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Self-assembled macromolecular nanocoatings to stabilize and control drug release from nanoparticles

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ABSTRACT

A layer-by-layer (LbL) nanocoat (<25 nm thick) of two polyelectrolytes, chitosan and chondroitin sulfate was self-assembled step-wise onto drug nanoparticles that were prepared by a solvent-evaporation emulsification method using eucalyptol as the oil phase. Four poorly water-soluble model drugs, furosemide, isoxyl, rifampin and paclitaxel were chosen to prepare these particles. Zeta potential, particle size measurements, and microscopic inspection of the coated particles were used to confirm the successful addition of each polyelectrolyte layer and the stability of the nanoparticles. This manufacturing process produced stable drug nanoparticles with volume mean diameters below 250 nm. Dissolution tests confirmed that although the nanocoat reduced the dissolution of nanoparticles proportional to the coat thickness; they still dissolved much faster than commercially available micronized powders of the drugs. In addition, increasing the layer thickness (still less than 50 nm thick) by adding more LbL bilayers produced sustained release nanoparticles. Ultimately, the LbL nanocoating stabilized these small particles against crystal growth and aggregation in suspension and resulted in nearly perfect controlled drug release.

1. Introduction

Powder agglomeration can significantly deter efficient mixing, dispersion and fluidization of particles into a medium of choice. Physical stability of small particles is significantly compounded since their large surface free energy can only be dissipated by agglomeration [1]. In the case of nanocrystals, a special phase definition states that the stable seed size will be where the interfacial free energy will be a minimum for a specific phase volume, otherwise known as Wulff's theorem [2]. In the case of drug nanoparticles, these are not necessarily presented as single phase, homogeneously sized particles. Most often these small particles incorporate an array of inhomogeneous particles of various size and shape distributions which will result in the well-known Ostwald-ripening effect [3]. Due to this ripening effect, particles with wide size distributions will continue to grow and redissolve to finally reach a thermodynamically stable size as predicted by Wulff's theorem.

The need for drug nanoparticles arose from the fact that approximately 95% of all new drugs have poor pharmacokinetic properties such as low water solubility and bioavailability [4]. The well-established and current dissolution theory argues that an optimum, small particle size will result in the highest rate of dissolution and solubility [5–7] and therefore proposes that these insoluble drugs would demonstrate significant improvements in their technological behavior if they were deagglomerized and if a small particle size, possibly in the nanoscale range, could be assured [8].

It is quite apparent that size reduction of these insoluble drugs would induce physical instability of the particles that will compromise any advantages that size reduction would have imparted. Therefore, several stabilization mechanisms were introduced to maintain the benefit of particle size reduction on release rate and solubility of drugs. Some examples include the encapsulation of poorly water-soluble drugs such as paclitaxel [9], furosemide [10] and nifedipine [11,12] in PAMAM dendrimers, interactive powder mixing which deposited minute particles of griseofulvin on a larger carrier particle [13], dispersion of poorly water-soluble drugs in highly soluble polymers such as poly(vinylpyrrolidone) [14], and poly(ethylene glycol) [15]. Perhaps the most robust and easiest method to ensure small particle stability is the layer-by-layer (LbL) self-assembly nanocoating technique [16]. The successive deposition of minute quantities of polyelectrolytes of alternating polarities has been shown to create a highly stable nanoshell around dexamethasone microparticles [17]. Additional successful applications of LbL coating were illustrated for the inhibition of surface crystallization of amorphous indomethacin [18], nanoencapsulation of furosemide microcrystals to control drug release [19], to improve the photostability and pharmacokinetic profile of nifedipine [20] and the stabilization of dexamethasone nanoparticles [21].

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In this study, several poorly water soluble drugs with diverse surface properties were subject to an LbL nanocoating procedure to investigate whether any beneficial properties could be imparted to these troublesome drugs.

2. Materials and methods

2.1. Materials

Chitosan (CHI) of molecular weight, 15,000 g/mol was purchased from Polysciences, Inc, (Warrington, PA, USA). Chondroitin sulfate sodium salt (CS, MW ~50,000 g/mol) from bovine trachea, cationic poly(dimethyldiallyl ammonium chloride) (PDDA, MW ~150,000 g/mol), anionic sodium poly(styrenesulfonate) (PSS, MW ~70,000 g/mol), fluorescein isothiocyanate (FITC), USP grade furosemide and nifedipine, eucalyptol FCC grade, and analytical grade organic solvents were purchased from Sigma Aldrich Chemicals (St. Louis, MO, USA). Isoxyl and paclitaxel were supplied by Cayman Chemicals (Sapphire, Redfern, NSW, Australia). Ultrapure water used for all experiments and cleaning steps was obtained from a Barnstead Nanopure Diamond RO system having a specific resistance of greater than 18 MΩ/cm. Except for CHI, the polyelectrolyte (PE) solutions were prepared in 0.1 M phosphate buffered saline solution (PBS, pH 7.40) consisting of 1.1 mM potassium phosphate monobasic, 3 mM sodium phosphate dibasic heptahydrate, and 0.15 M NaCl. CHI was dissolved in 0.01 M acetic acid solution.

The synthesis of FITC-labeled CHI was based on the reaction between the isothiocyanate group of FITC and the primary amino group of chitosan as reported in the literature [22,23]. Briefly, 20 mg FITC was dissolved in 20 mL anhydrous methanol and added to 20 mL 1% w/v CHI in 0.01 M acetic acid solution. After 3 h under light occlusion at ambient temperature, the FITC-labeled chitosan (FITC-CHI) was precipitated by raising the pH to 10.0 with 0.5 M NaOH. The unreacted FITC was removed by washing with distilled water and centrifugation (10,000 rpm for 5 min) until no fluorescence was detected in the supernatant.

2.2. Preparation of nanoparticles

Nanoparticles of the drugs were prepared by a modified solvent-evaporation emulsification method as previously reported in the literature [21]. Briefly, 30 mg/mL solutions of the drugs in acetone were emulsified with twice the volume of eucalyptol for 30 min under vigorous agitation and low temperature (ice bath with 2000 rpm stirrer, IKA Eurostar PWR, Wilmington, NC, USA). Prepared emulsions were then further processed using a microfluidizer (model 110-Y, Microfluidics, Newton, MA, USA) [24]. The drug particles were collected by centrifugation at 5 000 RPM for 10 min and resuspended in 0.1 M PBS. The nanoparticles were again collected by centrifugation at 5 000 RPM for 10 min. This process was repeated several times to remove all the eucalyptol. The nanoparticle powders were dried and stored in a refrigerator for future use.

2.3. Layer-by-layer absorption of polyelectrolytes

Except for CHI that was dissolved in 0.01 M acetic acid, the pH of the PE solutions was maintained at 7.40. The LbL assembly procedure was as follows: 1.0 mL of a 20 mg/mL of the PE solution was mixed with 1 mL of 30 mg/mL drug nanoparticles, followed by 5 minute incubation under gentle shaking [16]. After a layer was added, three washing cycles of centrifugation, removal of the supernatant, and re-suspension in 1.5 mL of pH 7.20 buffer solution were performed to ensure the removal of unbound PE. The centrifugation was performed at 5,000 rpm for 5 to 10 min. The process was continued by alternating the polycationic and polyanionic layers until the desired number of layers was attained. The first PE layer was either PDDA or PSS dependent on the ζ-potential of the uncoated drug nanoparticles (furosemide +22 mV, paclitaxel −39 mV, isoxyl +35 mV and nifedipine −32 mV).

2.4. Characterization of nanoparticles

2.4.1. Quartz crystal microbalance analysis

Prior to PE multilayer formation on the nanoparticles, the coating procedure was elaborated on gold electrode resonators of 5 MHz quartz crystal microbalance (QCM200, Stanford Research Systems, Sunnyvale, CA, USA). The resonators were immersed in a polyion solution for 15 min, removed, and dried. The added mass and the coating thickness (ΔL) can be calculated from the frequency shift (ΔF), according to the Sauerbrey equation [25], using a scaling factor. For the instrument used in this study, the scaling factor was $\Delta L\ (nm) = -0.022\ \Delta F\ (Hz)$. These experimental self-assembly conditions were then applied to the LbL shell assembly on the nanocrystals. In addition, after each PE coating

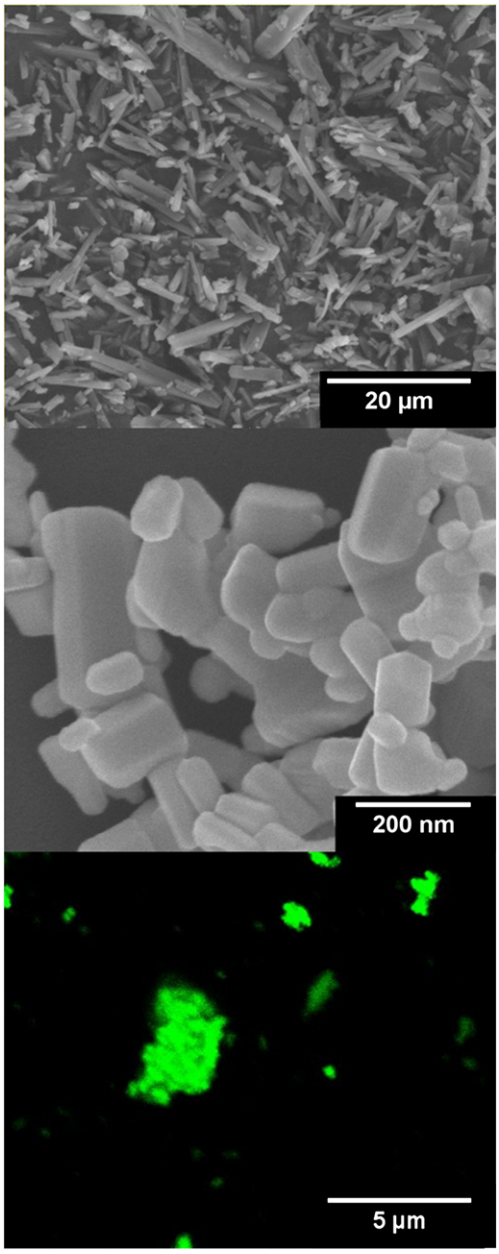

Fig. 1. SEM photomicrographs of furosemide microparticles (top), coated nanoparticles (middle) and nanoparticles LbL coated with FITC labeled chitosan (bottom). Particles were coated with 4 layers of PE's (see Fig. 7).