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Carboxylate cross-linked cyclodextrin: A nanoporous scaffold for enhancement of rosuvastatin oral bioavailability

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ABSTRACT

Cyclodextrins play an important role in supramolecular chemistry acting as building blocks than can be cross-linked by various linker molecules forming nano-porous structures called nanosponges (NS). NS have the ability to enhance the stability, solubility and bioavailability of various actives. This work aimed at elaborating rosuvastatin (ROS) loaded NS to improve its oral bioavailability. Carboxylate-linked NS were synthesized by reacting β-CD with pyromellitic dianhydride (PDA) at different molar ratios under specific conditions. ROS-loaded NS were prepared by lyophilisation technique and characterized for particle size, zeta potential, entrapment efficiency and drug release. Occurrence of cross-linking and ROS incorporation within the NS were assessed by DSC, FT-IR and SEM micrographs. NS prepared at a molar ratio of 1:6 of β-CD: PDA demonstrated the highest entrapment efficiency (88.76%), an optimum particle size of 275 nm, a narrow size distribution (PDI of 0.392), and zeta potential of − 61.9 indicating good colloidal stability. In vivo oral pharmacokinetics study in male Sprague Dawley rats showed that ROS-NS provided an outstanding enhancement in oral bioavailability compared to drug suspension and marketed tablets besides their physicochemical stability for 3 month. Accordingly, ROS-NS represent a superior alternative to the conventional marketed formulation for effective ROS delivery.

1. Introduction

Cyclodextrins are cyclic oligosaccharides consisting of (α-1,4-) linked D- glucopyranose units with a characteristic toroidal shape. The CD molecule is a truncated cone-shaped structure with primary hydroxyl groups extending from the narrow edge and secondary groups from the wide edge, which characterizes the molecule with a hydrophilic outer surface and a hydrophobic inner cavity of the glucopyranose monomers backbone. CDs have played an important role in supramolecular chemistry due to their unique ability to form inclusion complexes with a wide variety of compounds (Davis and Brewster, 2004; Loftsson et al., 2005), which results in enhancement of apparent water solubility and could provide steric protection against chemical and enzymatic degradation (Concheiro and Alvarez-Lorenzo, 2013). Amongst the natural cyclodextrins, β-CD is the most prominently used in pharmaceutical formulations owing to its ease of production, and low price (Kurkov and Loftsson, 2013).

However, CDs showed two major limitations, which are the ease of dissociation of the formed complexes on dilution, and the inability to form inclusion complexes with certain molecules (Vyas et al., 2008). This triggered the use of CDs and their derivatives as building blocks for supramolecular structures based on linking CD molecules in a regioselective manner with a variety of bi or polyfunctional cross-linking agent molecules which react with the polymer and become fitted as tiny grappling hooks that fasten different parts of the polymer together. This produces particles possessing interconnecting nanosized cavities, which form a three-dimensional porous non-collapsible structure called nanosponge (NS) (Trotta, 2011). This name (NS) is related to the property of forming a sponge-like structure when lyophilized and high capacity to entrap small molecules in its matrix. This peculiar structural nanoporous scaffold changes many properties of the native CD, offering various advantages and superiority in complex formation with guest molecules. The interaction sites available for complexation in NS are significantly increased compared to those in CD molecules. The highly cross-linked network being amphiphilic in nature, can simultaneously host hydrophobic molecules in the hydrophobic CD cavities, and hydrophilic molecules in the nanochannels between individual CD moieties (Chilajwar et al., 2014; Lembo et al., 2013). The NS form suspension when dispersed in water, providing a matrix-like structure in aqueous media and allowing a free transfer of entrapped drug molecules (Tejashri et al., 2013). NS showed promising solubility enhancing effect of hydrophobic drugs such as resveratrol, tamoxifen and

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paclitaxel (Ansari et al., 2011; Mognetti et al., 2012; Torne et al., 2013).

Furthermore, the incorporation of drug molecules within the nanoporous structure offers stability enhancement and reduction of side effects (Tejashri et al., 2013). The CD/crosslinker ratio can be modulated during their preparation offering a porous network with tunable channels size, to improve the drug loading and to obtain a tailored release profile (Ahmed et al., 2013). Moreover, changing the type of the cross-linker affects the polarity of the cavities and the swelling capability of the formed nanosponge. Safety of NS has been assessed by in vitro and in vivo cytotoxicity studies, which showed them to be biocompatible with negligible biotoxicity (Trotta et al., 2012).

Rosuvastatin calcium (ROS) is an orally administered lipid-lowering statin that acts by inhibition of hydroxyl methyl glutaryl coenzymeA reductase (HMG-CoA reductase), which is the rate controlling enzyme in cholesterol biosynthesis. It is used in the treatment of various blood lipid metabolic disorders, including mixed hyperlipidemia, mild to moderate hypercholesterolemia (Fredrickson's type IIa/IIb), severe hypercholesterolemia (heterozygous or homozygous familial hypercholesterolemia), and hypertriglyceridemia (Fredrickson's type IIb/IV) (Olsson et al., 2002). It is also used for primary and secondary prophylaxis of cardiovascular events in patients with multiple risk factors as diabetes mellitus and renal failure (Lennernäs and Fager, 1997). Beyond lipid lowering effects, statins showed promising antitumor effects by inducing growth suppression or apoptosis of malignant cells (Sassano and Platanias, 2008), in addition to immunomodulatory and anti-inflammatory properties (Greenwood and Mason, 2007). Moreover, statins are reported for their potential use in osteoporosis treatment (Jadhav and Jain, 2006), benign prostatic hyperplasia (Zhang et al., 2015) and Alzheimer's disease (Haag et al., 2009).

ROS structure is also characterized by a fluorinated phenyl group which, together with the methane sulfonamide group and the cromophore dihydroxyheptenoic acid group common to all statins, provides ROS with superior potency amongst other statins due to enhanced binding affinity to HMG-CoA reductase by increasing ionic interactions with the enzyme. Thus, ROS is a significantly more potent inhibitor of hepatic sterol synthesis compared to all currently available statins, with an IC_{50} (50% inhibitory concentration) value of 0.2 nM compared to other statins with IC_{50} ranging from 1.2 to 6.9 nM (Olsson et al., 2002).

However, ROS is BCS class II drug with a crystalline nature and low aqueous solubility (Potur et al., 2014). This accounts for its poor bioavailability (20%) where dissolution is the rate limiting step after oral administration.

The formation of inclusion complex between ROS and β-CD or its derivatives is reported to cause an enhancement in ROS solubility and rate of dissolution (Venkatesh et al., 2014; Vidya et al., 2016). However, in these studies assessment was based only on in vitro characterization, and neither ex vivo or in vivo studies was exploited. Moreover, the ease of dissociation of the formed CD complexes on dilution may result in failure to enhance drug dissolution and bioavailability upon dilution in the GIT (Ahmed et al., 2013). Thus, in vivo studies are crucial in assessing of the efficacy and performance of the developed formulations.

In view of the aforementioned characteristics of ROS and merits of NS as oral drug delivery carriers, the appraisal of developing ROS-loaded NS for improved oral delivery is a desirable approach with the possibility of achieving promising outcomes. Accordingly, this work aimed at elaborating ROS-loaded NS and evaluating their effect on the pharmacokinetics of ROS to assess the intrinsic effect of the NS for improving its oral bioavailability.

2. Materials and methods

2.1. Materials

Rosuvastatin calcium and ketoprofen were a gifts from El-Borg Co., and El-Amriya pharmaceutical Co., Alexandria, Egypt, respectively, β-CD (Kleptose®) was a gift from Roquette (Freres, Lesterm, France). Pyromellitic dianhydride (PDA) (Sigma-Aldrich, Co., St Louis, MO, USA). Triethyl amine (TEA) (Loba Chemie Pvt. Ltd., Mumbai, India). Dimethyl formamide (DMF) (Lab-Scan, Sowińskiego, Poland) Mannitol (Oxford Lab., Mumbai, India). Methanol and Acetonitrile, HPLC grade (Merck, USA). All other chemicals and reagents used were of analytical grade.

Table 1
Composition and % yield of NS prepared at different weight ratios of β-CD: PDA.

Formulation	β-CD:PDA molar ratio	β-CD	PDA	TEA	Yield[a]
NS1	1:4	0.25 g	0.190 g	0.363 g	95.97 ± 0.62%
NS2	1:6	0.25 g	0.288 g	0.508 g	96.80 ± 0.75%
NS3	1:8	0.25 g	0.388 g	0.870 g	96.98 ± 0.64%

Abbreviations: NS, nanosponges; PDA, pyrolmellitic dianhydride; TEA, triethyl amine.
[a] Mean ± SD, n = 3.

2.2. Methods

2.2.1. Synthesis of carboxylate nanosponges

Carboxylate-linked β-CD NS were prepared following previously described procedure with minor modifications (Mele et al., 2011). Briefly, β-CD was dissolved in DMF, and then a specific volume of TEA was added and stirred for 2 min. PDA was added to the solution in varying amounts with molar ratios of (1:4, 1:6, and 1:8) of β-CD: PDA (Table 1). The reaction mixture was stirred at ambient temperature for 3 h. The solid obtained was recovered by centrifugation and washed repeatedly with distilled water and acetone, then centrifuged at 4000 rpm for 5 min for removal of residual by-products or unreacted reagents in the supernatant. The clean residue obtained was left to dry in a desiccator and then ground in a mortar to obtain the crude NS. Several preliminary trials were carried out to optimize the proper amounts of ingredients required for the occurrence of the cross linking reaction. The solid product obtained after washing and drying was weighed for calculating the % yield, according to the following equation:

$$\%\text{yield} = \frac{\text{weight of nanosponges}}{\text{weight of}\,\beta\text{CD} + \text{weight of PDA}} * 100$$

2.2.2. Preparation of ROS-loaded NS

Nanosponges prepared at molar ratios 1:4, 1:6 and 1:8 of β-CD:PDA named NS1, NS2, and NS3 respectively (Table 1) were accurately weighed and suspended in 25 mL millipore-filtered distilled water while stirring. A calculated amount of ROS was added to form a drug:NS mixture of ratio 1:2 (w/w). The mixtures were sonicated for 15 min and stirred at 2000 rpm protected from light for 24 h. The suspensions obtained were lyophilized after adding 5% w/v mannitol. Finally the lyophilized ROS-loaded nanosponges named NSR1, NSR2, NSR3 prepared respectively at molar ratios 1:4, 1:6 and 1:8 of β-CD:PDA were stored in a desiccator at ambient temperature until further use (Osmani et al., 2016).

2.2.3. Characterization of ROS-loaded NS

2.2.3.1. Recovery yield and entrapment efficiency (EE). The recovery yield was calculated according to the following equation (Peter Christoper et al., 2014):

$$\text{Recovery yield} = \frac{\text{Mass of lyophilized powder}}{\text{Mass of (ROS + NS+mannitol)}} * 100$$

For calculating the EE, 200 mg of the lyophilized ROS-loaded NS were dispersed in water, vortexed for 30 s and centrifuged at 2000 rpm for 10 min to separate the unentrapped drug (Osmani et al., 2016). One