

# Formulation and In Vivo Evaluation of Effervescent Inhalable Carrier Particles for Pulmonary Delivery of Nanoparticles

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15 The purpose of this study was to evaluate the safety of a new  
inhalable effervescent carrier preparation containing model  
nanoparticles. Spray-freeze drying was used to prepare inhalable  
20 powders containing butylcyanoacrylate nanoparticles. The particle  
size of the nanoparticles before incorporation into the effervescent  
carrier and after dissolving the carrier powder was measured  
using laser light scattering. The particle size distribution of the  
effervescent carrier aerosol particles was measured using a  
cascade impactor. The prepared powder was tested in vivo using  
25 five Balb/c nude mice. The animals were treated with 1 mg of  
inhalable powder every week for 4 weeks. The body weight and  
morbidity score of the mice were observed over an 8-week period.  
The effervescent activity of the inhalable nanoparticle powder  
was observed when the powder was exposed to humidity. The  
30 particle size of the nanoparticles did not change significantly after  
spray-freeze drying. The mass median aerodynamic diameter  
(MMAD) of the prepared powder was  $4.80 \pm 2.12 \mu\text{m}$ , which is  
suitable for lung delivery. The animals that were treated with  
effervescent powder tolerated the administration without any  
35 changes in their morbidity scores. Our pilot study demonstrates  
that pulmonary nanoparticle delivery via effervescent carrier  
particles appears safe in the present animal model.

**Keywords** nanoparticle; pulmonary delivery; carrier particles;  
effervescent; cyanoacrylate

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## INTRODUCTION

The pulmonary route of administration is used for local and 40  
systemic delivery of active ingredients (Labiris & Dolovich,  
2003). Drugs can be delivered in the form of solutions, suspen-  
sions, gases, or dry powders. Dry powder delivery, however,  
must overcome many obstacles due to critical particle size  
45 range restrictions for sufficient lung delivery (3–5  $\mu\text{m}$ ). This is  
due to aggregation tendencies of many fine powders that may  
increase their mass median aerodynamic diameter (MMAD),  
and this affects optimized delivery to the alveolar region  
(Finlay, 2001).

Nanoparticles have been investigated recently through 50  
different routes of administration. Drug-loaded nanoparticles  
are normally between 100 and 300 nm in diameter. This size  
range is typically not suitable for pulmonary delivery due to  
low deposition probabilities and difficulties in dispersing parti-  
cles in this size range. However, such nanoparticles can be 55  
incorporated into carrier particles that have an appropriate par-  
ticle size. The pulmonary administration of nanoparticles was  
first proposed by Tsapis, Bennett, Jackson, Weitz, and Edwards  
(2002) for large porous particles and by Sham, Zhang, Finlay,  
Roa, and Löbenberg (2004) for regular carrier particles. 60  
Recently, Ely, Roa, Finlay, and Löbenberg (2007) introduced  
effervescent carrier particles with an active release mechanism  
for the pulmonary route of administration. These carrier parti-  
cles were made via spray drying, and they released incorporated  
nanoparticles actively. In another study (Azarmi et al., 2006),

we showed that drug-loaded doxorubicin nanoparticles can be incorporated into carrier particles with appropriate particle size range for pulmonary delivery and can maintain their cytotoxic activity. These particles were produced by a spray-freeze drying technique.

While environmental aerosol science is concerned about small doses of inhaled ultrafine nanoparticles (Oberdorster, Oberdorster, & Oberdorster, 2005) and their possible inflammatory reaction on lung tissues (Dailey et al., 2006), we propose to deposit rather large amounts of nanoparticles into the lungs. A careful safety assessment of inhalable nanoparticles as a drug delivery system is therefore necessary.

In this work, we spray-freeze dried carrier particles to prepare inhalable effervescent particles containing blank model nanoparticles. In this study, we assessed the in vivo safety of effervescent powders that contained these blank model nanoparticles.

## MATERIALS AND METHODS

### Materials

Butylcyanoacrylate was a gift from Loctite Ltd (Dublin, Ireland). Dextran 70 (~70 kDa), ammonium hydroxide, and citric acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Lactose monohydrate (FlowLac 100) was obtained from Meggle (Germany). Sodium carbonate anhydrous was obtained from BDH Inc. (Toronto, ON, Canada). Nuclepore filter paper was purchased from Whatman, USA. DP-4M insufflator was purchased from Penn-Century Inc. (Philadelphia, PA, USA). Hydrochloric acid was obtained from Caledon, Canada. All the chemicals were of analytical grade and used as received.

### Methods

#### *Preparation of Butylcyanoacrylate Nanoparticles*

Butylcyanoacrylate nanoparticles were prepared as described previously (Azarmi et al., 2006). In brief, 100  $\mu$ L of *n*-butylcyanoacrylate monomer was added to 1% dextran solution in 10 mL of 0.01 M HCl under constant stirring at 600 rpm. After 4 h, the nanoparticle suspension was filtered through a 0.8- $\mu$ m membrane filter (Nuclepore Track-Etch membrane, Whatman) and stored at 2–8°C until further use.

#### *Particle Size Analysis of Nanoparticles*

The particle size of the nanoparticles was determined by laser light scattering using a Zetasizer, model HSA 3000 (Malvern, Worcestershire, UK). For particle size analysis, 100  $\mu$ L of the nanoparticle suspension was dispersed in 4 mL deionized water. The mean particle size and polydispersity index were determined.

#### *Preparation of Effervescent Carrier Particles*

For the preparation of nanoparticle-loaded carrier particles, 250 mg sodium carbonate and 1,000 mg spray-dried lactose

monohydrate (FlowLac 100, Meggle) were dissolved in 3 mL distilled water and 300  $\mu$ L ammonium hydroxide 28–30% was added. The solution was kept in a tightly closed container. Before spray-freeze drying, 200 mg citric acid powder and 10 mL of nanoparticle suspension were added to the solution. The suspension was spray-freeze dried as described before (Azarmi et al., 2006). In brief, a two-fluid nozzle (Spraying Systems Co., Wheaton, IL, USA) utilizing gaseous nitrogen at a flow rate of 0.6 scfm was used to atomize the nanoparticle suspension, which was supplied at a flow rate of 37 mL/min using a peristaltic pump (CTP-A, Chem-Tech, Punta Gorda, FL, USA). The nozzle was placed ~15 cm above a 600 mL flask containing 300–400 mL of liquid nitrogen. Following spraying, the flask contents were transferred into a Pyrex vacuum beaker, and the liquid nitrogen was allowed to evaporate. The vacuum container was attached to a freeze dry system (Labconco Corp., Kansas City, USA) operating at 0.004 mbar with the collector at –52°C. The powder in the flask was held at subzero temperature for the initial 7 h, followed by 41 h at 23°C. After 48 h, the powder was collected and stored in a sealed vial at 4°C.

#### *Mass Median Aerodynamic Diameter Measurement*

The MMAD of the powder was measured as previously described (Azarmi et al., 2006) using a Mark II Anderson Cascade Impactor (Graseby Anderson, Smyrna, GA, USA) with effective cut-off points recalibrated at 60 L/min. A passive dry powder inhaler (Wang, Grgic, & Finlay, 2006) that utilizes cyclonic action as well as mechanical impaction to disperse powder particles was used to de-agglomerate and deliver the powder to the impactor. The flow rate was monitored with a pneumotachometer (PT 4719, Hans Rudolph Inc., Kansas City, MO, USA). Non-linear least squares regression was used to determine the MMAD and geometric standard deviation (GSD).

#### *In Vivo Evaluation of the Effervescent Carrier Particles Containing Nanoparticles*

The animal work was approved by the animal ethics committee of the Cross Cancer Institute at the University of Alberta. For assessing the in vivo tolerability of the prepared effervescent nanoparticle powder, female 4- to 5-week-old Balb/c nude mice were treated once a week with 1 mg of effervescent powder nanoparticles over a period of 4 weeks and were observed for 8 weeks. A DP-4M insufflator (Penn-Century Inc.) was used to administer the powder. The insufflator was calibrated by weighing before and after powder filling, as well as after administration of the powder to determine precisely the delivered dose. The tip of the insufflator tube was positioned near the carina (first bifurcation) so that the measured dose of effervescent powder could penetrate deep into the lung. The average body weight and morbidity score of the mice were monitored. The morbidity score entails five categories of physiologic parameters, such as appearance, body weight, food intake, clinical signs, and behavior, monitored daily (Table 1). Each category entails scores 0–3; higher morbidity score rate

TABLE 1

## Physiologic Parameters Used for Measuring Morbidity Score

Project/Animal Identification	Score
Appearance	
Normal	0
General lack of grooming	1
Coat staring, ocular or nasal discharge	2
Piloerection, hunched up	3
Body weight	
Normal <5%	0
Body weight drop 6–15%	1
Body weight drop 16–25%	2
Body weight drop 26–35%	3
Body weight drop >35%	4
Food intake	
Normal	0
Food intake drop 10–33%	1
Food intake drop 34–75%	2
Food intake drop >75%	3
Clinical signs	
Normal respiratory rate and hydration	0
Slight changes	1
Respiratory rates up or down 30%, measurable dehydration	2
Respiratory rates changes 50% or very low, severe dehydration	3
Behavior	
Normal	0
Minor inactivity or exaggerated responses	1
Moderate change in expected behavior, isolated or listless	2
Reacts violently, or very weak and pre-comatose	3
Total	

Humane endpoints and actions.

*Any individual score of 3 or more:* Terminate animal.

*Total score:* 0–5, normal; 6–10, Monitor carefully, consider analgesics (increase monitoring frequency of body weight and food intake if “2” is scored in either category); 11–15, suffering, provide relief, observe regularly (seek second opinion from animal care staff and Director of Animal Care as indicated. Consider termination).

170 shows that animal is in the situation that needs more observation and may needed to be euthanized.

## RESULTS AND DISCUSSION

175 The purpose of this study was to test the safety of a new inhalable carrier particle that is used to deliver nanoparticles deep into the lungs. Our previous studies showed that nanoparticles can be loaded into carrier particles either as blank (Sham et al., 2004) or

drug-loaded (Azarmi et al., 2006) nanoparticles. Recent research showed that spray dried effervescent carrier particles are able to release nanoparticles more favorably compared with carrier particles that only dissolve (Ely et al., 2007). The active release prevents the nanoparticles from agglomeration when the carrier particle dissolves. Because spray drying has the disadvantage that the applied temperature might compromise drug stability, in this work we introduced a new method of preparing effervescent carrier particles using a spray-freeze drying technique. Ammonium hydroxide was added to the effervescent formulation to keep the pH high enough to prevent a fast effervescent reaction between citric acid and sodium carbonate. The ammonium hydroxide evaporated during the spray-freeze drying process resulting in a stable powder that showed effervescent activity when exposed to humidity. The prepared carrier powder containing nanoparticles has spherical shape with geometric particle size of  $4.94 \pm 0.2 \mu\text{m}$  as demonstrated by SEM micrographs (Figure 1). The mean particle size of the nanoparticles, which was measured by laser light scattering method, was  $157 \pm 12 \text{ nm}$  ( $n = 3$ ) before spray-freeze drying. After re-dissolving the carrier particles in distilled water, the nanoparticle size was determined as  $162 \pm 16 \text{ nm}$  ( $n = 3$ ). No significant change in the particle size was observed after spray-freeze drying ( $p > .05$ ). The MMAD of the effervescent carrier

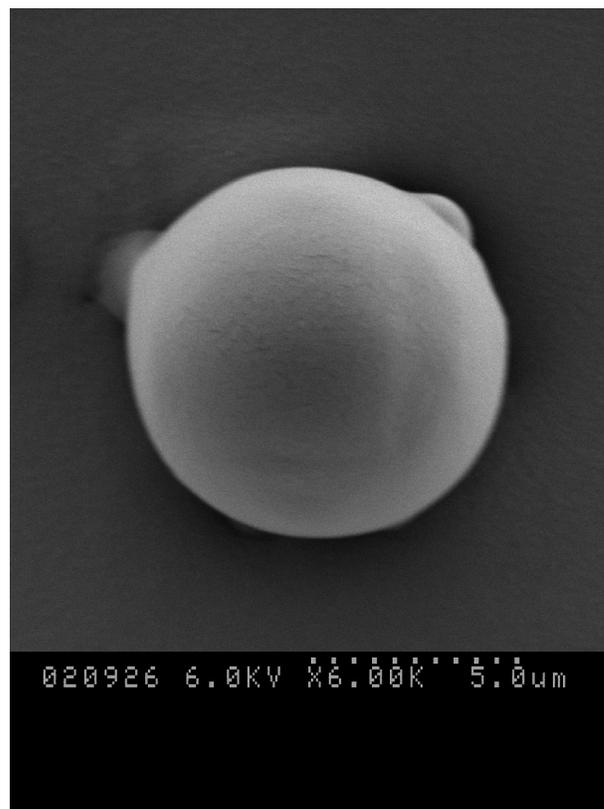


FIGURE 1. SEM micrograph of effervescent nanoparticle powder showing spherical shape of the carrier particle.

TABLE 2  
Particle Size of Nanoparticles and Carrier Powder Containing Nanoparticles Measured Using Different Techniques

	Measuring Method	Particle Size
Particle size of nanoparticles before spray freeze drying	Laser light scattering	$157 \pm 12$ nm
Particle size of nanoparticle after spray freeze drying and re-dissolving in water	Laser light scattering	$162 \pm 16$ nm
Geometric particle size of dry powder carrier	Scan electron microscopy	$4.94 \pm 0.2$ $\mu$ m
Mass median aerodynamic diameter (MMAD)	Cascade impactor	$4.80 \pm 2.12$ $\mu$ m

200 particles loaded with blank nanoparticles, measured using cascade impactor, was  $4.80 \pm 2.12$   $\mu$ m ( $n = 3$ ) and therefore suitable for lung deposition. Table 2 shows the results of different particle size measurements.

205 Whereas occupational and environmental sciences are concerned about low deposition rates of dust nanoparticles to the lungs (Oberdorster et al., 2005), pulmonary drug delivery of nanoparticles to the lungs requires high deposition rates. Therefore, the toxicity of any inhaled particles is an important safety aspect for designing a pulmonary delivery system for nanoparticles. Reports showing significant inflammatory responses of inhaled dust particles are mostly described for ultrafine particles that have sizes below 100 nm (Oberdorster et al., 2005). However, until today there are very few reports about the safety of pulmonary nanoparticle delivery for treatment purposes which investigated the compatibility and safety of inhalable nanoparticles. In an in vitro study performed by Brzoska et al. (2004), they showed that gelatine, albumin, and polyalkylcyanoacrylate nanoparticles have little or no toxicity and cause no inflammation on primary airway epithelium cells, 16HBE14o. Dailey et al. showed that biodegradable nanoparticles exhibit a less inflammatory response to the lungs compared with nonbiodegradable particles (Dailey et al., 2006). Another safety aspect of lung deposition is the interaction of nanoparticles with the alveolar environment and especially the lung surfactant film. This film is responsible for gas exchange and for preventing the collapse of alveolar space by decreasing the surface tension at the lung air interface. Compromising one or both of its main functions might have life-threatening consequences. A biophysical investigation by Stuart et al. showed that nanoparticles do not destabilize the monolayer film (Stuart et al., 2006). Although in vitro data are very important, only animal studies can answer the safe use in vivo. Therefore, this study was intended to test the safety of the prepared carrier powder in an animal model. The results of MMAD measurements showed that the carrier powder has proper characteristics

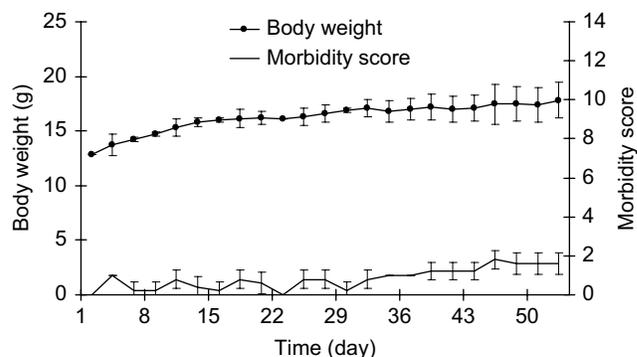


FIGURE 2. Body weight and morbidity score of animals treated once a week with 1 mg inhalable effervescent nanoparticle powder for 4 weeks ( $n = 5$ ).

for pulmonary delivery. One milligram of the effervescent particles was administered to five female 4- to 5-week-old Balb/c nude mice. The animals did not show any signs of harm after the administration and behaved normally after recovering from anesthesia. The delivery of the powder via the DP-4M insufflator seemed to be sufficient because no powder residuals were found in the device after administration. Figure 2 illustrates the average body weight and morbidity score of Balb/c mice treated with the inhaled effervescent powder. The average morbidity score for each mouse was less than 2 during the surveillance period of 8 weeks. Meanwhile, the body weight of the animals increased as can be see in Figure 2. This indicates that the treatment did not negatively impact their health.

## CONCLUSION

This study showed that the effervescent powder was well tolerated by the mice. Nanoparticle delivery via effervescent carrier particles appears to be a safe route of administration.

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