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Drug nanoparticles by emulsion-freeze-drying *via* the employment of branched block copolymer nanoparticles

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ABSTRACT

A large percentage of drug compounds exhibit low water solubility and hence low bioavailability and therapeutic efficacy. This may be addressed by preparation of drug nanoparticles, leading to enhanced dissolution rate and direct use for treatment. Various methods have been developed to produce drug nanocrystals, including wet milling, homogenization, solution precipitation, emulsion diffusion, and the recently developed emulsion freeze-drying. The drawback for these methods may include difficult control in particles size, use of surfactants & polymer, and low ratio of drug to stabilizer. Here, biocompatible branched block copolymer nanoparticles with lightly-crosslinked hydrophobic core and hydrophilic surface groups are synthesized by the direct monomer-to-particle methodology, characterized, and then used as scaffold polymer/surfactant to produce drug nanoparticles *via* the emulsion-freeze-drying approach. This method can be used for model organic dye and different poorly water-soluble drugs. Aqueous drug nanoparticle dispersions can be obtained with high ratio of drug to stabilizer and relatively uniform nanoparticle sizes.

1. Introduction

A report published in 1988 demonstrated that of all pharmaceutical drugs produced in the UK over a timeframe of 20 years, 40% exhibited poor bioavailability [1]. New and improved screening methods can now predict and eliminate some drug candidates with low bioavailability, before going into testing [2]. Still a report published in 2004 [3] reported that 17.1% of all *essential drugs* defined by the World Health Organization (WHO) could be classified as BCS II drugs (high permeability and low solubility) and 10.6% as BCS IV drugs (low permeability and low solubility), as defined by Amidon et al. [4]. Low bioavailability is the direct consequence of low water solubility for a large percentage of drugs, particularly for BCS II drugs. A promising approach to enhance solubility of poorly water-soluble drugs is nanosizing technologies, with the particle sizes in the range of 10 to 1000 nm [5]. Reducing particle size to nanoscale range enhances both the saturation solubility as described in the Oswald–Freundlich equation [6,7] and the dissolution rate as shown in the Noyes–Whitney equation [8].

Both top-down and bottom-up routes have been reported to form nanoparticles. In the top-down process, larger drug particles are downsized by mechanical methods, *e.g.*, grinding (wet-media milling) [9–12], or by the application of pressure (Piston-Gap) [13–15]. Top-down processes, although used more in industry [16], have disadvantages of being time and energy inefficient, difficult to produce small nanocrystals and control particle size distribution, and not applicable for hard crystalline drugs without pre-treatment. More than one cycle of operation is often required, prone to introducing impurities from solvent or milling material [17,18]. In bottom-up processes the nanoparticles are formed from solution, whereby a better control of the crystallization process can lead to smaller particles with narrower particle size distribution. The main obstacle in bottom-up approach is repressing and stabilizing against Ostwald-Ripening. A variety of bottom-up methods have been described and excellent reviews can be found accordingly [18,19]. Established and industrially applied approaches [19] include solvent–antisolvent precipitation (SAS) [20–22], with its variation of high gravity reactive precipitation (HGRP) [23,24], supercritical fluid precipitation [25–28] and spray drying [29–31]. Although these techniques have certain advantages, like easy handling (SAS), almost no solvent residue (supercritical fluid) and are more cost and energy efficient, problems in stabilizing particle suspensions remain. A solution to this problem, while still maintaining the advantages of bottom-up processes, is to rapidly freeze the drug solution and arrest particle growth due to freezing. In freeze-drying a solution is frozen and the solvent is subsequently removed in a freeze-dryer under vacuum [32–34]. In spray freeze drying, the dissolved material is sprayed into liquid nitrogen for downsizing and subsequently freeze dried. This is mainly used for preparation of protein particles, which would denature under harsh conditions [35–37].

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We previously reported the use of emulsion freeze-drying to form organic or drug nanoparticles *in situ* in water-soluble porous polymer. The polymer scaffold prevents the nanoparticles from aggregation in the solid state, ensuring a long storage time. The nanoparticles can be readily released by dissolving the polymer scaffold in water to produce aqueous nanoparticle dispersion [38]. Both polymer (*e.g.*, poly(vinyl alcohol)) and surfactant (*e.g.*, sodium dodecyl sulphate) are required to form the emulsions, produce the porous scaffold, and stabilize the nanoparticles in aqueous suspensions. It is possible to generate porous polymer by freeze drying and then employ a solvent evaporation approach to form organic/drug nanoparticles directly in the porous polymeric scaffold [39,40]. Aqueous nanoparticles dispersion can be prepared similarly. In both approaches, the use of both polymer and surfactant is important in forming stable aqueous nanoparticle dispersions. This, however, can result in low loading of drug compounds in the formulations. A formulation that utilizes a biocompatible polymer acting both as scaffold and surfactant would be advantageous in improving drug loading and reducing the formulation complexity (*e.g.*, in assessing biocompatibility).

We have also reported synthesis of super-lightly crosslinked branched copolymers and a new *direct* monomer-to-particle synthetic strategy based on these copolymers, which could be applied in drug delivery [41–43]. After a simple dialysis process to generate isolated *macromolecular species*, well-defined uni-molecular polymer nanoparticles can be obtained directly. This *de novo* synthetic approach differs significantly from the reported arm-first or core-first core-crosslinked star-polymer synthesis where the core is effectively a highly cross-linked microgel formed by the addition of a large volume of cross-linkers such as divinylbenzene at the end of the polymerization [44–46]. In contrast, polymer nanoparticles were prepared from discrete soluble molecular species (soluble branched copolymers) which have been synthesized by a controlled branching strategy. Utilizing this strategy, it was possible to prepare amphiphilic materials with defined nanoparticle shape by a one-pot, concerted growth process rather than joining of pre-formed spheres [41,42]. The lightly crosslinked core could offer the obtained polymer nanoparticles with larger loading capacity of guest compounds. And the stability of the nanoparticles was very high (*e.g.*, up to one year maintaining the size and shape). This synthetic methodology may be easily scaled-up as we demonstrated previously, even with the possibility to be extended in the synthesis of hyperbranched polydendrons [47].

Herein, we demonstrated for the first time that the branched copolymer nanoparticles (BCN) could be used to form stable emulsions without other additives. The branched copolymers applied here were the biocompatible poly(ethylene glycol)-b-(*N*-isopropylacrylamide) (PEG-PNIPAM). The formed oil-in-water (O/W) emulsions with hydrophobic dyes or drug compounds dissolved in the oil-droplet phase were freeze-dried to form nanoparticles *in situ* within the PEG-PNIPAM scaffold, which can then be readily dissolved in water to produce aqueous nanoparticles dispersions.

2. Experimental

2.1. Chemicals and reagents

Deionized water was prepared using an AquaMAX-Basic 321 DI water purification system. Oil Red O (OR) dye content ≥75%, ketoprofen ≥98% (TLC), ibuprofen ≥98% (HPLC), indomethacin ≥99% (TLC), *o*-xylene ≥98% (GC), sodium acetate, *N*-isopropylacrylamide (NIPAM, 97%), and dodecanethiol (98%) were purchased from Sigma-Aldrich. Macro-azo poly(ethylene glycol) initiator was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Cyclohexane (extra pure) and *o*-xylene were purchased from Fisher scientific and VWR international respectively. All other solvents were reagent grade and purchased from Sigma-Aldrich. All chemicals were used as received.

2.2. Synthesis of crosslinked branched poly(ethylene glycol)-b-poly(N-isopropylacrylamide) (PEG-PNIPAM)

2.2.1. Synthesis of ethylene diacrylamide

Ethylene diamine (1.2 g, 20 mmol, 1 eq) and sodium acetate (3.6 g, 40 mmol, 2 eq) were dissolved in $CHCl_3$ (50 mL) and the solution cooled to 0 °C in an ice bath. Acryloyl chloride (3.6 g, 44 mmol, 2.2 eq) in $CHCl_3$ (50 mL) was then added dropwise over 20 min. The reaction was left to stir for 1 h at 0 °C. The reaction was then refluxed for 1 h at 60 °C and the solution filtered while hot, upon cooling a white precipitate formed which was isolated by filtration. The crude white solid was further purified by recrystallization in hot $CHCl_3$ to afford the desired product ethylene diacrylamide as a white solid (1.2 g, 36%). ^{1}H NMR (DMSO-d_6): δ 8.19 (s, 2H), 6.19 (m, 2H), 6.07 (m, 2H), 5.58 (m, 2H), 3.21 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 164.9, 131.8, 125.2, 38.4. HRMS (ESI) *m/z*: $[M + H]^+$ calculated for $C_8H_{13}N_2O_2$, 169.0972; found: 169.0980 (ppm 4.77).

2.2.2. Synthesis of PEG-PNIPAM (**1**–**3**) and the corresponding nanoparticle dispersions

Typically, the radical macro-initiator poly(ethylene glycol) dimer (12 kDa, 1.2 g, 0.1 mmol, 1 eq), *N*-isopropylacrylamide (0.56 g, 5 mmol, 25 eq per PEG chain), ethylene diacrylamide (10.1 mg, 0.06 mmol, 0.3 eq per PEG chain) and dodecanethiol (10.1 mg, 0.05 mmol, 0.25 eq per PEG chain) were transferred into a small schlenk tube fitted with a magnetic stirrer bar and *N*,*N*′-dimethylformamide (DMF, 7 mL) added. The reaction mixture was degassed and the vessel was backfilled with N_2. The reaction mixture was then placed in an oil bath at 70 °C and the polymerization was quenched by rapid cooling after 16 h. The reaction mixture was dissolved in a minimal amount of tetrahydrofuran (THF) and added dropwise to a large excess of ice-cold diethyl ether. The precipitation was repeated once more before the desired branched copolymer was obtained as a white solid (0.94 g). The molar ratio of ethylene diacrylamide per PEG chain was varied as 0.3 (**1**), 0.6 (**2**), and 0.9 (**3**) eq per PEG change for the PEG-PNIPAM branched block copolymers. Corresponding nanoparticle aqueous suspension can be prepared by a simple solvent-removal process. Typically, 10 mg of branched block copolymer was dissolved in 5 mL of acetone, followed by addition of 5 mL of water and stirred for 0.5 h at room temperature. Acetone was removed by evaporation at room temperature, and final transparent nanoparticles aqueous suspension was obtained.

2.3. Formulation of nanoparticles by emulsion-freeze-drying approach

Stock solutions of 2 wt.% branched block polymer PEG-PNIPAM (0.3, 0.6 and 0.9 cross-linkages as synthesized by ethylene diacrylamide of 0.3, 0.6, 0.9 eq per PEG chain) in deionized water and 0.5 wt.% Oil Red O (or indomethacin, ibuprofen, ketoprofen) in cyclohexane (*o*-xylene) solutions were prepared. Cyclohexane and *o*-xylene were chosen as the organic solvents to dissolve the hydrophobic dye/drugs because they are Class 2 solvents for pharmaceuticals with high concentration limits (3880 ppm and 2170 ppm, respectively) [48]. Both solvents are volatile with high melting points (~4 °C for cyclohexane and −25 °C for *o*-xylene), which makes them suitable for a freeze-drying process. Furthermore, both solvents could be readily emulsified to form stable oil-in-water emulsions [38,49]. Solvent residuals after freeze-drying could be within the limit as defined by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) as shown by freeze-drying organic solvents with similar vapour pressure [50]. Under stirring at 1000 rpm with an overhead stirrer (Eurostar digital, IKA-WERKE), the cyclohexane solution was added dropwise over a period of 2 min to the aqueous PEG-PNIPAM solution at room temperature (also once at 50 °C to investigate the temperature effect because the NIPAM block is known to be temperature sensitive). The emulsions with the volume ratios of aqueous phase to organic phase (W/O) of 1:4; 1:3 and 1:2 were prepared. After continuously