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Near-critical fluid micronization of stabilized vaccines, antibiotics and anti-virals

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Abstract

Fine powder preparations of vaccines hold promise in resolving many issues encountered in the transport and delivery of vaccines such as loss of potency during transport through the 'cold chain' and needle free delivery. We have demonstrated the efficacy of a new powder-generating technique, Carbon dioxide Assisted Nebulization with a Bubble Dryer® (CAN-BD), for producing dry, active powders of vaccines and small molecule pharmaceuticals. A hepatitis B surface antigen (HBsAg) protein vaccine and a live-attenuated measles vaccine were stabilized in various formulations, then processed into fine powders by nebulizing and drying them at near ambient temperatures (50 °C). Full preservation of HBsAg ELISA activity was achieved for formulations containing sufficient amounts of stabilizing trehalose. The powders were stored for 43 days either at −20 °C or at +66 °C without loss of potency. Commercial live-attenuated virus measles vaccine was further stabilized by adding trehalose or sucrose to retain full potency through CAN-BD drying. Powders had a mass median aerodynamic diameter (MMAD) of 1.9 μm and respirable mass fraction of 94%. A formulation of the anti-viral zanamivir was micronized to give 73% respirable mass fraction and MMAD of 2.4 μm. The antibiotic rifampin was processed by CAN-BD to yield powder with an MMAD of 1.2 μm and 86% respirable mass fraction.

Keywords: Supercritical CO_2; Vaccine; Antibiotic; Aerosol delivery

1. Introduction

Preparation of biopharmaceuticals and vaccines in solid dry formulation is often desirable or necessary to avoid freeze and thermal damage and to meet shelf-life storage stability requirements. In addition, fine powder formulations may be desired for greater storage stability and/or alternatives to the traditional subcutaneous injection delivery route, such as delivery to the lungs. Freeze-drying is the most common process for producing parenteral products as dry formulations. However, there are a large number of vaccines that cannot be lyophilized because they are damaged by the freezing step. These products are vaccines using aluminum hydroxide gel ("alum") as an adjuvant—the only type of vaccine adjuvant now in commercial use within the United States. It has been shown that alum-conjugated vaccines can undergo serious damage and deactivation when frozen during processing, shipping, or storage [1–6]. Indeed, it has only recently been recognized that in developing countries, the cold-chain vaccine distribution networks had often been accidentally freezing vaccines [7]. Another drawback of freeze-drying is that further processing (*e.g.*, jet-milling) would be required to transform the freeze-dried cake into a powder for alternative delivery methods such as inhalation.

Carbon dioxide Assisted Nebulization with a Bubble Dryer® (CAN-BD) is a patented [8–16] technique for producing fine, dry powders from solutions or suspensions, using either aqueous or non-aqueous solvents [17–37]. This paper covers

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Table 1
Liquid formulation details

Formulation	Composition before drying[a]
Hepatitis B vaccine formulations	
•Trehalose	27% vaccine suspension + 9% trehalose in water
•Trehalose/PVP	27% vaccine suspension + 5% trehalose + 4% PVP in water
•Lactose	40% vaccine suspension + 9% lactose in water
Live-attenuated measles vaccine	4.9% trehalose in reconstituted vaccine which contains Minimal Essential Medium (MEM) culture supernatant plus gelatin, sorbitol, L-histidine, L-alanine, tricine, L-arginine hydrochloride and lactalbumin hydrolysate
Relenza®	1% zanamivir + 4% lactose in water
Rifampin	2% rifampin in ethyl acetate

[a] All concentrations are w/w.

the formulation and micronization of four diverse products: recombinant hepatitis B surface antigen protein, live-attenuated measles vaccine virus, an 823 molecular weight antibiotic (rifampin) and a 332 molecular weight anti-viral (zanamivir).

2. Materials and methods

Hepatitis B vaccine, composed of hepatitis surface antigen (HBsAg) adsorbed onto aluminum hydroxide ($Al(OH)_3$) gel, was provided by Shantha Biotechnics Limited (Hyderabad, India). Measles vaccine consisting of a lyophilized preparation of Edmonston-Zagreb live, attenuated measles virus was provided by the Serum Institute of India (Pune, India). The anti-viral Relenza® was manufactured by GlaxoSmithKline, and rifampin was purchased from Sigma (St. Louis, MO). High purity trehalose and sucrose were purchased from Ferro Pfanstiehl (Waukegan, IL). Ethyl acetate, polyvinylpyrrolidone (PVP), lactose and surfactants Tween 80 and 10% Pluronic F68 where purchased from Sigma–Aldrich (St. Louis, MO). Table 1 shows the composition of the formulations reported in this paper.

The CAN-BD process equipment is shown in Fig. 1. The reader is also referred to previous papers for more details of

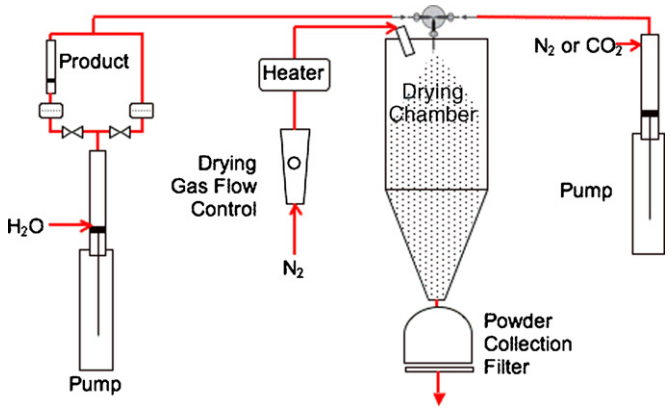

Fig. 1. Schematic of CAN-BD system.

CAN-BD processing [21,24,31]. Solutions containing the pharmaceutical were pumped through 1/16 in. outer diameter (OD) stainless steel tubing into one inlet of an Alltech 0.25 mm bore stainless steel tee (p/n 30771) at room temperature and 80 bar pressure. The nebulization fluid, either liquid carbon dioxide or liquid nitrogen, was pumped into the other inlet at the same temperature and pressure, also *via* 1/16 in. OD stainless steel tubing. Either Isco piston pumps for CO_2 or conventional HPLC pumps were used. The two-phase flow of product solution and nebulizing fluid exited the third tee connection, flowing into a 75 μm inner diameter (ID), 10 cm long fused silica restrictor (Polymicro TSP075375), connected to a glass drying chamber maintained at atmospheric pressure, into which was also pumped heated dry nitrogen gas. Rapid decompression from the restrictor to the drying chamber from 80 to 1 bar caused vaporization of liquid and dissolved nebulizing fluid (CO_2 or N_2), forming a fine aerosol plume of the product fluid, which was dried into a powder in the drying chamber by the heated nitrogen gas. The resulting powder was collected on a 0.45 μm pore size filter downstream of the drying chamber. After collection and placement in sample tubes with loose caps, powders were dried further with at least 24 h in a vacuum dessicator at room temperature. Table 2 shows additional experimental details by product.

Images of micronized powders were taken by a scanning electron microscope (ISI, model SX-30). Samples were prepared by adhering them to aluminum stubs using double-sided carbon

Table 2
Process Conditions

	Rifampin	Live Measles Vaccine	Hep B Vaccine	Relenza®
Product solute	Ethyl acetate $CH_3CH_2OC(O)CH_3$	H_2O	H_2O	H_2O
Nebulizing fluid	N_2	CO_2	CO_2	CO_2
Sample chamber (in-line, downstream of the water pump)	50 mL floating piston column	32 mL floating piston column	7 mL reservoir	7 mL reservoir
Drying chamber dimensions	2.4 L glass chamber	2.4 L glass chamber	3.5 L glass chamber	3.5 L glass chamber
Mixing tee pressure	1200 psig	1200 psig	1200 psig	1200 psig
Product and nebulizing fluid temperatures	Room Temperature	Room Temperature	Room Temperature	Room Temperature
Product flow rate	0.3–0.5 mL/min	0.3–0.5 mL/min	0.3–0.5 mL/min	0.3–0.5 mL/min
Nebulizing fluid flow rate	8 mL/min	1.5 mL/min	2 mL/min	2 mL/min
N_2 drying gas flow rate	30 LPM	30 LPM	30 LPM	30 LPM
Drying chamber outlet temperature	30 °C	50 °C	50 °C	50 °C