Summer Scholar Report

Synthesis and Characterization of Functional Polymeric Nanoparticles

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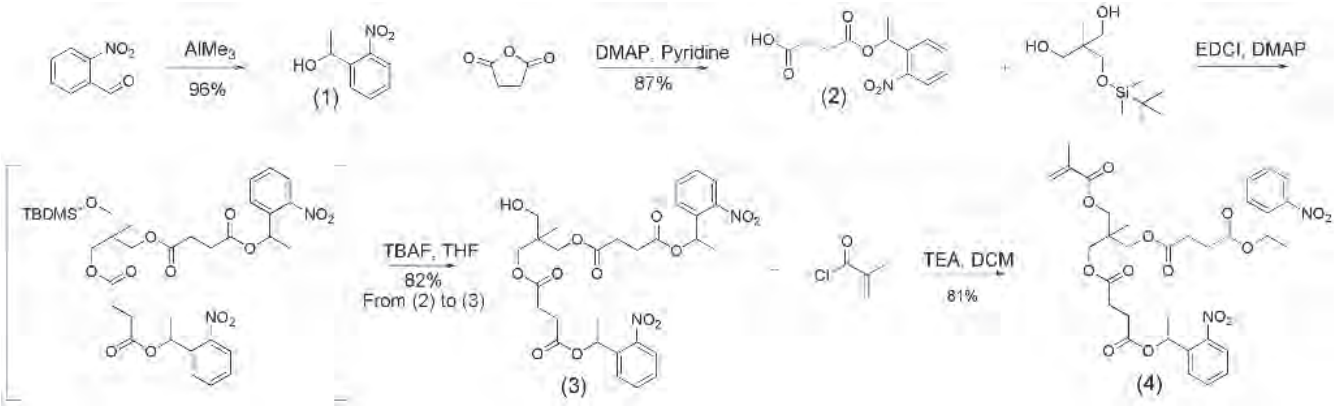
Nanoparticles are colloidal particles ranging in size from approximately 5 – 900 nm. These particles can be synthesized from a variety of materials, depending on the desired application.[1-9] In the area of drug delivery, nanoparticles are useful for addressing many of the difficulties encountered when administering therapeutic compounds. Nanoparticles can increase the solubility of hydrophobic drugs, provide a more consistent level of drug in the body through sustained release, protect sensitive drugs from low pH environments or enzymatic alteration, and, in some cases, provide local delivery or targeting of the drug to the desired tissues.[1] There are four main types of nanoparticle systems receiving considerable attention in drug delivery: drug nanocrystals, liposomes, dendrimers, and polymeric nanoparticles. To date, protein-stabilized drug suspensions, along with nanocrystals and liposomal systems, are the only nanocarriers that have received FDA approval. As our interest lies with polymeric nanoparticles, we will focus on this system.

Polymeric nanoparticles tend to be more stable than other carriers, such as liposomes, and their delivery properties can be adjusted by manipulating the structure and composition of the polymer used to prepare the particles. Thus polymeric nanoparticles may be a more favorable means to deliver chemotherapeutic agents in a post-operative setting. Although several natural and synthetic polymers have been investigated (including chitosan,[10] methacrylic acid copolymers,[11] and polycaprolactone[12]) poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) are the most widely studied due to availability, biocompatibility, and FDA-approved status. While PLA and PLGA systems are relatively safe, simple to synthesize, and have been explored for the delivery of many agents, including anti-cancer drugs, these particles afford relatively rapid "burst" release of the encapsulated drug (> 50% release in 10-48 hrs) regardless of nanoparticle location and thus may negate or reduce the benefit of using a drug delivery system.[8,13-15]

Therefore, functional systems in which delivery of therapeutic compounds can be tailored and even triggered by specific stimuli are being pursued in order to improve local drug delivery and anti-tumor efficacy.[16,17] Nanoparticles which respond to a wide array of stimuli are being investigated, including those that respond to pH[18-24], temperature[25-28], light[29-33], and ultrasound[34-37]. The nanoparticles described herein utilize a novel mechanism for drug release where the nanoparticle swells in response to the particle on going from a hydrophobic to a hydrophilic composition. This transition occurs in response to a lowering of physiological pH to 5, such as that found in the endosome. The first example of such expansile nanoparticles was reported by Griset *et. al.*, who used the deprotection of masked hydroxyl group at a mildly acidic pH of 5 to trigger this change in polymer structure and release of an entrapped drug.[3] Although the release is triggered by an environmental signal of low pH, there is not complete spatial and temporal control over payload release. In our attempt to solve this challenge of payload delivery, we have engineered a new polymeric expansile nanoparticle possessing carboxylic acids masked as photo-labile ester moieties. The esters can be cleaved upon irradiation with long-wave UV light ($\lambda \geq 365$ nm). The synthesis of the monomer and preparation of both polymeric core and aqueous-filled nanoparticles are discussed herein.

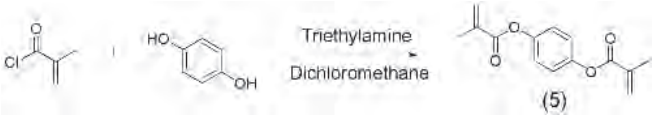
The preparation of the photo-sensitive monomer, **4**, for nanoparticle synthesis is shown in Scheme 1. Briefly, 1-(2-nitrophenyl)ethanol was added to a stirring solution of succinic anhydride to afford 4-(1-(2-nitrophenyl)ethoxy)-4-oxobutanoic acid (**2**). This was then re-suspended in dichloromethane, along with 2-((tert-butyldimethylsilyloxy)methyl)-2-methylpropane-1,3-diol and DMAP (catalytic) at 0 ºC. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) was then added to the reaction solution. After 12 hours of stirring at room temperature, the reaction solution was extracted 3 times with 0.1 M HCl and dichloromethane. The organic phases were collected, dried over sodium sulfate and concentrated. The crude product was used without further purification. The yellow viscous oil was added to a THF solution at 0 ºC, upon which it was treated with 1.0 M tetrabutylammonium fluoride (4 mmol) for 6 hours to afford '2-(hydroxymethyl)-2-methylpropane-1,3-diyl bis(1-(2-nitrophenyl)ethyl) disuccinate (**3**). Compound **3** was then treated with methacryloyl chloride and triethylamine to afford the desired photo-sensitive monomer (**4**). The composition of **4** was confirmed by ^{13}C NMR [(400 MHz, $CDCl_3$): δ17.1, 18.3, 22.0, 28.8, 29.1, 68.6, 124.5, 126.0, 127.2, 128.4, 133.6, 135.9, 137.8, 147.7, 166.9, 171.0, 171.8], ^{1}H NMR [(400 MHz, $CDCl_3$): δ 0.82 (s, 6H), 0.93 (s, 9H), 0.96 (s, 3H), 1.63 (d, 6H, J = 6.12), 1.95 (s, 3H), 2.64 (m, 8H), 3.35 (s, 2H), 3.99 (s, 4H), 5.59 (d, 1H, J = 1.2 Hz), 6.07 (d, 1H, J = 1.2 Hz), 6.38 (m, 2H), 7.42 (m, 2H), 7.67 (m, 4H), 7.85 (m, 2H)], and HR-Mass Spectrometry via a Waters QT of (hybrid quadrupolar/time-of-flight) API US System by ESI (Empircal Formula: $C_{33}H_{38}N_2O_{14}$; Exact mass: 686.23 Theoretical: 709.2221 [M+Na]; Experimental: 709.2220 [M+Na]; Error: 0.1410 ppm).

The synthesis of nanoparticles containing an aqueous core was performed in a manner adopted from Hillereau *et. al.* and these nanoparticles will be referred to as type A nanoparticles.[38] Briefly, a 14% w/w Span 80 (Aldrich) in glyceryl octanoate (Aldrich) solution was prepared (total amount of oil used was 1 gram). The oil mixture was stirred and allowed to become homogeneous, upon which 20 μL of



Scheme 1. *Synthetic scheme to monomer **4**.*

0.2 M ammonium persulfate dissolved in 80 μL of 0.1 PBS solution (Dulbecco's) was added to the oil mixture. The oil-water suspension was vortexed for 30 seconds to create a water-in-oil emulsion. Then, 40 mg of (**4**) and 1% w/w crosslinker (**5**) were dissolved in ethyl acetate was added to the mixture. The crosslinker was synthesized as shown in Scheme 2.



Scheme 2. *Synthetic scheme to the crosslinker **5**.*

Tetramethylethylenediamine (co-initiator) was also added with brief vortexing, and the emulsion was allowed to stir overnight to allow polymerization at room temperature. The emulsion was then partitioned into 350 μL portions. Each portion was re-suspended in 650 μL of 0.1 PBS buffer and centrifuged at 13000g for 40 minutes. The oil layers were aspirated. The aqueous layer and nanoparticle pellet were combined and dialyzed for 48 hours. As these photo-sensitive nanoparticles were prepared using a water-in-oil emulsion, they contain a polymeric shell and an aqueous core. By dynamic light scattering (Brookhaven Instruments, Inc. 90 Plus), there were two major populations of nanoparticles in solution one centered with a diameter of 145 nm

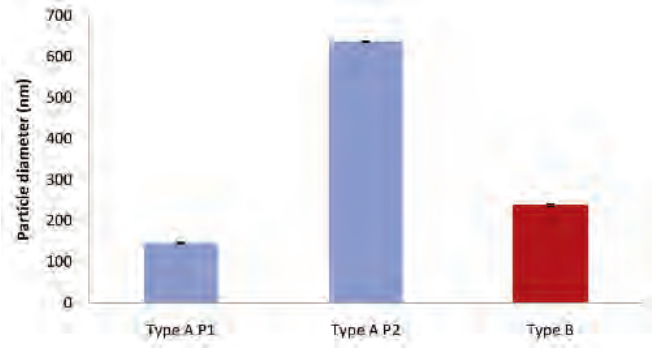


Figure 1. *Average diameter of the nanoparticles as determined by DLS. The solution of type A nanoparticles (blue) contained two populations (P1 & P2) whereas a single population was observed for type B nanoparticles (red).*

(PDI 0.19) and the other with a diameter of 637 nm (PDI 0.24) (Figure 1). As shown in Figure 2 top, scanning electron microscopy of the nanoparticles revealed spherical structures between 100 and 300 nm.

Next, we prepared nanoparticles with a solid hydrophobic polymeric core following the protocol from Griset *et. al*.[39] and these particles are referred to as Type B. Briefly, the photo-sensitive monomer, **4**, (50 mg) and crosslinker (**5**) (1% w/w) were dissolved in 0.5 mL of dichloromethane. The crosslinker was synthesized as shown in Scheme 2. This "oil" phase was suspended in 2 mL of 5 mM Tris buffer containing 10% w/w sodium dodecylsulfate. The oil-in-water suspension was subject to 10 minutes of sonication at 80 W with a 1 second pulse, 2 second delay under argon atmosphere. Upon completion of sonication, the emulsion was subject to vigorous stirring while 20 μL of 0.2 M ammonium persulfate and 4 μL of tetramethylethylenediamine were added. The system was left stirring overnight, upon which it was subject to dialysis for 24 hours in 5 mM PBS buffer to remove excess surfactants. Dynamic light scattering measurements showed nanoparticles with an average diameter of 238 nm (DPI 0.02) (Figure 1). Scanning electron microscopy showed spherical as well as elongated particle structures of approximately 250 nm in size (Figure 2-bottom).

To investigate the chemical reaction occurring with photolysis, LCMS was performed on the Type B nanoparticle solution after irradiation (Agilent LC/MSD VL system by electrospray (ESI) using a reverse-phase C18 Zorbax Eclipse 2.1 × 50 mm column (Agilent). Mobile phases were water and acetonitrile with 0.1% formic acid. Both an irradiated and non-irradiated nanoparticle suspension (40 μL of NP in 2 mL of 0.1 PBS), after filtration through a 0.02 μm syringe filter, were subject to LCMS analysis and only the irradiated sample showed the photo-degraded byproduct 1-(2-nitrosophenyl)ethanone [M_2+H]. Such mass peak was not evident in the control, non-irradiated sample. The observed photo-degradation product is consistent with that reported in the literature for this reaction.[40] Similar studies with Type A nanoparticles are ongoing.

In summary, two polymeric nanoparticles have been

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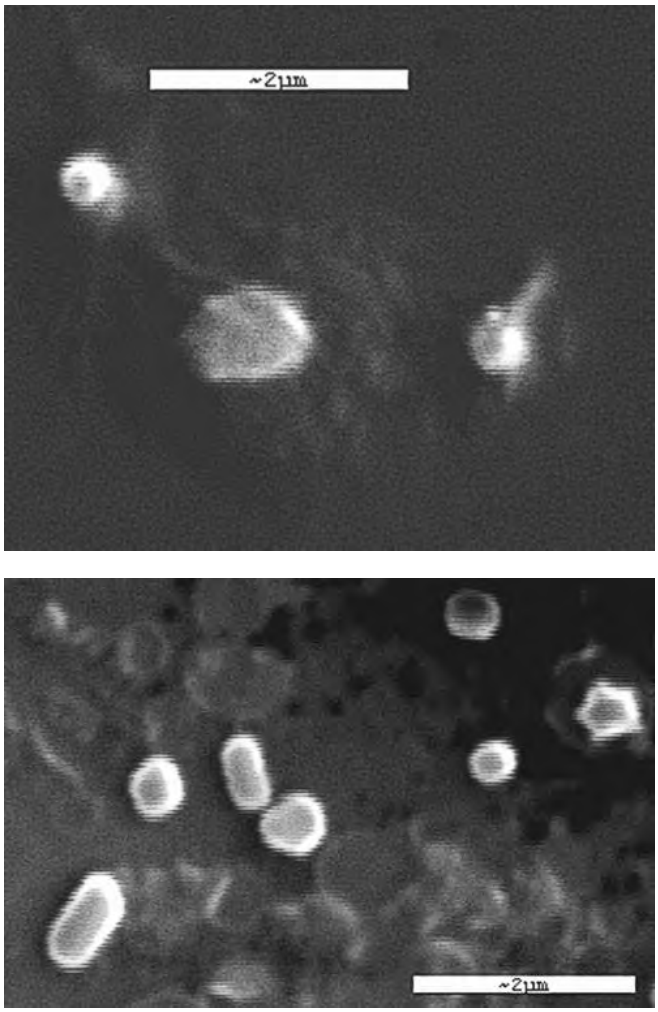

Figure 2. *Scanning electron micrograph of type A (**top**) and type B (**bottom**) nanoparticles.*

synthesized and characterized by dynamic light scattering and scanning electron microscopy. The nanoparticles were synthesized using two different emulsification/polymerization methods, with the first giving aqueous core nanoparticles (or nanocapsules) and the second giving solid polymeric core nanoparticles. The mass spectrometry studies showed the photolysis products were being formed. Further experiments are planned to characterize the nanoparticles and to examine the effects of irradiation on nanoparticle size and structure, as well as to monitor the release of an entrapped drug. Continued research on different types of nanoparticles will provide new materials for evaluation in *in vitro* and *in vivo* models of biological and clinical relevance.

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