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Formation and stability assessment of self-assembled nanoparticles from large Mw chitosan and sulfobutylether-β-cyclodextrin

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ABSTRACT

Stable self-assembled nanoparticles were prepared by mixing aqueous solutions containing chitosan and either sulfobutylether β-cyclodextrin or sulfobutylether γ-cyclodextrin at pH 4. The nanoparticle formation was monitored by UV-VIS spectroscopy operated at 700 nm. The stability of the nanoparticle system was tested under different conditions, for example upon storage, upon dilution, at elevated temperatures and upon salt addition. The effect of media pH on the formation and stability of the nanoparticles was also tested. Stability studies were followed by dynamic light scattering measurements and showed that the nanoparticles are stable at all conditions tested except in basic media. The nanoparticles were investigated by transmission electron microscopy which confirmed that the diameter of the nanoparticles is between 100 and 200 nm. Drug release from the particles was studied using hydrocortisone as a model drug.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides, typically consisting of 6–8 α-glucopyranose units; they have toroid structure, hydrophilic outer surface and relatively lipophilic central cavity. They became important pharmaceutical excipients due to their ability to form inclusion complexes with lipophilic molecules, thus increasing the aqueous solubility of various poorly soluble drugs [1–3]. CDs tend to form nano- and micro-sized aggregates [4,5]; this phenomena is enhanced upon formation of inclusion complexes with hydrophobic drug molecules [6–8]. However, these self-associates are generally not stable and dissociate upon dilution [9,10], but still they can be applied topically as drug delivery systems where media dilution is negligible [11–14]. Several synthetic and physicochemical techniques can be applied to form stable self-assembled CD nanoparticles, including addition of water-soluble polymers [15,16].

Chitosan (CS) is a linear polysaccharide consisting of β-(1 → 4)-linked D-glucosamine and N-acetyl-D-glucosamine. CS is produced by deacetylation of chitin (poly[β-(1 → 4)-N-acetyl-D-glucosamine]), the main component of arthropod exoskeletons, e.g. shrimps and insects. CS has been used for some time as pharmaceutical excipient and more recently to form micro- and nanoparticulate systems [17,18]. Various methods can be applied to prepare CS nanoparticles but one of the most promising technique is ionotropic gelation that is based on ionic interactions between the polycationic CS and a polyanionic source such as sodium tripolyphosphate (TPP) [19]; in some cases nonionic CDs are incorporated into CS/TPP nanoparticles [20]. Higher ratio of CD incorporation can be achieved by replacing TPP by polyanionic CD derivatives; these derivatives are most commonly sulfobutylether β-cyclodextrin (SBEβCD) [21–26] or carboxymethyl-β-cyclodextrin (CMβCD) [27,28], sulfobutylether and carboxymethyl γCD derivatives can also be used.

The focus of this paper is nanoparticles (NPs) formed by CS and SBEβCD. It has been shown that nano- and microparticles with the diameter between 250 and 1000 nm can be formed by using lower

Abbreviations: CD, cyclodextrin; CS, chitosan; SBEβCD, sulfobutylether β-cyclodextrin; SBEγCD, sulfobutylether γ-cyclodextrin; NP, nanoparticle.

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Nomenclature of NPs

NP the type of CD used (β or γ) – Mw of CS in kDa – drug loaded (L) or unloaded (–)

Types of NPs used
NP(β110), NP(β110L) Most studies were performed using these types of NPs
NP(γ110) NP(β10)

molecular weight (approx. 10 kDa) CS, although the NPs were only metastable [29]. Here the aim is to describe the preparation parameters of the CS/SBEβCD NPs using higher molecular weight CS (110 kDa), estimate the NP composition, measure their diameter, examine their drug release capabilities and to test their stability under various conditions.

2. Materials and methods

2.1. Materials

Sulfobutylether β-cyclodextrin sodium salt (SBEβCD) with average molar substitution of 0.9, average molecular weight (Mw) 2163 Da, and sulfobutylether γ-cyclodextrin sodium salt (SBEγCD) with average molar substitution 0.61, Mw 2072 Da, were kindly donated by CyDex Pharmaceuticals (Lawrence, KS, USA). Higher molecular weight chitosan hydrochloride salt (CS110, Chitoclear, 96% deacetylation degree, Mw ≈ 110 kDa) was kindly donated by Primex (Siglufjörður, Iceland), chitosan mesylate salt (CS10, 95% deacetylation degree, Mw ≈ 10 kDa) was prepared according to previously described method [30,31]. Hydrocortisone (HC) was purchased from Fagron (Nieuwerkerk aan den Ussel, The Netherlands). Methanol and tetrahydrofuran were purchased from Sigma–Aldrich (St. Louis, MO, USA). All solutions and the HPLC mobile phase were prepared using Milli-Q water (Millipore, Billerica, MA, USA).

2.2. Quantitative determination of hydrocortisone

Quantitative determination of HC was performed on a reversed-phase high performance liquid chromatographic (HPLC) Ultimate 3000 system from Dionex Softron GmbH (Germany) consisting of LPG-3400A pump with a built-in degasser, WPS-3000-TSL autosampler, 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.

2.3. Nomenclature of nanoparticles

NPs were formed by mixing SBEβCD or SBEγCD with lower or higher Mw chitosans (CS10 or CS110). To differentiate between the different NP formulations the NPs were named according to as follows: NP – the type of SBECD used (β or γ) – Mw of CS in kDa – loaded or unloaded (L or nothing). NPs prepared from SBEβCD and CS110 are the main focus of this article and are named NP(β110) if they are unloaded or NP(β110L) of they are loaded with HC. The stability parameters of NP(β110) were compared with those of two other NPs, that is NP(β10) [29] to examine the effect of shorter CS chains on the NPs and NP(γ110) to see the effects of utilizing a larger CD cavity (note that the DS of SBEγCD is not identical to that of SBEβCD).

2.4. Nanoparticle preparation and loading; titration of chitosan with SBEβCD followed by UV-VIS spectroscopy

Aqueous solutions containing 0.25%, 0.5%, 0.75% and 1% w/v of SBEβCD, SBEγCD, CS110 or CS10 were prepared by dilution of pH 4 stock solutions with an aqueous pH 4 hydrochloric acid solution. Then a CD solution was mixed into a CS solution of identical weight concentration to form self-assembled NPs. The mixing ratios of the CD and CS solutions were determined by monitoring NP formation (i.e. the turbidity) by UV/VIS spectroscopy (Perkin Elmer Lambda 35 UV/VIS spectrometer, USA). The CD solution was added to a 2 ml CS solution in 100 μl portions. After each addition, the solution was mixed for about one minute and the absorbance at 700 nm was determined. This wavelength was selected since none of the components (i.e. water, SBEβCD, CS and HC) have absorbance in that region, but the intensity of light scattering from the NPs was still small enough to be measured by the UV/VIS spectrometer.

In order to compare the different NP concentrations, mixing ratios of SBEβCD and CS solutions that gave 0.5 absorbance were determined. When a desired mixing ratio had been determined, the nanoparticle solutions were prepared by slowly adding the right amount of the SBEβCD solution to the CS solution and stirring the mixture for one hour. The nanoparticle solutions were used for measurements within 1–2 days although later studies showed that they were stable for much longer time periods. Most frequently NPs were prepared by mixing aqueous 0.5% (w/v) SBEβCD solution into a 0.5% (w/v) CS solution.

To prepare loaded nanoparticles the initial SBEβCD and CS solutions were saturated with HC by adding an excess amount of drug to the solutions followed by treatment in an ultrasonic bath for 60 min at 60 °C (Cole-Palmer Instrument Company, Niles, IL, USA) and then shaking the drug saturated SBEβCD and CS solutions for a week at room temperature. Then the NPs were prepared as previously described, mixing together CS and HC/SBEβCD solutions of identical concentration at pH 4, stirring for one hour and using the NP solutions for measurements within a week.

2.5. Viscosity measurements

Since, according to the Stokes–Einstein equation, determination of the particle diameter by dynamic light scattering (DLS) requires that the medium viscosity is known, the viscosity of the CS and NP solutions were determined at the various excipient concentrations at 25.0 ± 0.1 °C with a Brookfield DV-I Prime digital viscometer (Middleboro, MA, USA).

Viscosity measurements were also used to estimate the amount of CS bound to the NPs. This was performed by preparing NPs from aqueous 0.5% (w/v) SBEβCD and 0.5% (w/v) CS solutions at various mixing ratios. It was observed that at low SBEβCD concentrations the SBEβCD solutions have the same viscosity as water and that the viscosities of the NP solutions were lower than expected by the amount of CS present. According to Einstein's equation for viscosity: