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Self-assembly and emulsification of dopamine-modified hyaluronan

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ABSTRACT

Dopamine modified hyaluronan (HA-DOPA) with different grafting degree were synthesized and self-assembled into nanoparticles. The effects of pH and salinity changes on the structure of the HA-DOPA nanoparticles were investigated. Nanoparticles swelled under increased pH values, and disassociation started at pH values above 6.20. At a fixed pH of 6.20, the size of the nanoparticles decreased with increased salinity, and flocculation occurred when the salt concentration was higher than 0.3 M. The emulsification performance of HA-DOPA nanoparticles at different pH values and salt concentrations demonstrated that nanoparticles with moderately swollen structures possess better emulsifying efficiency and emulsion stability. Additionally, HA-DOPA nanoparticles were able to stabilize various types of oils. These results suggest that HA-DOPA nanoparticles have promising potential as Pickering emulsifiers for applications in the cosmetics, medical, and food industries.

1. Introduction

The development of "Ramsden" or "Pickering" emulsions (Pickering, 1907; Ramsden, 1903) has received much attention since they were discovered a century ago. Compared with conventional surfactant systems, particulate emulsifiers have enhanced oil/water interface stability and lower toxicity (Binks, 2002; Aveyard, Binks, & Clint, 2003). One popular particulate emulsifier is inorganic nanoparticles, including silica nanoparticles (Binks & Lumsdon, 2000), barium sulfate nanoparticles, and calcium carbonate nanoparticles (Schulman & Leja, 1954). The other is polymeric particles, which includes polymer-grafted silica nanoparticles (Sarbu, Sirk, Lowry, Matyjaszewski, & Tilton, 2005; Tan, Gautrot, & Huck, 2010; Wu, Zhang, Qu, Liu, & Yang, 2010), polymer-stabilized latexes (Binks & Rodrigues, 2005; Dupin, Armes, Connan, Reeve, & Baxter, 2007; Fujii et al., 2011), polymeric microgels (Brugger & Richtering, 2008; Morse et al., 2013; Ngai, Behrens, & Auweter, 2005), and polymeric micelles (Fujii, Cai, Weaver, & Armes, 2005; Fujii, Read, Binks, & Armes, 2005; Yi, Liu, Zheng, Jiang, & Liu, 2012; Yi, Sun, et al., 2012; Yi et al., 2014). Among these materials, polymeric micelles, because of their controllable structure and tailorability, have been recognized as one of the most promising candidate particulate emulsifiers.

The earliest study on polymeric micelle-based particulate emulsifiers was focused on block copolymers. For example, poly{(ethylene oxide)-*block*-(glycerol monomethacrylate)-*block*-[2-(diethylamino) ethyl methacrylate]} (PEO-PGMA-PDEA) triblock copolymer-based shell cross-linked micelles have been used as pH-responsive particulate emulsifiers for the stabilization of 1-undecanol-in-water emulsions (Fujii, Cai, et al., 2005; Fujii, Read, et al., 2005). Recently, stable water-in-chlorobenzene emulsions were obtained using single-chain nanoparticles composed of poly(methyl methacrylate)-*block*-poly(4-vinylpyridine) ($PMMA_{2250}$-*b*-$P4VP_{286}$) (Xu et al., 2014). However, the practical application of block copolymer-based particulate emulsifiers may be limited by their complicated and time-consuming synthesis processes. Therefore, micelles self-assembled from amphiphilic random copolymers with simple synthesis procedures and low costs have been developed as attractive particulate emulsifiers in recent years. Previously, we reported the synthesis and utility of amphiphilic random copolymer poly-(styrene-*alt*-maleic anhydride)-*co*-poly(7-(4-vinylbenzyloxyl)-4-methylcoumarin-*alt*-maleic anhydride) (PSMVM)-based photo-cross-linked micelles for the stabilization of Pickering emulsions (Liu et al., 2010). More recently, a poly{(styrene-*alt*-maleic acid)-*co*-[styrene-*alt*-(*N*-3,4-dihydroxyphenylethyl-maleamic acid)]} (SMA-DOPA) copolymer was synthesized through hydrophobic modification of SMA by

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DOPA, and the SMA-DOPA micelles show excellent emulsifying performance (Yi, Liu, et al., 2012; Yi, Sun, et al., 2012).

However, until now, most of the polymeric micelle-based particulate emulsifiers were prepared from synthetic polymers. Considering their potential application in the food, cosmetics, and pharmaceutical fields, it is essential to develop environmentally friendly and nontoxic natural materials-based particulate emulsifiers. Although scientists have tried using naturally occurring materials, such as viruses (Russell et al., 2005) and protein particles (Fujii et al., 2009), to stabilize Pickering emulsions, the large-scale application of these materials will be limited by their high cost and complicated synthesis process. Therefore, much interest has been shown for investigation of particulate emulsifiers using natural macromolecular polysaccharides such as chitosan, cellulose, and starch (Kalashnikova, Bizot, Cathala, & Capron, 2011; Liu, Wang, Zou, Wei, & Tong, 2012; Tan et al., 2012).

Hyaluronan (HA), a well-known linear polysaccharide involved in many biological processes, has been intensively exploited for potential applications in the fields of tissue engineering and drug delivery (Gerecht et al., 2007; Kurisawa, Chung, Yang, Shu, & Uyama, 2005). However, the liquid–liquid interface behavior of HA is seldom discussed. In this study, we developed a new kind of dopamine-modified HA-based particulate emulsifier, which was expected to combine both the advantages of an amphiphilic random copolymer-like structure and the biocompatible nature of natural macromolecules. It should be noted that dopamine is a biocompatible and naturally occurring compound found in mussel adhesive proteins. Previous research on dopamine has shown that its catechol group can form strong bonds with various organic and inorganic surfaces (Lee, Dellatore, Miller, & Messersmith, 2007); thus, it might improve the interfacial stabilization performance of HA. Herein, the preparation and characterization of dopamine–HA micelles and the effects of various environmental stimulations on micelle structural transitions and emulsification performance were carefully investigated.

2. Experimental

2.1. Materials

Sodium hyaluronate (HA, M_n = 310 kDa,) was obtained from Zhenjiang Dong Yuan Biology Technology Co. Ltd. (Zhenjiang, China). 3,4-dihydroxyphenethylamine hydrochloride (DOPA), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl), *N*-hydroxysuccinimide (NHS), and other chemicals were purchased from Aladdin Reagent (Shanghai, China). White oil, silicone oil, isooctyl palmitate, dicaprylyl carbonate, and propylheptyl caprylate were supplied by Wenhua Chem. Co., Ltd. (Shanghai, China). All chemicals were used as received without further purification.

2.2. Synthesis of DOPA-modified HA (HA-DOPA)

Briefly, the HA-DOPA were synthesized by a coupling reaction carried out at room temperature under nitrogen atmosphere in pH 5.5 aqueous solutions to avoid the irreversible oxidation of DOPA (Lee et al., 2007). First, HA (5 unit mmol), EDC·HCl (10 mmol), and NHS (20 mmol) were dissolved in 100 mL deionized water, and the pH of the solution was adjusted to 5.5 using HCl. Then, DOPA (5–50 mmol) was added into the reaction mixture. After 24 h, the reaction mixture was dialyzed using dialysis tube (MWCO: 3000 Da) against acidic water (pH ~5.5) water until all unreacted DOPA was removed, and then dialysis against deionized water for further purification. The DOPA outside of the dialysis tube was monitored by UV absorption. After purification, samples were lyophilized until dry. The obtained HA-DOPA were characterized by ^{1}H NMR analysis (Bruker DMX500 NMR 400 MHz spectrometer, at 70 °C) in D_2O.

2.3. Preparation and characterization of HA-DOPA nanoparticles

HA-DOPA nanoparticles were prepared by a direct dispersion method according to a previous report (Akagi, Piyapakorn, & Akashi, 2012). HA-DOPA were directly dissolved or dispersed in deionized water with stirring overnight and then sonicated (AS ONE corporation, VS-150) at 150 W for 2 min. The final concentration of the stock solution was 10 mg/mL. HA-DOPA nanoparticle aqueous solutions (5 mg/mL) at various pH values were prepared by diluting the stock nanoparticle solution with deionized water, and then adjusting the pH using 0.1 M HCl or NaOH. HA-DOPA nanoparticle aqueous solutions (5 mg/mL) at pH 6.20 with different salt concentrations were prepared by dissolving a calculated amount of NaCl into the nanoparticle solutions. The hydrodynamic radius (R_h) and size distribution of the HA-DOPA nanoparticles were characterized by dynamic light scattering (DLS) using an ALV-5000 light scattering spectrometer equipped with a He–Ne laser with a wavelength of 632.8 nm as the light source. The measurement was performed with a fixed scattering angle of 90° at 25 °C, and the scanning time was 300 s. The zeta potential of the HA-DOPA nanoparticles was measured by a Malvern Zetasizer Nano ZS90. All samples were filtered through 0.8 μm Millipore filters before measurement, and all data were averaged over three measurements. The morphology of HA-DOPA nanoparticles was observed by a JEOL JEM-2100 (HR) LaB6 transmission electron microscope (TEM) operating at an accelerating voltage of 200 kV. A drop of each diluted nanoparticle aqueous solution was placed on a carbon coated copper grid. After drying, the samples were directly observed without further staining.

2.4. Critical aggregation concentration of HA-DOPA

The critical aggregation concentrations (CAC) of HA-DOPA were determined as described elsewhere (Akagi et al., 2012). Briefly, 10 μL of pyrene in acetone solution at a concentration of 1×10^{-4} M was added into a series of glass vessels. After solvent evaporation, 1 mL of copolymer stock solutions at various concentrations was added into the vessels. Then, the resulting mixtures were stirred at room temperature for 24 h before measurement. The final concentration of pyrene in the mixtures was 1×10^{-6} M. The steady-state fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorometer. The emission spectrum of pyrene was measured at an excitation wavelength of 335 nm at room temperature.

2.5. Emulsion preparation and characterization

Equal volumes of HA-DOPA nanoparticles and oil (white oil, silicone oil, isooctyl palmitate, dicaprylyl carbonate, propylheptyl caprylate, and toluene) were mixed and homogenized at 8000 rpm for 2 min by a XHF-D H-speed dispersator homogenizer with a 10 mm dispersing tool. In the study of the effect of pH or salinity on emulsifying performance, the pH or salt concentration of the nanoparticle aqueous solution was first adjusted using 0.1 M HCl/NaOH or adding NaCl, respectively. Then, the resulting solutions were homogenized with an equal volume of oil as described above. As determined by the drop test, reported elsewhere (Morse et al., 2013), all emulsions in this study were oil-in-water type. Emulsion droplets were observed by a Motic DM-BA450 optical microscope.