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Investigation of coumarin functionality on the formation of polymeric nanoparticles

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ABSTRACT

The effect of coumarin molecules on the formation of polymeric nanoparticles is examined using a model polymer, poly(methyl methacrylate) (PMMA), functionalized with varying amounts of coumarin pendant groups (PCM). PCM nanoparticles are prepared in a continuous manner by Flash NanoPrecipitation (FNP). PCM forms spherical nanoparticles in water, while the PMMA without coumarin functionality fails to form nanoparticles. As the amount of coumarin functionality increases, the nanoparticle size and size polydispersity are decreased and the nanoparticle stability in water is enhanced. In particular, well-isolated spherical nanoparticles are generated from PCM with 20 mol% coumarin side chain functionality. These results can be explained by an observed increase in the negative surface charge with increasing coumarin content in the polymer.

1. Introduction

Over the past decade, polymeric nanoparticles have attracted significant attention due to their promising applications in the fields of drug transport and medical imaging [1]. A major issue in drug delivery is maintaining the stability of nanoparticles in the human body for the effective delivery of a drug to the target site without reticuloendothelial (RES) clearance [2]. In fact, significant progress towards this end has already been achieved by introducing hydrophilic moieties to the nanoparticle surface, which can inhibit the adsorption of serum proteins and hemocytes, thus maintaining stable nanoparticle circulation in the blood [3–5]. Recently, interest in the controlled release of drug agents to the specific site of action at the therapeutically optimal rate and dose regimen has grown as an approach to improve drug effectiveness and limit side effects [3,6]. Several approaches have been investigated to control the rate of drug release from nanoparticles, including the use of biodegradable polymers with slow erosion [7–9], the chemical crosslinking of nanoparticles to increase their durability and hinder free-release of the drug [10–13], and the introduction of stimuli-sensitive moieties to cause burst-like drug release in response to specific triggers, such as pH [14–17], temperature [18–21], and light [22,23]. Especially, the combination of both crosslinking of polymer chains and introduction of stimuli-responsive moieties in nanoparticle systems is quite attractive because high nanoparticle durability and controlled drug release can be achieved simultaneously.

From this point of view, nanoparticle systems containing coumarin functionality are highly fascinating because reversible chemical fixation and light response can be realized concurrently. It is well-known that coumarin molecules are photodimerized via [2 + 2] cyclobutane ring formation upon photoirradiation with over 300 nm wavelength light, and the cyclobutane ring is photocleaved upon irradiation with under 290 nm wavelength light [24–27]. Zhao et al. showed that micelles composed of amphiphilic block copolymers with coumarin pendant groups were reversibly stabilized by light-induced crosslinking of the coumarin moieties [28,29]. Jiang and coworkers also studied the stabilization and morphology switching of polymer vesicles using the photoresponsibility of coumarin [30]. Yin and coworkers reported stimuli responsive copolymeric nanoparticles containing coumarin moieties and observed a sharp change in the morphology of the nanoparticles in response to light and temperature [31]. Furthermore, the intrinsic fluorescence of coumarin molecules permits the facile detection of nanoparticles containing coumarin within cells [32]. However, in spite of the usefulness of coumarin for creating advanced nanoparticle systems, the influence of coumarin functionality on the fundamental properties of polymeric nanoparticles, such as shape, size, size dispersity, and stability, has not been systematically studied. Indeed, most studies, in which coumarin was employed, have focused on the photoreversible chemical fixation of nanoparticles by coumarin dimerization and cleavage. Recently, we reported that coumarin moieties at the end of a biodegradable polyester, poly(ε-caprolactone), led to the formation of smaller, more stable nanoparticles in aqueous medium compared to poly(ε-caprolactone) without coumarin end functionality [33]. Moreover, these nanoparticles showed reversible interparticular

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assembly via the photodimerization and photocleavage of coumarin moieties on the nanoparticle surface. To the best of our knowledge, this was the first report to show that coumarin moieties can affect the formation of polymeric nanoparticles. However, further detailed and controlled studies are required to determine how coumarin affects the formation of nanoparticles with different polymer composition and structure.

Here, we investigate the effect of coumarin on the formation of polymeric nanoparticles using a traditional, non-biodegradable polymer, poly(methyl methacrylate) (PMMA) with coumarin side chain functionality. We show that coumarin has distinct effects on nanoparticle formation and morphology. Furthermore, we explore how the amount of coumarin functionality influences the size, size distribution, stability (self-life), and zeta potentials of the generated nanoparticles.

2. Experimental

2.1. Materials

Methylmethacrylate (MMA), methacryloyl chloride and 2,2-azobisisobutyronitrile (AIBN) were purchased from Sigma Aldrich Chemical Company. 7-Hydroxycoumarin, dimethylsulfoxide (DMSO, >99.7%), ethanol (99.5%), tetrahydrofuran (THF, 99.5%) and pyridine (99.5%) were purchased from Acros Organics. Prior to use, the inhibitor in MMA was removed using monomethyl ether hydroquinone (MEHQ) remover; other chemicals were used as received without any additional purification.

2.2. Synthesis of 7-methacryloyloxycoumarin (CM-MA)

Coumarin functionalized monomer, 7-methacryloyloxycoumarin (CM-MA), was prepared according to a previously reported method [34]. Briefly, a THF solution of 6.0 g (57.4 mmol) of methacryloyl chloride was slowly added to a solution of 6.0 g (37 mmol) of 7-hydroxycoumarin in 40 mL of pyridine, and the solution was stirred at room temperature for 1 h. The reaction mixture was poured into 600 mL of water, followed by collection of a white precipitate that was recrystallized from ethanol to give a colorless powder in 67% yield. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.01 (s, 3H, CH3), 5.96 (s, 1H, ethylene), 6.32 (s, 1H, ethylene), 6.49 (d, 1H, enone), 7.22 (d, 1H, aromatic), 7.36 (s, 1H, aromatic), 7.79 (d, 1H, aromatic), 8.09 (d, 1H, enone).

2.3. Polymerization of PMMAs with coumarin side functionality (PCM)

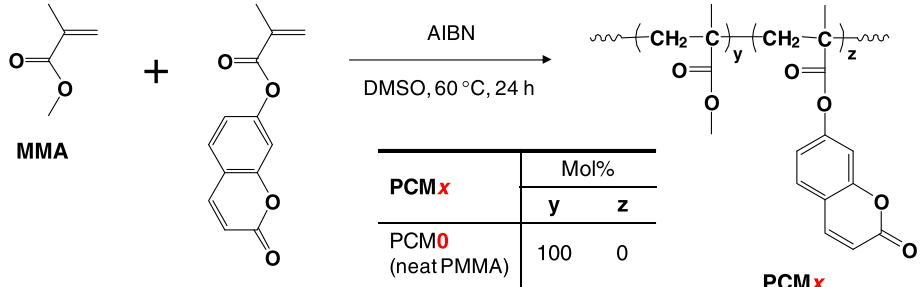
A typical procedure for the preparation of PMMA with coumarin side functionality (PCM) is as follows. 0.95 g (9.5 mmol) of MMA, 0.12 g (0.5 mmol) of CM-MA, 36 mg (0.22 mol) of AIBN, and 40 mL of DMSO were quickly added to a 50 mL round bottom flask. Then, the mixture was gently stirred at room temperature with nitrogen purging and the flask was placed in a preheated oil bath (60 °C) for 24 h. The PCM was then precipitated twice in methanol and dried *in vacuo* at 60 °C for 24 h, resulting in PCM containing about 5 mol% coumarin functionalized repeat units (Yield: 70%). PCMs with various coumarin contents were synthesized by controlling the amount of MMA and CM-MA in the feed and following the same procedure. These are denoted as PCM*x* ("*x*" indicates the mol% of CM-MA added for the reaction. For example, "PCM5" means the PCM prepared by the addition of 5 mol% of coumarin functionalized monomer). Fig. 1 shows the synthetic route for the preparation of PCM*x*.

2.4. Preparation of PCM nanoparticles

PCM*x* nanoparticles were generated using the Flash NanoPrecipitation (FNP) method. 5, 10, 20, 30, and 50 mg of PCM*x* were individually dissolved in 10 mL THF. 3 mL of each PCM*x* solution was mixed with 3 mL water in the jet stream mixer, and the mixing stream was then allowed to flow into a beaker containing 27 mL of water with mild stirring. The inner diameter of the jet stream line was 1 mm and the volumetric flow rate was approximately 0.6 mL s^{-1}. The PCM*x* nanoparticle suspensions were then collected in a glass testing jar. THF was subsequently removed through air evaporation, resulting in the aqueous suspended PCM*x* nanoparticles, denoted as PCM*x*–*m*. ("*m*" indicates the amount, in mg, of PCM*x* dissolved in 10 mL THF.)

2.5. Characterization

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded in DMSO-d_6 on a Bruker Avance-II 500 MHz spectrometer to verify the synthesis of CM-MA and PCM*x*. Gel permeation chromatography (GPC) was carried out on a Waters chromatograph connected to a Waters 410 differential refractometer. A PLgel 10 μm Mixed-B column (Agilent technologies) connected in series was used with THF as the mobile phase. The molecular weight and polydispersity values were calculated from the resulting GPC data based on calibration with polystyrene standards of known molecular weights and polydispersity indexes. Differential scanning calorimetry (DSC) analysis was performed using a TA DSC Q2000 under N_2 flow. All of the samples were first heated from 25 °C to 180 °C at 20 °C min^{-1} to erase the thermal history, then cooled to 25 °C at 20 °C min^{-1}, and finally heated to 180 °C at 20 °C min^{-1}. The morphology of the nanoparticles was visualized by field-emission scanning electron microscopy (FE-SEM, XL30 FEG-SEM). Prior to



PCM*x*	Mol%	
	y	z
PCM0 (neat PMMA)	100	0
PCM5	94.4	5.6
PCM10	89.3	10.7
PCM20	78.7	21.3

Fig. 1. Synthetic scheme for the preparation of PCM*x*.