Self –Assembled Nanoparticles of Dextrin Substituted With Hexadecanethiol

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ABSTRACT

A new amphiphilic molecule, dextrin-VA-SC_{16} ($dexC_{16}$) was synthesized and analysed in this work. $DexC_{16}$ has a hydrophilic dextrin backbone with grafted acrylate groups (VA), substituted with hydrophobic 1-hexadecanethiol (C_{16}). A versatile synthetic method was developed allowing to control the dextrin degree of substitution with the hydrophobic chains (DS_{C16}, number of alkyl chains per 100 dextrin glucopyranoside residues). Materials with different DS_{C16} were prepared and characterized using ^{1}H NMR. $DexC_{16}$ self assembles in water through association of the hydrophobic alkyl chains, originating nanoparticles. The nanoparticles properties were studied by dynamic light scattering (DLS).

Keywords: nanoparticles, dextrin, micelles, amphiphilic, self-assembling

1 INTRODUCTION

Amphiphilic molecules, such as surfactants or lipids, spontaneously self-assemble in water, forming self-aggregates, such as micelles, bilayer membranes, tubes and vesicles. Amphiphilicity of biopolymers is one of the most important factors for their self-organization in water. Among the different types of amphiphilic polymers, water-soluble polymers with hydrophobic molecules grafted on side chains have received special attention. By self-assembling, the hydrophobic segments are segregated from the aqueous exterior, to form an inner core surrounded by hydrophilic chains. Polymeric micelles or nanoparticles with hydrophobic core and hydrophilic shell are thus prepared. This kind of structure is suitable for trapping hydrophobic substances, such as fluorescent probes [1], proteins [2], and hydrophobic pharmaceuticals [3]. Size, density and colloidal stability of nanoparticles can be controlled, by changing the degree of substitution of hydrophobes and its hydrophobicity [4]. The association mechanism is mainly governed by the alkyl side chain concentration and length, and is little influenced by the molecular weight of the polymer backbone [5]. However, with low-molecular weight polymers, the hydrophobic aggregates are not connected via the polymer backbone [6]. The study of nanogel (hydrogel nanoparticles) has intensified during the last decade due to enormous potential applications in the development and implementation of new environmentally responsive materials, biomimetics, biosensors, artificial muscles and drug delivery systems [7]. Solid nanoparticles made from biodegradable polymers have been widely investigated for long-term delivery of drugs [8]. They can potentially provide benefits such as increased therapeutic effect, prolonged bioactivity, controlled release rate, and finally decreased administration frequency, thereby increasing patient compliance.

In this work, dextrin derivatives were used for the preparation of self-assembled hydrogel nanoparticles, a new system reported in a previous work. The hydrophobized polysaccharide form relatively monodisperse and colloidally stable nanoparticles (5-20nm), in water, upon self-aggregation. The nanoparticles characteristics depend on the polymer concentration, degree of substitution with hydrophobic chains (C_{16}), ionic strength and additives. In the present work, the influence of the degree of substitution is studied.

2 EXPERIMENTAL SECTION

2.1 Materials

Dextrin-VA was synthesized as described by Ferreira *et al.* [9], with few modifications. In this work, dextrin-VA with 20 acrylate groups per 100 dextrin glucopyranoside residues (DS_{VA} 20%) was used. Dimethylsulfoxide (DMSO), triethylamine (TEA) and deuterium oxide (D_2O) were from Aldrich. Regenerated cellulose tubular membranes, with 3500 MWCO, were obtained from Membrane Filtration Products.

2.2 Synthesis of $DexC_{16}$

Dextrin-VA and 1-hexadecanethiol were dissolved in dimethylsulfoxide (equivalent VA = 0.058 M). Different

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molar percentages of 1-hexadecanethiol (10, 20, 40, 60, 100 % relatively to VA) were added to the reaction mixture in order to obtain different levels of grafting. Triethylamine (1 molar to VA) was added to the reaction mixture. The medium was stirred for 24h, at 50ºC. The mixture was dialysed for 48h against water, with frequent water change. After freezing, the mixture was lyophilized and stored.

2.3 Sample Preparation

Lyophilized dexC_{16} was dissolved in water under stirring at 50ºC, and then further sonicated for 20min until a clear solution was obtained. The degree of solubility of dexC_{16} depends on the degree of substitution. Increasing the degree of substitution reduces the solubility.

2.4 Dynamic Light Scattering (DLS)

The size distribution, zeta potential and nanoparticle weight were determined with a Malvern Zetasizer, MODEL NANO ZS (Malvern Instruments Limited, UK), using a He-Ne laser (wavelength of 633 nm) and a detector angle of 173º. Size distribution and zeta potential were determined by dynamic light scattering (DLS) and the nanoparticle weight was determined by static light scattering (SLS).
For size distribution measurements, a dispersion of nanoparticles in ultra-pure water or PBS buffer (1 mL) was analysed at 25ºC in a polystyrene cell. The concentration of nanoparticles was adjusted by dilution with ultra-pure water of concentrated nanoparticle dispersion.

For zeta potential measurements, the aqueous solutions of nanoparticles at different pH values were obtained by dissolving dexC_{16} DS_{C16} 6.1% (0.1 g/dL) in phosphate-citrate buffer (pH 2.2-8.0). Each sample was analysed in a folded capillary cell. The zeta potential values were calculated using the Smoluchowski equation. Repeated measurements were performed (3 times) and the values reported are average values.

The DLS cumulants analysis provides the characterization of a sample through the mean value (z-average) for the size, and a width parameter known as the Polydispersity, or Polydispersity Index (PdI). The z-average diameter is the mean hydrodynamic diameter, determined from the intensity of scattered light. It is calculated using the cumulants analysis as defined in the International Standard ISO13321. It is comparable with other techniques only when the sample is monomodal, spherical and monodisperse, and when the sample is prepared in the correct dispersant. For samples with moderately high width (PdI>0.1), the z-average and polydispersity can be used only for comparative purposes. For broader distributions, where the polydispersity is over 0.5, it is unwise to rely on the z-average mean, and a distribution analysis should be used to determine the peak positions. Although the fundamental size distribution generated by DLS is an intensity distribution, this can be converted, using Mie theory, to a volume distribution. This volume distribution can also be further converted to a number distribution. However, number distributions are of limited use as small errors in gathering data for the correlation function will lead to huge errors in distribution by number.

2.5 ^{1}H NMR

Lyophilized dexC_{16} was dispersed in deuterium oxide (10mg/mL). Solutions were transferred to 5 mm NMR tubes. 1D ^{1}H NMR measurements were performed with VARIAN UNITY PLUS 300 spectrometer operating at 299.94 MHz. 1D ^{1}H NMR spectra were measured at 298 K with 80 scans, a spectral width of 4800 Hz, a relaxation delay of 1 s between scans and an acquisition time of 3.75 s.

3 RESULTS AND DISCUSSION

3.1 Production of the amphiphilic dextrin

The reaction between the thiol moiety and the acrylate group of dextrin-VA is a conjugate addition, with thiol acting as a nucleophile. Although the reaction was detected in the absence of base, the addition of a base (e.g. TEA) substantially increases the reaction rate, as described ahead. ^{1}H NMR was used to analyse the structure of the reaction product (Figure 1).

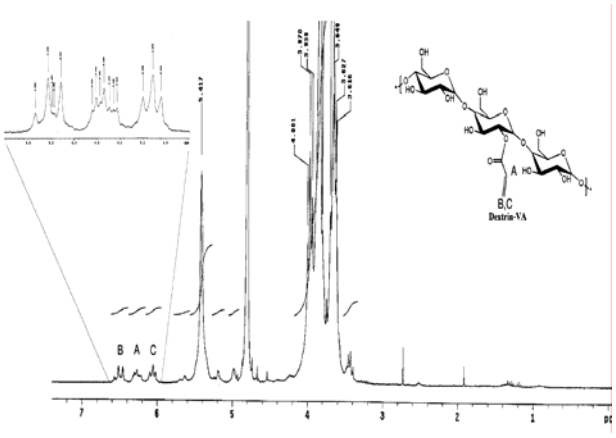

Figure 1: ^{1}H NMR spectra of dextrin-VA (DS_{VA} 20%) in D_2O at 25ºC.

The signals between 5.8 and 3.0 ppm, in the ^{1}H NMR spectrum of dextrin-VA, are assigned to protons from the dextrin scaffold. The protons from the acrylate group, attached to the dextrin backbone are observed between 6.6 and 6.0 ppm. The presence of two different positional isomers in dextrin-VA was revealed by the ^{1}H-^{1}H COSY

and ^{1}H-^{13}C HMQC spectra. The two positional isomers are located at positions 2 and 3 in the glucopyranosyl residues, in the main dextrin backbone. Due to the limited mobility of the alkyl hydrophobic chains inside of the nanoparticles, the shape and the width of the ^{1}H NMR signals assigned to the alkyl chains protons (2.0 - 0.6 ppm) are dependent on the solvent (D_2O/$DMSO_d$) used to record the ^{1}H NMR spectra. The ^{1}H NMR signals assigned to the methyl (0.8 ppm) and methylene (1.1 ppm) groups are sharp in DMSO, but progressive broadening at the base is noticeable as the percentage of water in D_2O/DMSO mixtures increases. In pure D_2O extensive broadening is noticeable. The shape of the ^{1}H NMR alkyl chain signals in deuterated water is characteristic of a superposition of peaks representing a collection of chemically identical species, yet possessing various degrees of mobility.

This result suggests that alkyl chains have different environments when dispersed in water. Some chains might be involved in hydrophobic microdomains (low mobility), others remaining exposed to the hydrophilic solvent (high mobility). In DMSO, all hydrophobic chains are exposed to the solvent, having the same mobility.

The synthesis of $dexC_{16}$ with different degrees of substitution was accomplished by varying the molar ratio 1-hexadecanethiol/VA in the reaction mixture. The ^{1}H NMR spectra of $dexC_{16}$, in deuterated water, was used to determine the degree of substitution obtained (DS_{C16}, amount of alkyl chains per 100 dextrin glucopyranoside residues). DS_{C16} values in the range from 0-70% were obtained.

3.2 Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) studies were done using a Nano-ZS (Malvern) instrument. Solutions with different concentrations of $dexC_{16}$ (DS_{C16} 7%) were analysed. DLS provides valuable information on the homogeneity of the dispersion. A single sharp peak in the DLS profile implies the existence of a single population of particles, with a size in the range 12-20nm.

As can be seen in Figure 2, $dexC_{16}$ dispersion is neither monomodal nor monodisperse. Therefore, the z-average can not be interpreted as an absolute value. In order to obtain a representative size value, we will consider the main peak of the volume distribution analysis as the best approach to the actual nanoparticles size. As a matter of fact, the intensity distribution is rather influenced by the presence of larger particles, while the volume distribution provides a better approach to characterize the more representative fraction of the samples.

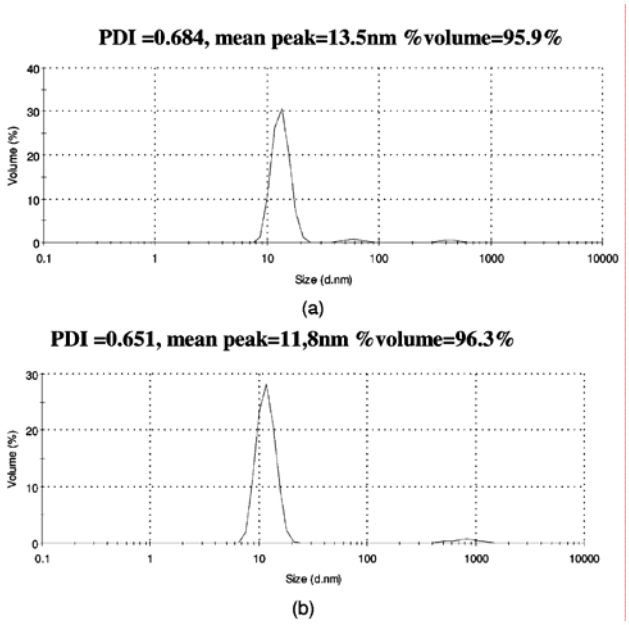

Figure 2: Size distribution of dexC16 (DSC16 7%) aqueous dispersion (a) 0.01 and (b) 0.1 g/dL.

3.3 Influence of pH, urea and ionic strength

The magnitude of the zeta potential gives an indication of the stability of the colloidal system. If all the particles in suspension have a large, negative or positive, zeta potential then they will repeal each other and the particles do not aggregate. However, if the particles have a low zeta potential (close to zero), then there is no electrostatic force to prevent the particles to aggregate. The most important factor that affects zeta potential is pH. To explore the influence of pH conditions on the particle properties, a study was performed by varying the solution pH. The variation of hydrodynamic diameter and zeta-potential of nanoparticles with the pH of mediums was studied.

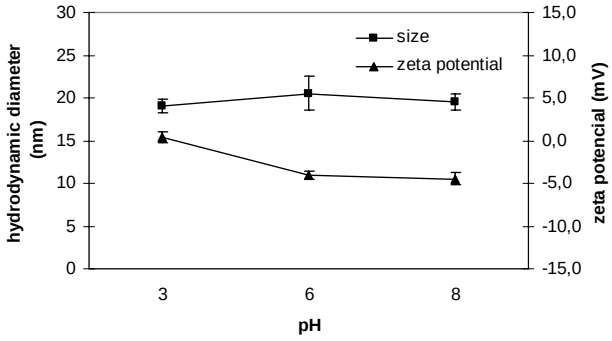

Figure 3: Particle size and zeta potential of $dexC_{16}$ 6.1% (0.1g/dL) as a function of solution pH.

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In the present study we report the synthesis of a hydrophobized dextrin polymer, dexC_{16}. The synthesis method is versatile, as it allows controlling the degree of substitution with hexadecanethiol, and therefore fine tuning the properties of the materials. DexC_{16} self-aggregates in water, originating colloidally stable (over 2 months) nanoparticles with a narrow size distribution. A diameter of about 20 nm was determined by DLS and AFM. Reaction of dextrin-VA with other (thiol) nucleophiles might open access to a variety of amphiphilic materials with tailored properties.

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