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New shell crosslinked micelles from dextran with hydrophobic end groups and their interaction with bioactive molecules

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ABSTRACT

Micelles formed in aqueous solution by dextran with hydrophobic (alkyl) end-groups were stabilized through divinyl sulfone crosslinking of the dextran shell. The efficacy of the crosslinking reaction was influenced by the divinyl sulfone amount, the pH and micelle concentration. Crosslinked micelles with a moderate crosslinking degree were further functionalized by attachment of 10 and 17 moles% *N*-(2-hydroxypropyl)-*N*,*N*-dimethyl-*N*-benzylammonium chloride groups along the dextran chain. The size and shape of both crosslinked micelles and their cationic derivatives were analyzed by DLS and TEM. The prepared micelles were able to bind anionic diclofenac (60–370 mg/g), hydrophobic anionic indometacin (70–120 mg/g), and hydrophobic alpha-tocopherol (170–220 mg/g) or ergocalciferol (90–110 mg/g) by hydrophobic or/and electrostatic forces. The release experiments and the antioxidant activity of bound alpha-tocopherol highlighted the potential of the new nano-sized micelles mainly as carriers for prolonged and controlled delivery of hydrophobic drugs.

1. Introduction

Amphiphilic polymers can self-assemble in selective solvents with formation of aggregates of various morphologies, the properties of which recommend them for application in biotechnology, pharmacy, cosmetic or painting industries. Nano-sized aggregates of amphiphilic polymers have been extensively studied as carriers for the controlled drug delivery systems (dos Santos, Medronho, dos Santos, & Antunes, 2013) due to their small particle size which allows the circulation in the blood stream without immobilization at capillaries, and permeate into the target cells through blood vessels. Moreover, these aggregates can solubilize hydrophobic drugs and deliver them to targeted tissues/organs. Amphiphilic block-copolymers represent the main classes of amphiphilic polymers used as hydrophobic drug carriers. Single end hydrophobically modified polymers (amphiphilic semi-telechelic polymers) can associate into spherical micelles, similarly to the amphiphilic block copolymers, and can also be used as drug carriers (Kuskov et al., 2010).

The use of block copolymer or block-like end modified polymers as drug delivery systems can be hampered by the low stability in the blood stream, where the micelles are prone to extensive dilution, which can go below the polymer critical aggregation concentration. Intramicellar crosslinking of either the shell or the core of the core–shell micelles can lead to more stable nanostructured materials (Wooley, 2000). Thus, micelles of triblock poly(propylene oxide)–*b*-poly(glycerol monomethacrylate)–*b*-poly[2-(dimethylamino)ethyl methacrylate] were inner shell crosslinked with divinyl sulfone (DVS) (Pilon, Armes, Findlay, & Rannard, 2006), obtaining shell-crosslinked micelles with amine-functional coronas; micelles of triblock poly(propylene oxide)–*b*-poly[(2-(dimethylamino)ethyl methacrylate]–*b*-poly (glycerol monomethacrylate) were crosslinked in their inner shell with 1,2-bis(2-iodoethoxy)ethane, obtaining shell-crosslinked micelles with hydroxyl-functional coronas (Pilon, Armes, Findlay, & Rannard, 2005). Also, micelles of poly(propylene oxide)–*b*-poly(glycerol monomethacrylate)-(or 2-hydroxyethyl methacrylate)–*b*-2-(diethylamino)ethyl methacylate inner shell crosslinked with DVS can serve as nanoreactors for synthesis of gold nanoparticles (Liu, Weaver, Sauve, & Armes, 2002).

Micelles formed by dextran-block-polystyrene block copolymers were core- or shell-crosslinked with divinyl sulfone (DVS), using different solvent mixtures (Houga et al., 2009). DVS was the crosslinker of choice for preparation of hydrogels from various polysaccharides, such as dextran (Zhang, Bowyer, Eisenthal, & Hubble, 2007), mixture of carboxymethyl cellulose and hydroxyethyl cellulose (Capitani, Del Nobile, Mensitieri, Sannino, & Segre, 2000), hydroxypropyl cellulose (Lu, Hu, & Gao, 2000), hyaluronic

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acid (Ibrahim, Kang, & Ramamurthi, 2010), K-carrageenan (Sagbas, Butun, & Sahiner, 2012) or proteins (Sereikait et al., 2003).

The present study continues our previous work on the synthesis and characterization of dextran modified with hydrophobic (alkyl) end groups, which forms in aqueous solution micelles-like associations above a critical aggregation concentration. The stability of these micelles to dilution could be significantly improved by crosslinking (Nichifor, Mocanu, & Stanciu, 2014). Our aim is the preparation of stable nanoparticles with potential biomedical applications, by the shell crosslinking of the end-modified dextran micelles. As far as we know, the synthesis of crosslinked micelles formed by end-group hydrophobically modified polymers was not reported yet. By varying crosslinking degree one can obtain different shell porosities, which will influence the capacity to encapsulate/release biomolecules. In order to improve the nanoparticle capacity to retain different biologically active molecules, the DVS crosslinked nanoparticles were further chemically modified by attachment of quaternary ammonium groups to the dextran main chain. Applicability of the synthesized nanoparticles as drug carriers was evaluated by encapsulation/release experiments performed with biomolecules of different hydrophobicity.

2. Experimental

2.1. Materials

Dextran with octadecyl end groups (D10-C18) was obtained from dextran with Mr 9000–11,000 (Sigma) by reductive end group amination, according to the procedure described in detail elsewhere (Nichifor et al., 2014). DVS, indometacin (IND), diclofenac (DCF) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were from Sigma Aldrich, alpha-tocopherol (α-TF) and ergocalciferol (ERG) were supplied by Fluka.

2.2. Methods

2.2.1. Synthesis of shell-crosslinked micelles (CM)

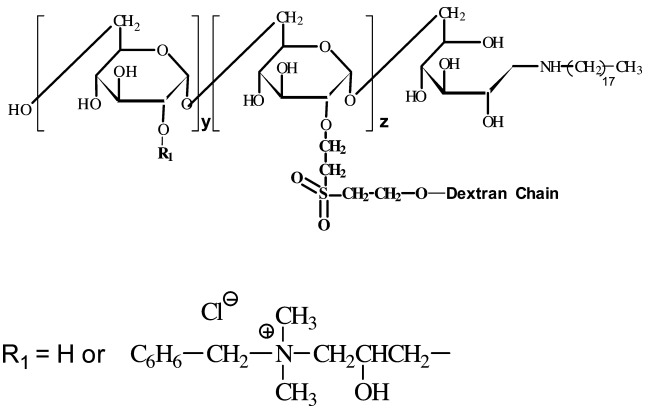
Crosslinking of micelle dextran shell was performed as follows: dry D10-C18 (0.1 g) was dispersed in an aqueous solution of pH 12 (50 mL) at room temperature. The reaction vessel was immersed in an ice bath and the mixture was stirred for 30 min at 700 rpm, then, the calculated amount of DVS was added and the reaction continued for 24 h at room temperature. The reaction mixture was purified by dialysis with a cellulose membrane tube (cut-off 10,000), under conductometric control. The purified reaction product was recovered by lyophilization. Crosslinking extent of the reaction was monitored by sulfur analyses, performed by an EDX—energy dispersive X-ray method. The degree of substitution (DS) with DVS units was calculated with the following equation:

$$DS = \frac{162 \times S\%}{3200 - (118 \times S\%)} \quad (1)$$

where 162: molecular weight of glucopyranosic unit; 118: molecular weight of DVS; *S*%: sulfur content (g%).

Chemical modification of CMs was performed by a procedure described elsewhere (Nichifor, Stanciu, & Simionescu, 2010). Two cationic derivatives were obtained, namely CMQ10 and CMQ17, having 10 and 17 moles% *N*-(2-hydroxypropyl)-*N*,*N*-dimethyl-*N*-benzylammonium chloride groups, respectively.

Chemical structure of the obtained crosslinked micelles is presented in Scheme 1.



Scheme 1. Chemical structure of dextran (or its cationic derivative) hydrophobically modified with octadecyl end groups, crosslinked with divinyl sulfone.

2.3. Characterization of crosslinked micelles

Hydrodynamic diameter and size distribution of crosslinked micelles were determined by dynamic light scattering (DLS) measurements (Malvern Zetasizer NS-Malvern Instruments, UK), using solutions/suspensions with 0.2 g/dL concentration. The reported data are means of 5 separate measurements.

2.3.1. Transmission electron microscopy (TEM)

The size and morphology of dried micelles were evidenced by a HITACHI T7700 transmission electron microscope operated at 120 kV in high resolution mode. Samples with a concentration of 0.2 mg/mL were dropped onto a carbon-coated copper grid, letting the polymer be absorbed for 3 min, followed by filter paper blotting to remove the excess solution. Staining was performed by exposing the samples to 2 wt% aqueous phosphotungstic acid (PTA) for 30 s, followed by filter paper blotting. The samples were dried overnight under ambient conditions and imaged the following day.

2.4. Biomolecules retention/release

Retention of biologically active substances was performed using different retention media, as a function of drug solubility. DCF was dissolved in water, IND in ethanol/water mixture (8/2, v/v), while pure ethanol was used for the preparation of α-TF and ERG solutions. The drug solution with a known concentration (20 mL) was added to 20 mg of either dry particles (in case of DCF and IND) or pre-swollen particles in 2 mL water (for α-TF and ERG). The mixtures were stirred for 48 h at ambient temperature, then they were introduced in dialysis tubes and dialyzed against water (for DCF) or against water/methanol (1/1 v/v) mixture (for IND, α-TF, ERG), until no drug was detected in external fluid by UV measurements performed at 276 nm (DCF), 319 nm (IND), 292 nm (α-TF), and 265 nm (ERG). Finally, the particles were recovered by freeze–drying and the amount of loaded drug was determined by UV measurements of ethanol/water (8/2) suspensions. Drug content was expressed as mg drug/g support. Schematic representation of drug loaded micelles is presented in Scheme 2.

Drug release rate was followed by dialysis. A mixture of drug-loaded particles (30 mg) and PBS 0.1 N, pH 7.4 (3 ml) was placed in a dialyzing tube (cellulose acetate membrane, cut-off: 10,000) and 30 mL PBS was used as an external solution. At pre-determined time intervals, the external solution was removed and replaced with an equal volume of fresh buffer. Each removed solution was analyzed by UV for quantification of the released drug. Cumulative amount of drug release was reported for each drug. For hydrophobicdrugs: IND, α-TF and ERG PBS contained 0.1 g% Tween 80, an emulsifying