Published in final edited form as:

J Drug Deliv Sci Technol. 2015 October 1; 29: 143–151. doi:10.1016/j.jddst.2015.06.013.

Responsive Hydrogel Nanoparticles for Pulmonary Delivery

Nathanael A. Stocke^a, Susanne M. Arnold^b, and J. Zach. Hilt^{a,*}

^aDepartment of Chemical and Materials Engineering, University of Kentucky, Lexington, KY 40506, USA

^bCollege of Medicine, Department of Internal Medicine, University of Kentucky, Lexington, KY 40506, USA

Abstract

Nanoparticles represent one of the most widely studied classes of advanced drug delivery platforms in recent years due to a wide range of unique properties and capabilities that can be utilized to improve upon traditional drug administration. Conversely, hydrogel nanoparticles (HNPs) – also called nanogels – represent a unique class of materials that combine the intrinsic advantages of nanotechnology with the inherent capabilities of hydrogels. Responsive hydrogels pose a particularly interesting class of materials that can sense and respond to external stimuli and previous reports of inhalable hydrogel particles have highlighted their potential in pulmonary delivery. Here, we synthesized two different pH-responsive HNPs, designated HNP120 and HNP270, by incorporating functional monomers with a common crosslinker and characterized their physicochemical properties. One of the HNP systems was selected for incorporation into a composite dry powder by spray drying, and the aerodynamic performance of the resulting powder was evaluated. The HNP120s displayed a hydrodynamic diameter of approximately 120 nm in their fully swollen state and a minimal diameter of around 80 nm while the HNP270s were approximately 270 nm and 115 nm, respectively. Electron microscopy confirmed particle size- and morphological uniformity of the HNPs. The HNP120s were spray dried into composite dry powders for inhalation and cascade impaction studies showed good aerosol performance with a mass median aerosol diameter (MMAD) of 4.82 ± 0.37 and a fine particle fraction > 30%. The HNPs released from the spray dried composites retained their responsive behavior thereby illustrating the potential for these materials as intelligent drug delivery systems that combine the advantages of nanotechnology, lung targeting through pulmonary delivery, and stimuli-responsive hydrogels.

Graphical abstract

*Corresponding author: J. Zach Hilt, Department of Chemical and Materials Engineering, University of Kentucky, 177 F. Paul Anderson Tower, Lexington, KY. 40506-0046, Phone: (859) 257-9844, Fax: (859) 323-1929, zach.hilt@uky.edu.

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Keywords

Responsive Hydrogels; nanoparticles; drug delivery; pulmonary delivery; inhalation; aerosol performance

1.0 Introduction

Over the last few decades, exhaustive efforts have focused on developing advanced drug delivery systems for improved therapeutic efficacy, enhanced patient compliance, and optimal drug safety profiles [1]. Nanoparticles represent one of the most widely studied classes of advanced drug delivery platforms with more than 25,000 publications over the last 10 years [2]. Local delivery, environmental sensing, and responsive behavior are a few properties of nanoparticulate drug delivery systems that can be utilized to improve upon traditional drug administration [3-7]. Conversely, hydrogel nanoparticles (HNPs) – also called nanogels – represent a unique class of materials that combine the intrinsic advantages of nanotechnology with the inherent capabilities of hydrogels [8, 9].

Generally, hydrogels are defined as three-dimensional polymeric networks composed of hydrophilic moieties and possess the ability to imbibe large quantities of water [10]. Though traditionally made at the macroscopic scale, hydrogels possess unique characteristics that are promising for nanoformulations. The high water content of hydrogels results in materials that resemble living tissues and contributes to the success of many hydrogel applications in research and medicine [11-13]. While many of these applications center around gels at equilibrium (e.g. contact lenses), researchers have elicited a variety of mechanisms by which to control hydrogel behavior resulting in intelligent materials with great potential in drug delivery.

Responsive hydrogels have been examined thoroughly over the past several years and researchers have used a variety of approaches to achieve desired functionality [14]. By incorporating various monomers, crosslinkers, and synthesis conditions one can tune the hydrogel to respond in a variety of manners including degradation of the hydrogel network or swelling/shrinking of the gel [15, 16]. Additionally, the response of the hydrogel can be initiated by a variety of external stimuli such as heat, specific analytes, and changes in pH and these particles have potential in pulmonary administration, which has shown promise for nanoparticle and drug delivery to the lungs [13, 17, 18].

Previous reports of delivering hydrogel particles to the lungs via pulmonary administration demonstrated the ability to engineer micron-sized hydrogel particles that can be delivered to

the lungs as stand-alone carriers [19-21]. Liquid aerosols of hydrogel micro- and nanoparticle suspensions were also accomplished using metered dose inhalers and nebulizers [22, 23]. Additionally, the *in vivo* administration of 220 nm nanogels showed sustained release of a model peptide after pulmonary administration, thereby illustrating the potential of HNPs administered via inhalation [24].

Here, we synthesized pH-responsive HNPs, characterize some of their physicochemical properties, and incorporate them into dry powder composites for pulmonary delivery applications. The responsive behavior of these HNPs was achieved by incorporating functional monomers with a common crosslinker. Specifically, pH responsive HNPs were formed by polymerizing methacrylic acid (MAA) with poly(ethylene glycol) (n=400) diacrylate (PEG400DA). Dynamic light scattering was used to examine the change in hydrodynamic diameter of two different HNP systems as a function of pH and electron microscopy confirmed particle size- and morphological uniformity. One of the HNP systems was then selected for incorporation into a composite dry powder by spray drying and the aerodynamic performance of the resulting powder was evaluated. The characteristics of the HNPs released from this inhalable powder were examined and compared to the HNPs as synthesized.

2.0 Material and methods

2.1 Materials

Acrylic acid (AA), poly(ethylene glycol) 400 (PEG400DA) diacrylate, 9-anthracenylmethyl methacrylate (9AM), sodium dodecyl sulfate (SDS). Hydrochloric acid (HCL), Sodium Hydroxide (NaOH). Phosphate buffer saline (PBS) and acetic acid buffer (AAB) stock solutions were made at a concentration of 100 mM and an ionic strength of 1540 mM with sodium phosphate glacial acetic, respectfully. Ionic strength of buffers were adjusted with sodium chloride (fisher). For pH adjustments hydrochloric acid (Fisher) and sodium hydroxide were mixed with DI water to a final concentration of 0.1 M. Sigma-Aldrich (St Louis MO), Fisher Scientific (Pittsburgh, PA). Scott-Gross (Lexington, KY). ATTC (Manassas, VA). Life Technologies (Grand Island, NY).

2.2 Synthesis of responsive hydrogel nanoparticles

Responsive hydrogel nanoparticles were prepared with acrylic acid (AA), poly(ethylene glycol) 400 (PEG400DA) diacrylate, and 9-anthracenylmethyl methacrylate (9AM) in an aqueous solution of sodium dodecyl sulfate (SDS). The molar ratio of AA:PEG400DA was 80:20 and 1mol% 9AM was incorporated based on total monomer weight. Briefly, Acrylic acid was weighed into a 15 mL centrifuge tube and 9AM was dissolved into this liquid monomer. Subsequently, PEG400DA was weighed into a separate 15 mL centrifuge tube. SDS was dissolved in 100 mL of DI water and added to a 500 mL round bottom flask. A mechanical stir bar and a MODEL 260 J KEM Digital Temperature Controller were used during the experiment, which was carried out for 45 mins at 100 rpm and 45°C. The reaction mixture was via dialysis for 72 hours. Two different HNP systems were synthesized by changing the SDS concentration and were labeled HNP120s and HNP270s corresponding to SDS concentrations of 3.0 mM and 1.5 mM, respectively.

2.3 Electron microscopy and particle sizing

Scanning electron microscopy (SEM) was performed with a Hitachi S4300 microscope (Tokyo, Japan). Three milliliters of HNP solutions were transferred to a weigh boat and allowed to evaporate under mild heating on a hot plate. Double-sided adhesive carbon tabs were adhered to aluminum studs (Ted Pella, Redding, CA) and blotted on dried HNPs. Samples were coated with a thin film of a gold/palladium alloy using an Emscope SC400 sputter coating system at 20 mA for 1 minute under argon gas. For all electron microscopy experiments at least 3 independent samples were examined and multiple images were examined for each sample. ImageJ® software (NIH, Bethesda, MD) was used to measure particle diameter in multiple ($n>3$) representative images.

2.4 Colloidal stability of HNPs

The colloidal stability of the HNPs was determined via UV/vis spectroscopy. HNPs were dispersed in 10 mM PBS at 0.5 mg/mL and adjusted to desired pH with 0.1 M HCl or NaOH solutions. After adjusting the pH, the suspensions were transferred to a quartz cuvette and scanned from 200 nm to 800 nm in order to insure no maxima or minima occurred near desired wavelength of 600 nm, hence absorption is due to light scattering from nanoparticles. A separate quartz cuvette was filled with 10 mM PBS and used as a blank for the measurements, which were taken every 5 minutes for 12 hours at 600 nm. The stability was calculated by normalizing the absorption at time t to the initial absorption and the data were plotted in excel.

2.5 pH response of HNP diameter with dynamic light scattering

Dynamic light scattering was used to measure the hydrodynamic diameter of HNPs at varying pH conditions. HNPs were first dispersed in 10 mM PBS at a concentration of 0.5 mg/mL and the pH was titrated to a range of values with 0.1 M HCl and 0.1 M NaOH. At each pH value a sample of the mixture was placed in a disposable cuvette and placed into a [model] Malvern ZetaSizer®. The z-averaged diameter of these particles was recorded

2.6 pH response of HNP diameter with dynamic light scattering

Dynamic Light Scattering (DLS) was performed to determine the hydrodynamic diameter of the HNPs at varying pH values. Readings were performed in disposable cuvettes using a Malvern Instruments ZetaSizer® (Malvern Instruments, Malvern, United Kingdom). Aqueous dispersions were made with HNP and PBS concentrations of 200 μ g/mL 10 mM, respectively and the pH was measured using a pH probe. For each measurement the pH was adjusted using 0.1 M HCl and NaOH, recorded, and then placed in ZetaSizer®.

2.7 Inhalable HNP composites from spray drying

Spray drying was accomplished with a Büchi Mini Spray Dryer B290 coupled with a B-295 Inert Loop and high-performance cyclone (all from Büchi Labortechnik, Flawil, Switzerland) using parameters previously optimized by our lab [25]. Spray drying experiments were carried out in closed mode using UHP dry nitrogen gas at a flow rate of 600 L/h, an inlet temperature of 150°C, and an aspirator rate of 35 m³/h. Once the inlet temperature of 150°C was reached, pure methanol was fed to the spray dryer until the outlet

temperature was stable. For feed mixtures, D-mannitol was dissolved in methanol and iron HNPs were subsequently dispersed such that the combined mass of these two components resulted in a dilute concentration of 0.1% wt/vol. The HNP concentration in the feed was 10wt% based on the combined mass of D-mannitol and HNPs. Five hundred milliliters of this mixture was fed to the spray dryer at a pump rate of 50% (15 mL/min) through a stainless steel nozzle with a diameter of 0.7 mm. Powders were collected and transferred to a glass desiccator over Drierite™ and stored in the freezer at -23°C.

2.8 Aerosol performance with the Next Generation Impactor™ (NGI™)

Impactor studies were carried out with a MI70 Next Generation Impactor™ (MSP Corporation Shoreview, MN) in accordance with United States Pharmacopeia (USP) Chapter <601> specifications on aerosols [26]. The conditions for these experiments were previously reported [27, 28]. The NGI was equipped with a stainless steel induction port (USP throat adaptor) attachment and specialized stainless steel NGI™ gravimetric insert cups (NGI Model 170, MSP Corporation, Shoreview, Minnesota). Type A/E glass fiber filters with diameter 55mm (Pall Corporation, NY) were placed on each stage of the impactor and weighed before and after the experiment to determine the particle mass deposited. Approximately 10 mg of powder was loaded into each clear hydroxypropyl methylcellulose, size-3 capsule (Quali-V®) (QUALICAPS, Spain) and these capsules were placed into a HandiHaler® DPI™ device (Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, CT) attached to a customized rubber mouth piece connected to the NGI™. Three capsules were loaded and released for each sample and experiments were performed in triplicate. The total dose for each sample was approximately 30 mg. A Copley HCP5 vacuum pump (Copley Scientific, United Kingdom) and a Copley TPK 2000 critical flow controller (Copley Scientific, United Kingdom) were used to produce a flow rate (Q) of 60 L/min in order to model the flow rate in a healthy adult lung. This flow rate was adjusted before each experiment using a Copley DFM 2000 flow meter (Copley Scientific, United Kingdom).

For Q=60 L/min, the aerodynamic cutoff diameters (D_{a50}) for each stage of the impactor were given from the manufacturer as: stage 1 (8.06 µm); stage 2 (4.46 µm); stage 3 (2.82 µm); stage 4 (1.66 µm); stage 5 (0.94 µm); stage 6 (0.55 µm); and stage 7 (0.34 µm). The emitted dose (ED), respirable fraction (RF), and fine particle fraction (FPF) were calculated as follows:

$$ED\% = \frac{\text{Initial mass in capsules} - \text{Final mass in capsules}}{\text{Initial mass in capsules}} \times 100\% \quad (1)$$

$$RF\% = \frac{\text{Mass of particles on stages 2} - 7}{\text{Total particle mass on all stages}} \times 100\% \quad (2)$$

$$FPF\% = \frac{\text{Mass of particles on stages 2} - 7}{\text{Initial mass in capsules}} \times 100\% \quad (3)$$

Additionally, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) for the particles were determined using a Mathematica® program written by Dr. Warren Finlay [29].

3.0 Results and Discussion

Figure 1 includes a representation of the structure and responsive of the HNPs at varying pH conditions, and the responsive behavior of the synthesized HNPs was examined by measuring the hydrodynamic diameter of the particles as a function of pH (Figures 2 & 3). At higher pH values, the carboxylic groups in MAA along the hydrogel backbone are deprotonated providing a negative charge that stabilizes the particles and thus prevents agglomeration as illustrated in Figure 1. As pH is lowered the protonation of these functional groups results in less electrostatic repulsion between chains and intrastrand hydrogen bonding. Both of these mechanisms lead to the collapse, or deswelling, of the three dimensional network in the HNPs and therefore a reduction in the hydrodynamic diameter of the HNPs. Eventually, increasing the proton concentration results in a transition of particle behavior wherein interparticulate hydrogen bonds result in large agglomerates of particles leading to a massive increase in the hydrodynamic diameter of the particles. Interestingly, this behavior is reversible wherein raising the pH above the transition value thus giving these HNPs the unique ability to reversibly sense and respond to changes in their environment.

The HNP120s (Figure 2) have a hydrodynamic diameter of approximately 120 nm in their fully swollen state and a minimal diameter of around 80 nm while the HNP270s (Figure 3) have a swollen diameter of approximately 270 nm with a minimum diameter of approximately 115 nm. Interestingly, the point at which these systems started agglomerating, the so-called transition pH, was different for the two HNPs systems as seen more clearly in Figure 4. The transition pH for both the HNP120 and HNP270 particles occurred near the pKa of methacrylic acid (4.65); however, the HNP120s began to agglomerate at a higher pH than the HNP270s suggesting the ability to tune this transition based on both the monomer and the size of the HNPs. The shift in the transition pH is possibly explained by the larger ratio of surface area to volume in the HNP120s thereby making an agglomerated state more energetically favorable relative to the HNP270s. A more energetically favorable agglomerated state would result in less protonation of carboxylate groups, and therefore less interparticulate hydrogen bonding, necessary to overcome electrostatic repulsion of deprotonated carboxylic groups.

Below the transition pH the HNP120 and HNP270 particles were extremely polydisperse with all of their PDI values > 0.35. These PDI values suggest agglomeration of the particles and support our hypothesis of interparticulate hydrogen bonding as the mechanism driving agglomeration. However, above the transition pH the both HNP systems were very monodisperse with the PDI of all measurements > 0.09. Interestingly, lowering the pH and introducing agglomerates is completely reversible by raising the pH of the system and presents a novel responsive behavior of these HNP systems that is both precise and reproducible. This reversible behavior further supports our hypothesis that the large agglomerates are indeed due to reversible and noncovalent interactions between HNPs.

Both HNP systems were examined with electron microscopy and these images confirmed their uniform size distribution (Figures 5 and 6). Figure 5 are representative images of individual HNPs showing a high degree of uniformity. Higher resolution SEM images (Figures 5b and 6b) show smooth surfaces on these nanoparticles and spherical particle morphology; however, upon drying the flexible HNPs pack together into large fibers causing the primary particles to slightly deviate from spherical morphology. ImageJ® was used to analyze the SEM images and measure the diameter of the HNPs in the dry state. The HNP120 and HNP270 particles displayed diameters in the dry state of $82.6 \text{ nm} \pm 4.3 \text{ nm}$ and $133.4 \text{ nm} \pm 12.2 \text{ nm}$, respectively and these values agree with the hydrodynamic diameter profiles of these systems.

After characterizing the size and responsiveness of two different HNP systems, the HNP120s were selected for incorporation into inhalable composites and were further characterized. Representative TEM images of the HNP120s (Figure 7) confirmed the spherical morphology of particles when allowed to attach to the TEM grid in dilute suspensions and thereby avoid morphological deformation due to surrounding particles upon drying. Additionally, high resolution TEM images (Figure 8) confirmed the amorphous character of these particles evident by the diffused peak in the selected area diffraction pattern (inset of Figure 8).

As a proof of concept, the HNP120s were loaded with a model chemotherapeutic agent, cisplatin, by imbibition with a cisplatin solution (supplemental Figure S1). In solution, cisplatin is solvated with water molecules and carries a slightly positive charge [30]. Hence, we hypothesized that cisplatin would accumulate in HNPs above their transition pH and not below it. Figure S1 shows the reduction in cisplatin concentration for HNPs at pH = 7 while no such decrease was seen at pH = 4 and the calculated loading of cisplatin $3.1\% \pm 0.2\%$ (mass cisplatin per mass of HNPs) for the HNP120s at pH = 7. These data indicate that cisplatin selectively accumulates in these composites based on pH and suggests that this is due to electrostatic interactions. Additionally, these findings illustrate the potential for triggered drug release by reducing the pH and thereby eliminating the electrostatic interaction between cisplatin and the HNPs although these studies have not yet been performed.

The colloidal stability of these HNPs was examined above and below their transition pH in 10 mM PBS at an ionic strength of 154 mM, which is physiologically relevant [31]. The data are presented in Figure 8 and show that above the transition pH the HNPs display excellent colloidal stability with no significant change in their relative absorption. Below the transition pH the HNP suspension is a relatively opaque and chalky mixture that continues to get more opaque with HNP agglomerate. This leads to an increase in relative absorption for approximately 2 hours. Eventually, these agglomerates become too large to remain in suspension and begin to crash out of suspension at approximately 120 mins.

After characterizing the size, responsiveness, and stability of the HNP120s, these particles were spray dried (SD) into a composite powder for inhalable purposes and this powder was designated SD HNP120. Figure 9 shows representative SEM images of these composites. The SD HNP120 composites exhibited spherical particle morphology and relatively uniform

size distribution. The inset of an individual particle illustrates a relatively rough surface for these particles that could potentially affect the aerodynamic performance of this powder.

Cascade impaction studies were used to evaluate the aerodynamic performance of the HNP composite powders using the Next Generation Impactor®. Initial attempts at spray drying these composites in purely aqueous systems resulted in powders dissolving in the collection vial presumably due to diffusion of water still retained within the HNPs after spray drying. Hence, the HNPs were dispersed in methanol and the resulting powders showed no sign of dissolution in the collection vial. The aerosol dispersion performance of the HNP composites (Figure 10) shows deposition of particles on all stages of the impactor and illustrates the potential of these materials to reach all regions of the lungs, even to the deepest alveolar region.

The mass median aerodynamic diameter (MMAD) of the composite HNPs was $4.82 \mu\text{m} \pm 0.37 \mu\text{m}$, which falls within the generally accepted range of $1\text{--}5 \mu\text{m}$ for optimal aerodynamic performance [32]. Additionally, the respirable fraction and fine particle fraction for this dry powder formulation were $54.8 \pm 4.4\%$ and $31.7 \pm 6.8\%$, respectively. These values show good agreement with former reports from our lab group as well as other very recent reports in the literature using a variety of DPI devices [33, 34]. Additionally, these formulations are absent of a lactose carrier which can further improve the aerosol performance of dry powder formulations [32, 35]. The aerodynamic performance parameters of the composite dry powder are reported in Table 1.

The responsiveness of the HNPs released from the composite HNP powders was examined by again measuring their hydrodynamic diameter as a function of pH. Figure 11 shows a slight increase in the z-averaged diameter of the HNPs released from the spray dried composites. This increase in z-averaged diameter is accompanied by an increase of the PDI of these particles. For all measurements above the transition pH the PDI varied between 0.1-0.2 indicating a slightly more polydisperse system relative to the HNPs before spray drying. Importantly, the HNPs released from inhalable composites still respond to environmental changes in pH and these responses continue to be reversible. The increase in z-averaged diameter and PDI suggested a population of particles that are in a permanently agglomerated state so we examined their colloidal stability.

Figure 12 compares the stability of the HNP120s to those released from the SD HNP120 composites. The HNPs released from the spray dried composites have a subpopulation of particles that fall out of suspension with time. This fraction of particles likely represents permanent agglomerates that were introduced during the spray drying process but only represents approximately $12.6\% \pm 0.3\%$ of the HNPs (assuming Beer's law).

Previous reports of delivering HNPs directly to the lungs, via pulmonary delivery, have focused mainly on liquid based aerosols or large HNP carriers [19-24]. However, the advantages of dry powder aerosols, coupled with the ease of incorporating multiple species through spray dried composites, provide a unique platform for advanced drug delivery applications. Additionally, the ability to control parameters such as the primary particle size, density, shape and surface roughness allow for a component of particle engineering for

improved aerosol behavior that is not possible in liquid-based aerosols. To our knowledge, this is the first report of dry powders for inhalation that contain pH-responsive hydrogel nanoparticles. These materials have great potential in diseased states that display local changes in pH such as the reduced pH in the microtumor environment due to increased formative metabolism and poor perfusion [36, 37]. The evidence of cisplatin loading in these particles and the ability to alter the transition pH indicates the potential to develop intelligent drug delivery vehicles that can be targeted to the lungs via pulmonary delivery of aerodynamically sufficient dry powders.

4.0 Conclusions

Responsive HNPs were successfully synthesized by incorporating MAA as a functional monomer and PEG400DA as a crosslinker. DLS showed a reduction in hydrodynamic diameter of the HNPs due to protonation of the carboxylic acids groups in MAA with decreasing pH. The transition pH of these materials can be altered by changes in the particle synthesis conditions suggesting the ability to tune this value even for a given monomeric system. HNPs were successfully incorporated into inhalable composites and retained their nanometer size as well as their responsive behavior. These findings highlight the great potential for these materials as intelligent drug delivery systems that combine the advantages of nanotechnology, lung targeting through pulmonary delivery, and stimuli-responsive hydrogels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work would not have been possible without the efforts of Marjorie L. Guy and financial support from the National Institute of Health's National Cancer Institute on grant number R25CA153954.

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Figure 1.
Representation of responsive hydrogel nanoparticles at varying pH conditions from low (left) to high (right).

Figure 2. Hydrodynamic diameter of HNP120s a) over a large range of pH values and b) zoomed in above transition pH

Figure 3. Hydrodynamic diameter of HNP270s a) over a large range of pH values and b) zoomed in above transition pH

Figure 4. Hydrodynamic diameter of HNP120 (diamonds) and HNP270 (squares) showing difference in transition pH

Figure 5.
SEM images of HNP120s at a) $\times 100k$ and b) $\times 180k$. ImageJ® size analysis shows $82.6 \text{ nm} \pm 4.3 \text{ nm}$

Figure 6.
SEM images of HNP270s a) $\times 35k$ and b) $\times 150k$. ImageJ® size analysis shows $133.4 \text{ nm} \pm 12.2 \text{ nm}$

Figure 7.
Representative TEM images of representative HNP120s on copper TEM grid illustrating spherical morphology and size uniformity. The diameters of two representative particles are:
a) 80.7 nm and b) 88.7 nm

Figure 8. High mag ($\times 500k$) TEM image of individual HNP120 with fast Fourier Transform inset showing amorphous character of HNPs

Figure 9. Stability of HNP120s above and below transition pH

Figure 10.
SEM image of SD HNP120 composite dry powder with individual particle shown in top right corner inset.

Figure 11.
Aerosol dispersion performance plot for inhalable responsive HNPs.

Figure 12. Hydrodynamic diameter of HNP120s and SD HNP120s a) over a large range of pH values and b) zoomed above transition pH

Figure 13. Stability of HNP120s and SD HNP120s above transition pH shows population of SD HNP120s that fall out of suspension

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Table 1

Aerosol performance of HNP composites for inhalation (mean $\pm$ std. dev.).

	MMAD (μm)	GSD (μm)	FPF (%)	RF (%)	ED (%)
HNPs	4.82 $\pm$ 0.37	2.51 $\pm$ 0.18	31.7 $\pm$ 6.8	54.8 $\pm$ 4.4	78.5 $\pm$ 5.3