detector (RI-101, Shodex, Japan) with a sensitivity of 600 μ RIU. UV detector operated at 241 nm was used to detect Dex, the mobile phase consisted of 80% (v/v) CH_3OH, 29% (v/v) water and 1% (v/v) tetrahydrofuran. The column was 5 μ Luna C18 100A (150 × 4.6 mm; Phenomenex, UK). The retention time for γCD and Dex was 11.7 and 3.5 min, respectively. Data integration was performed with CHROMELEON software (Dionex, Germany) version 6.80 for LC integration.

Results and discussion

The PEG/γCD polymer (Fig. 1) was obtained by reaction of the parent γCD with PEG-diacyl-chloride [12]. The polymer was purified by dialysis against water using membranes with a molecular cut-off of 6000–8000 Da to ensure removal of monomers, oligomers, DMAP and residual solvents. The chemical composition of the polymer was verified by ^{1}H NMR spectroscopy and the γCD content determined to be 46% (w/w). The molecular weight obtained from SEC was determined to be 25.1 kDa. The biodegradability of the PEG/γCD polymer was enforced through ester-linkages between the γCD molecules and the PEG bridges. The EPI/γCD polymer (Fig. 2) was obtained by reacting γCD with epichlorohydrin (EPI) in an alkaline medium resulting in formation of glyceryl bridges between the parent γCD molecules [13]. Again the composition was verified by ^{1}H NMR and the γCD content determined to be

Fig. 1 Representative structure of PEG/γCD



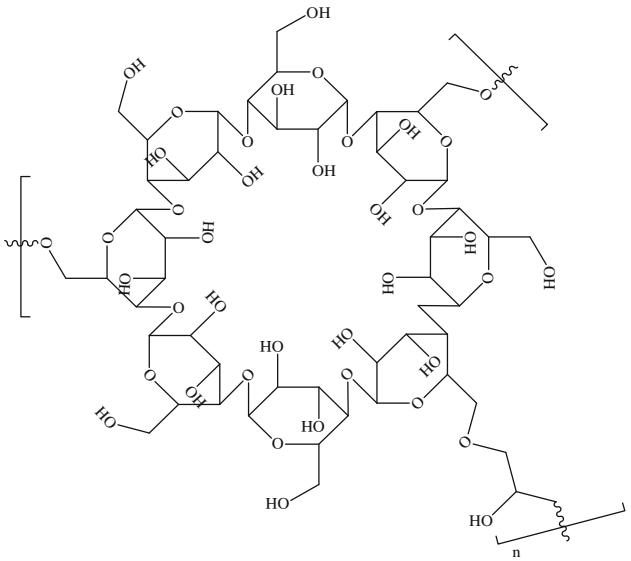


Fig. 2 Representative structure of EPI/γCD

73%. The molecular weight obtained by SEC was 21.0 kDa.

It is essential to know the stability of Dex, γCD and the CD-polymers in aqueous solutions prior to studies involving aqueous solutions of these excipients. Previous studies had shown that dexamethasone is chemically stable in aqueous γCD solutions [8, 9]. Also, γCD is known to be chemically stable in aqueous solutions [1]. For γCD-polymers we tested the stability in pure water as well as under acidic and basic conditions by monitoring the γCD release from the degrading polymer. The results of the stability testing are shown in Table 1. Although the PEG/γCD polymer network contains ester linkages it was relatively stable under these extreme experimental conditions, showing less than 10% degradation at acidic conditions and

Table 1 Stability of the polymers expressed as % of γCD remaining in the EPI/γCD and PEG/γCD networks after heating aqueous solution (pure water, 0.1 N HCl, 0.01 N NaOH solutions)

	PEG/γCD	EPI/γCD
Pure water	85.6	94.5
HCl 0.1 N	91.0	93.4
NaOH 0.01 N	86.2	94.7

14% degradation under neutral and basic conditions. This relatively high stability is most probably due to the steric hindrance of the network against nucleophilic attack of water and hydroxy ions on the ester linkage. The EPI/γCD polymer was more stable under these same conditions, most probably due to the chemical stability of the ether linkages of the polymeric network.

Table 2 shows the solubility of Dex and solubilization efficacy of γCD in aqueous 1% (w/v) γCD solutions, with and without presence of water soluble polymers, as well as in aqueous γCD-polymer solutions. At this concentration, the change in solubility up on addition of HPMC and CMC is negligible, while HDMBr increases the solubilization efficacy by 15%. The solubilization efficacies of the CD-polymers were 7 and 44% higher than that of the parent γCD for EPI/γCD and PEG/γCD, respectively. This indicates that Dex is mainly solubilized by the γCD moieties in the EPI/γCD polymer but that the PEG bridges might be participating in the solubilization by the PEG/γCD polymer.

The viscosity of aqueous 1% (w/v) γCD, 1.26% (w/v) EPI/γCD and 2% (w/v) PEG/γCD solutions was determined in a rotational spindle viscometer. The viscosity results are shown in Table 3. At this low concentration,

Table 2 Solubility and solubilization efficacy of γCD and γCD-polymer solutions

Solution	Solubility (mg/ml)	Solubilization efficacy
1% γCD	2.7 ± 0.4	–
1% γCD + HPMC*	2.8 ± 0.2	1.04
1% γCD + CMC*	2.4 ± 0.2	0.89
1% γCD + HDMB*	3.1 ± 0.1	1.15
1.26% EPI/γCD	2.9 ± 0.1	1.07
2% PEG/γCD	3.9 ± 0.2	1.44

The concentration of the polymers are equivalent to 1% γCD based on the γCD content of the polymers

* 0.25% (w/v)

Table 3 Viscosity determination of γCD, EPI/γCD and PEG/γCD in aqueous solutions at 25 ± 2 °C and 37 ± 2 °C

	γCD	EPI/γCD	PEG/γCD
η (mPa s) (25 °C)	1.49 ± 0.02	1.49 ± 0.03	1.19 ± 0.03
η (mPa s) (37 °C)	1.22 ± 0.02	1.09 ± 0.04	0.92 ± 0.05

γCD and the polymers had negligible effect on the viscosity.

From the solubility studies of Dex in the aqueous CD-polymers solutions the drug loading was calculated (Table 4) and expressed as the amount of drug per mg of γCD content of the polymers. When calculated this way, the polymers were able to load greater amount of Dex than the parent γCD with Dex loading 19 and 63% higher for EPI/γCD and PEG/γCD than for γCD, respectively. Table 5 shows the flux of Dex from aqueous solutions through a single layer of semi-permeable cellophane membrane with MWCO 12–14 kDa. The semi-permeable cellophane membranes are size exclusion membranes, where molecules permeate through pores in the membrane. According to Fick's first law

$$J = D\frac{(C_1 - C_2)}{h} \approx \frac{D \cdot C_1}{h} \tag{5}$$

the flux (J) through a porous membrane is proportional to the diffusion coefficient of the penetrating drug molecules (D) and the drug concentration difference between the donor phase (C_1) and the receptor phase (C_2), divided by the effective thickness of the membrane (h). Under sink conditions, C_2 can be omitted since $C_1 - C_2 \approx C_1$. The flux values were determined from aqueous polymer solutions containing different Dex concentration. The steady state flux was calculated as the slope (dq/dt) of linear section of the drug release profiles, where the amount of drug in the receptor chamber (q) is shown versus the time (t),

Table 4 Drug loading expressed in mg of Dex per mg of γCD obtained by the solubility and permeability methods

	mg Dex per mg γCD or γCD equivalents		
	γCD	EPI/γCD	PEG/γCD
Solubility method	0.27 ± 0.03	0.32 ± 0.01	0.44 ± 0.02
Permeability method	0.26 ± 0.01	0.38 ± 0.05	0.50 ± 0.05

Table 5 Fluxes obtained from different solutions containing different amounts of Dex

[Dex] (mg/ml)	J (μg cm^{-2} min^{-1})		
	γCD	EPI/γCD	PEG/γCD
1	0.85 ± 0.06	0.21 ± 0.03	0.45 ± 0.00
2	1.61 ± 0.04	0.47 ± 0.03	0.85 ± 0.06
4	2.05 ± 0.24	0.90 ± 0.16	0.90 ± 0.00
10	2.03 ± 0.06	0.90 ± 0.11	1.22 ± 0.12

$$J = \frac{dq}{A \cdot dt} \tag{6}$$

where A is the surface area of the mounted membrane (1.77 cm^2) and C_d is the initial concentration of the drug in the donor phase. Since the flux is proportional to the concentration in the donor phase, the flux should increase with increasing the concentration of dissolved Dex. The flux is constant for the solutions with 0.4 and 1.0% (4 and 10 mg/ml) of Dex that means that these solutions have been saturated with Dex and consequently the maximum drug solubility in the polymer solutions has been reached representing the maximum amount of Dex that can be loaded into the polymer. The determined viscosities are shown in the Table 3. The relationship between the viscosity (η) of the diffusion medium and the diffusion coefficient is given by Stokes–Einstein equation:

$$D = \frac{RT}{6\pi\eta r N} \tag{7}$$

where R is the molar gas constant, T is the absolute temperature, r is the radius of the diffusing molecule (assuming it can be represented by a spherical particle) and N is Avogadro's number. Combining Eqs. 6 and 7 and rearrangement gives [15, 16]:

$$J\eta = \frac{RT}{6\pi r N}\frac{C}{h} \tag{8}$$

Since the viscosity of the solutions does not differ, the diffusion of the drug depends on the radius of particles which is larger in the case of CD-polymers. Consequently, the decrease in the flux between the native γCD and CD-polymers solutions could be due to the entrapment of the

drug molecules into the polymeric network or due to the low MWCO of the membrane used. Using the results thus obtained, the drug loading of the polymers could be studied and calculated as was previously explained (Eq. 1). The results are shown in Table 4 and are comparable with those obtained from the solubility studies.

Conclusions

Novel water soluble CD-polymers were synthesized. The polymers are sufficiently stable in aqueous solution to be heated in autoclave (121 °C, 20 min). Both CD-polymers studied were able to form water soluble complexes with Dex. The drug loading was higher than that of the native γCD and the effect on the solubility of Dex was more remarkable than when water soluble polymers were added to aqueous solutions containing the parent γCD. These preliminary studies show that it is possible to utilize these CD-polymers as drug delivery vehicles.

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