Application of supercritical fluid to preparation of powders of high-molecular weight drugs for inhalation[☆]

Hirokazu Okamoto *, Kazumi Danjo

Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan

Abstract

The application of supercritical carbon dioxide to particle design has recently emerged as a promising way to produce powders of macromolecules such as proteins and genes. Recently, an insulin powder for inhalation was approved by authorities in Europe and the USA. Other macromolecules for inhalation therapy will follow. In the 1990s proteins were precipitated with supercritical CO_2 from solutions in an organic solvent such as dimethylsulfoxide, which caused significant unfolding of protein. Since 2000, aqueous solutions of proteins and genes have generally been used with a cosolvent such as ethanol to precipitate in CO_2. Operating conditions such as temperature, pressure, flow rates, and concentration of ingredients affect the particle size and integrity of proteins or genes. By optimizing these conditions, the precipitation of proteins and genes with supercritical CO_2 is a promising way to produce protein and gene particles for inhalation.
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Contents

1. Introduction

Macromolecules such as proteins and genes having biological functions in the body are promising therapeutic agents if they can be delivered to target tissue or cells without losing their biological activity. Peroral administration is most convenient for patients; however, the peroral bioavailability of proteins and genes is generally low due to high-molecular weight and susceptibility to enzymes in the gastrointestinal tract. Intravenous, intramuscular, and subcutaneous injections are so far the most practical routes for administration of these macromolecules for systemic therapy.

The lungs have been used as a site for drug administration for the local treatment of respiratory diseases, such as bronchial asthma. There have been a number of basic studies in which protein solutions were administered to animals via the lungs. These studies showed that proteins, which are little absorbed from the small intestine, could be absorbed after intratracheal administration [1,2]. Recently, the pulmonary route has attracted attention as a noninvasive route of administering proteins and/or genes for systemic therapy as well as local therapy. For effective inhalation therapy, tiny particles with an aerodynamic diameter of less than 7 µm are required to deposit deep in the lung [3,4].

Pressurized metered-dose inhalers (MDIs), nebulizers, and dry powder inhalers (DPIs) are the three major delivery systems that produce tiny droplets or particles for aerosol inhalation in humans [3]. Among these, DPIs appear to be the most promising for future use because the device is small and relatively inexpensive, no propellants are used, and breath-actuation can be used successfully by many patients with poor MDI technique [3,4].

Proteins and genes can be formulated in micron-sized particles by several methods [5,6]. Milling is a simple method; however, the mean particle size and size distribution are relatively large for use in inhalation therapy. This process tends to denature proteins. Fluid energy grinding can produce 1–10-µm particles; however, the particles tend to be charged electrostatically. The precipitation of proteins from an aqueous solution can be achieved by the addition of an organic solvent to reduce the solubility or an acid or base to move the solution's pH to its isoelectric point. These methods have the problem of residual solvents or salts. Lyophilization is one of the most practical ways to produce protein and gene particles suitable for inhalation. However, it is time consuming and the particles obtained have a broad size distribution.

Spray drying is a useful and widely applied method of preparing powders for inhalation. However, it is likely that proteins are susceptible to degradation upon spray drying due to relatively high temperatures [7]. Spray drying of a 5-mg/mL aqueous insulin solution caused significant degradation of insulin at outlet temperatures above 120 °C [8]. β-Galactosidase activity is susceptible to the spray drying temperature and only half of the activity remained after spray drying without additives at an outlet temperature of 50 °C [9].

Fluids at temperatures and pressures above the critical values are known as supercritical fluids (SCFs). SCFs have unique thermophysical properties, i.e. a liquid-like density, large compressibility, and viscosity intermediate between that of a gas and liquid. In addition, the mass transfer rate is higher in a supercritical fluid than in a liquid, and the dielectric constant and solubility can be changed markedly with a small change of pressure around the critical level [10]. Carbon dioxide is the most widely used supercritical solvent, because it is cheap and nontoxic and has an easily accessible critical point (31.1 °C and 73.8 bar). Recently, the precipitation of particles using supercritical carbon dioxide has attracted much attention as an alternative way to prepare protein and gene powders suitable for inhalation. By optimizing the operating conditions, protein and gene powders having a suitable size for inhalation can be produced with little loss of activity and a high yield.

2. Drug absorption from the lung

2.1. Features of the lung as a site of drug absorption

The lung is an attractive site for drug absorption because of its wide surface area, thin epithelial membrane, large blood supply, and low levels of enzymatic activity. The total cross sectional area of respiratory bronchioles is about 10 m^2 and that of the alveoli in human is more than 100 m^2 as large as that of the small intestine [11]. The alveolar epithelium is so thin that drugs in alveoli only have to travel 0.5 to 1.0 µm to enter the blood stream. The total volume of fluid in the human lung is estimated to be approximately 10 mL [12]. A comparison of the pulmonary absorption of several electrolytes from buffered and unbuffered solutions in rats indicated the lung pH at the site of absorption to be about 6.6 [13]. The average weight of the human lung is as little as 0.6 kg; however, the blood flow is as rich as 5700 mL/min because the lung receives the entire cardiac output. This flow is more than 5 times that of the portal system (1125 mL/min) including the stomach and small and large intestines [14]. The systemic bioavailability of budesonide for the oral route is 11%, whereas that for the inhaled route is 73%. Fluticason propionate, which has 99% hepatic first-pass metabolism, has no pulmonary first-pass metabolism [15]. Although the metabolic activity of the lung is much weaker than that of the intestinal wall and liver, peptides such as insulin are subjected to enzymatic degradation in the lungs [16,17]. However, avoiding the hepatic first-pass effect by using the pulmonary route would overcome the disadvantage of pulmonary metabolism.