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Self-assembly in aqueous solution of amphiphilic graft copolymers from oxidized carboxymethylcellulose

Eduardo Bordallo[a,c,d,*], Jacques Rieumont[b], Marcio José Tiera[c], Manuel Gómez[d], Massimo Lazzari[d,*]

[a] Cuban Research Institute of Sugar Cane, Havana, Cuba
[b] Faculty of Chemistry, Havana University, Havana, Cuba
[c] Department of Chemistry and Environmental Science, Institute of Biosciences, Humanities, and Exact Science (IBILCE), San Paulo University (UNESP), San Paulo, SP, Brazil
[d] Center for Research in Biological Chemistry and Molecular Materials (CIQUS), University of Santiago de Compostela, A Coruña, Spain

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ABSTRACT

A series of oxidized carboxymethylcellulose-*graft*-poly(ethylene glycol)-dodecylamine (OCMC-*g*-PEG-DDA) was prepared by using an appositely prepared PEG with terminal amino groups and different amounts of DDA. The nanoaggregates formed in aqueous solution were characterized by surface tension measurements, fluorescence spectroscopy, dynamic light scattering (DLS) and scanning electron microscopies (SEM and TEM). The micelles showed narrow hydrodynamic size distributions and diameters varying from 163 to 193 nm depending on the ratio of DDA to PEG chains. The DDA content in the graft copolymers also affected the core–shell interfacial compactness.

1. Introduction

Block and graft copolymers with amphiphilic character are known to assemble in selective solvents into polymeric micelle-type aggregates. In comparison with surfactant behavior, polymers generally form more stable micelles with remarkable lower critical micellar concentration (cmc). In aqueous solution the hydrophobic moieties of the polymer self-aggregate themselves by means of intra and intermolecular association giving clusters of hydrophobic mesodomains (Savic, Eisenberg, & Maysinger, 2006), i.e. the inner core, surrounded by a palisade of hydrophilic segments (Soo & Eisenberg, 2004). However, depending on the length of the hydrophilic block, the molecular architecture and the ratio between the number of hydrophobic and hydrophilic segments the morphology can vary from spherical micelles to rods, and, for higher molecular weight, bilayer spherical vesicles which, on the contrary, offer a hydrophilic reservoir (Hadjuk, Kossuth, Hillmeyer, & Bates, 1998; Kataoka, Harada, & Nagasaki, 2001).

Self-assembled micellar nanoparticles based on amphiphilic copolymers may permit the encapsulation of a small amount of hydrophobic bioactive agents such as specific therapeutic compounds, genes, imaging agents, etc. (Qutachi, Shakesheff, & Buttery, 2013; Li, Chen, & Liu, 2013). In addition, the use of polymers as carriers in clinical applications entails several advantages with respect to lower molecular weight delivery systems, going from a general reduced renal clearance for molecular weight >40 kDa (Yamaoka, Tabata, & Ikada, 1994) to a more specific accumulation in highly permeable vascular systems, e.g. tumor tissues, for <400 nm nanoparticles (Hashizume et al., 2000). In this context amphiphilic polysaccharide-based systems have potential in different areas because of the biocompatibility and biodegradability of the polymeric backbone. In particular, cellulose derivatives such as carboxymethylcellulose are cheap and widely used in many industrial sectors (Ali, El-Rehim, Kamal, & Hegazy, 2008), and are approved as excipient, e.g. by the U.S. Food and Drug Administration, in a variety of pharmaceutical products including oral, transcutaneous and parenteral drug administration. The design of amphiphilic polysaccharide systems has been sought through grafting, e.g.,

* Corresponding authors at: University of Santiago de Compostela, Center for Research in Biological Chemistry and Molecular Materials (CIQUS), C. Jenaro de la Fuente s/n Campus, 15706 Santiago Compostela, A Coruña, Spain.
Tel.: +34 88115723.
E-mail addresses: bordallolopez@gmail.com (E. Bordallo), massimo.lazzari@usc.es (M. Lazzari).

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hexadecylamine, cholesterol, *N*-hexadecylacrylamide, acid chloride, acyl chlorides, vinyl laurate or fatty acids anhydrides to carboxymethylcellulose (Xun et al., 2011; Rosilio, Albrecht, Baszkin, & Merle, 2000; Wei, Cheng, Hou, & Sun, 2008; Yang et al., 2008a; Yang, Kuang, Wang, Li, & Zhang, 2008b; Regia, Balaban, & Borsali, 2008; Sroková, Tomanová, Ebringenová, Malovíková, & Heinze, 2004), and polylactide or poly(ethylene glycol) (PEG) to chitosan and carboxymethyl chitosan, respectively (Wu et al., 2005; Lv et al., 2014). With regards to the use as drug nano-carrier, it is well known that PEGylation improves water solubility and stability of the system in clinical applications, minimizing clearance of the particles in the reticulo-endothelial system (Harris & Zalipsky, 1997), whereas grafting with short hydrophobic chains may facilitate the encapsulation of therapeutic agents and control micelle dimension.

In this paper we propose a novel copolymer obtained by grafting oxidized carboxymethylcellulose (OCMC) with PEG and dodecylamine (DDA), with potential application as drug delivery system. In order to improve its synthetic capability, carboxymethylcellulose was modified introducing more reactive groups, i.e. aldehydes by controlled oxidation, which may facilitate further conversion e.g. to imines (Schiff bases) with primary amines. A series of oxidized carboxymethylcellulose-*graft*-poly(ethylene glycol)-dodecylamine (OCMC-*g*-PEG-DDA) was prepared by using a 5 kDa PEG and different amounts of DDA, whereas the aggregation behavior and the characteristics of the polymeric nanoparticles formed in aqueous solution were explored by surface tension measurements, fluorescence spectroscopy, dynamic light scattering (DLS), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

2. Experimental

2.1. Materials

Sodium carboxymethylcellulose (molecular weight ~ 90 kDa, degree of substitution of 0.65–0.90, degree of polymerization around 400), was purchased from Sigma-Aldrich, Brazil. Sodium cyanoborohydride ($NaBH_3CN$), sodium periodate ($NaIO_4$), dodecylamine (DDA), methoxy-terminated PEG 5 kDa (Sigma Aldrich, USA), dimethyl sulfoxide (DMSO; Fluka) were used as received. Prior to use, the carboxymethylcellulose was purified by polymer dissolution in water for 24 h and then precipitated in methanol. All other reagents were purchased from Sigma Aldrich and used as received.

2.2. Oxidation of carboxymethylcellulose

Carboxymethylcellulose (5 g, 24 mmol of anhidroglucose units) was dissolved in 100 ml of deionized water for 24 h. $NaIO_4$ (4.9 g, 23 mmol) was then added to this solution and the mixture was vigorously stirred in the dark at 25 °C during 48 h. The resulting polyaldehyde derivative (OCMC) was precipitated from methanol, centrifuged and washed several times with methanol/water (90/10, v/v). Purified OCMC was obtained as white powder after drying overnight under vacuum at 50 °C. Aldehyde content was determined according to the literature (Zhao & Heindel, 1991).

2.3. Synthesis of amine methoxy poly(ethylene glycol)

PEG (10 g, 3.3 mmol) was dissolved in 100 ml of dry CH_2Cl_2 and 7.0 ml of dry $(CH_3CH_2)_3N$. Then 1.0 ml (13.3 mmol) of CH_3SO_2Cl was added dropwise under stirring, cooling the reaction mixture in an ice-water bath. At the end of the CH_3SO_2Cl addition, the ice bath was removed, and the reaction was stirred overnight at room temperature. The reaction mixture was washed with 100 ml of 50 mM $NaHCO_3$. The organic fraction was dried over $MgSO_4$ and filtered. After solvent evaporation the reaction product, PEG-SO_3CH_3, was immediately dissolved in 50 ml of a concentrated solution of aqueous ammonia and was left to stir for 48 h in a sealed flask. The product was extracted twice with CH_2Cl_2, dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. PEG-NH_2 was finally recrystallized from ethanol and dried at 50 °C for 24 h (Aronov, Horowitz, Gabizon, & Gibson, 2003).

2.4. Synthesis of amphiphilic copolymers

In a typical reaction 0.5 g of OCMC, corresponding to 2.44 mmol of anhidroglucose units were dissolved in 25 ml of deionized water and completed to 50 ml with DMSO in a round-bottom flask. A mixture of 0.61 g of PEG-NH_2 (0.12 mmol) and 0.068 g (0.36 mmol) of DDA previously dissolved in 5 ml DMSO/$CHCl_3$ (50/50, v/v) was added dropwise during 30 min to the OCMC solution. The mixture was kept under stirring during 2 h, cooled to 0 °C using an ice-water bath and treated with an excess of $NaBH_3CN$ with respect to the amino groups (0.46 g, 7.3 mmol). The mixture was allowed to reach room temperature and kept under stirring for 48 h. The product was first purified with extensive dialysis against deionized water for three days using Spectra/Pore® 3500 D cut off tubing and freeze-dried to obtain a slightly yellow powder. Unreacted PEG and DDA were extracted by washing the polymer in a Soxhlet system during 2 days, using chloroform as solvent. Finally the purified copolymer was dried under vacuum for 24 h. Following this procedure four syntheses were carried out by adjusting the DDA/OCMC molar ratios to 15, 25, 35 and 45%. The code of the copolymers refers to the initial concentration of the hydrophobic monomer (DDA) used in the grafting reaction, while maintaining a constant PEG-NH_2/OCMC molar ratio of 5% in all the series.

2.5. Characterization techniques

Fourier transformed infrared (FTIR) spectra were recorded on a Perkin-Elmer Spectrum RX-1 using KBr pellets. Five scans were recorded for each sample with a resolution of 1 cm^{-1}. Nuclear magnetic resonance (^1H NMR) spectra in D_2O/D_2SO_4 were recorded at 25 °C on a Bruker Inova-500 operating at 500 MHz, preparing the samples as described in the literature (Ho & Klosiewict, 1980). Molecular weight and their distributions were determined by size exclusion chromatography (SEC) in phosphate buffer, pH 7.4 and ionic strength 150 mM aqueous solutions. A PL-GPC 50 integrated system (Agilent) equipped with a refractive index detector and a manual injector via integrated Velco six port, two position valve with a 100 μl loop volume, and two PL aquagel-OH 40 and 30 columns in series (300 × 7.5 mm, particle size 8 μm) was used to optimize the separation conditions. The flow rate was set at 1 mL min^{-1} and the analysis were performed at room temperature injecting 1% (w/v) sample solutions filtered with 0.45 μm membrane syringe filters (Millex-GV, Millipore). Column calibration was carried out with PEG narrow distribution standards, using a third order polynomial equation obtained from regression analysis.

Surface tension measurements were conducted using the Du Noüy ring technique in a semi-automatic tensiometer Tensiomat 21 (Fischer Scientific) at 25 °C. The derivatives from stock solutions at 5.0 g L^{-1} were added to deionized water under magnetic stirring and the surface tension measured after each addition. The reported values correspond to the average of three independent experiments.

Fluorescence measurements were performed in a Cary Eclipse fluorescence spectrophotometer. Pyrene from a stock solution (1×10^{-3} M, in methanol) was added to the polymer solutions (1.0 g L^{-1}) to obtain a 5.0×10^{-7} M solution, vigorously shaken and allowed to equilibrate for 24 h, before measurements were obtained. The I_1/I_3 ratio of pyrene fluorescence spectra was used