

Full Length Research Paper

Development of novel preparation method for rapid release of paclitaxel as a dry powder for inhalation: A preparation method for dry powder inhalers

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The purpose of this experiment was to develop a novel dry powder inhalation preparation method. Paclitaxel dry powder for inhalation was prepared by spraying a paclitaxel-ethanol solution directly onto an inhalable lactose carrier. This process was carried out under a relatively low temperature, avoiding potential damage to the drug from exposure to high temperatures. After preparation, the particle morphology, size, flowability, emitted dose, respirable fraction, and *in vitro* drug release were investigated. The results indicated that paclitaxel dry powder for inhalation showed a suitable flowability, and that the emitted dose and respirable fraction were more than 90 and 20%, respectively. The *in vitro* drug release also showed that there was greater than 95% release of paclitaxel from the dry powder within 5 min of inhalation. These results, along with the easily implementable and facile nature of preparation for this newly developed method, support the idea that fabricating dry powder for inhalation using this method is a useful and promising approach.

Key words: dry powder inhalation (DPI), spray drying, paclitaxel, lung cancer, immediate-release dosage form, pulmonary administration.

INTRODUCTION

Lung cancer is one of the most malignant cancers, that continues threaten the health and longevity of the

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population (Navani et al., 2022). In the past years, numerous countries have reported a significant increase in the incidence and mortality of lung cancer (Bade and Dela Cruz, 2020). These rapidly growing rates in both incidence and mortality, can be chiefly attributed to metastasis, as the lungs have the highest tumor metastasis rate of any organ (Romaszko and Doboszyńska, 2018).

To prevent metastases and treat lung cancer, surgery, chemotherapy, radiotherapy, or any combination of these, may be necessary (Miller and Hanna, 2021). Of these treatment options, chemotherapy remains the standard of treatment for lung cancer (Yang et al., 2021). However, current chemotherapeutic drugs for lung cancer have many inherent limitations (Duma et al., 2019), and these treatments generally carry significant potential for toxicities and adverse effects (Skribek et al., 2022), consideration of the most optimal route of administration is prudent.

Intravenous injection and lung inhalation are two common administration routes for lung cancer treatment. Injections are frequently utilized, but can be less favorable if they lack proper targeting. However, inhalation techniques allow drug to be introduced at the site of the malignancy (Wang et al., 2021). If a chemotherapeutic preparation can be made in the form of dry powder, it can directly carry drug through the bronchus to reach the alveolus pulmonis, making it a high-efficiency method of pulmonary administration (Lee et al., 2018). Unfortunately, many of the anti-tumor drugs, such as paclitaxel, docetaxel, doxorubicin, methotrexate, etc., have very poor water solubility, thus it would be advantageous to increase the dissolution rate of these drugs, and formulating a dry powder for inhalation using our developed method can be used to accomplish this task (Su et al., 2019).

Currently, there are a number of ways to fabricate dry powders for inhalation (Mehta, 2019). The classic way of preparation is to micronize the drug and use a carrier, like lactose, for the inhaler. There are many methods to micronize the drug, and some are more feasible than others. Examples of these methods include: solvent evaporation, spray drying, spray freeze drying, controlled crystallization, and the use of supercritical fluid technology.

Spray drying is a commonly utilized method that has been widely employed by the drug industry (Baumann et al., 2021). With this method, it is easy to produce dry powder for inhalation (DPI) by spray drying the mixture of drug and carrier (typically α -lactose monohydrate). This method is promising due to the feasibility for large scale production. However, because spray drying requires high temperatures, the drug may be altered using this method. In addition, the process of spray drying can be further complicated by the nuances which may affect the final product, such as, inlet temperature, atomizing pressure, air speed of drying, etc.

Spray freeze drying (SFD) may be more suitable for

drugs or molecules that become destabilized when subjected to high temperatures (such as proteins, peptides, DNA, insulin, etc.) (Rostamnezhad et al., 2021). SFD also controls the particle diameter down to even potentially the nanometer level, well within the range for inhalable particles (less than 5 μm diameter). Particles prepared by SFD have better stability and dispersibility. Unfortunately, the operation of SFD is complicated, time-consuming and costly.

Another useful method for micronizing drugs, especially those which are hydrophobic, is the controlled crystallization method (Gao et al., 2015). This entails adding stabilizers (such as polymer material or other surfactants) into an anti-solvent, causing the hydrophobic drug to be immediately crystallized. However, in order to obtain a suitable and controlled particle size, a high concentration of crystal growth inhibitors, stabilizers, and other ancillary products are generally required. Use of such products can negatively impact the purity, and may lead to increased toxicity.

Taken together, micronization itself is a complicated process, and though there are numerous different preparation methods for micronization, most of them lack feasibility for chemotherapeutic agents because of the complexity and/or costly preparation processes involved for DPI. So, in this study, to circumvent the shortcomings of the existing preparation technology for DPI, we have developed a novel DPI preparation method that does not rely on micronization for success. A Paclitaxel DPI was prepared by using a nano atomizer, and then characterized by the physicochemical properties, *in vitro* deposition characteristics, and *in vitro* drug release. Using this method, the particle size of the powder for inhalation was easy to control, and the drug dissolution rate was highly improved. This will likely provide increased bioavailability and drug efficacy. Additionally, using a nano atomizer is straightforward, negating the need for complicated technology and costly machinery, making it suitable for scaling of mass production.

MATERIALS AND METHODS

Paclitaxel (Energy Chemical, China, Products NO. DDZ7004, Purity 97%); Lactose (Inhalation 400, Meggle Pharma-Excipients and Technology, Shanghai, China). All other reagents were analytical grade with no processing before use.

Preparation of paclitaxel DPI

Paclitaxel solution, 2 mg/ml in 100% ethyl alcohol, was sprayed onto lactose using a nano atomizer (KH1080, Liangdian Technology Co., Ltd., Tianjin, China). Then, the lactose carrier with paclitaxel was dried at 80°C for 2 h, followed by passing the formulation through a 120 mesh sieve once, and a 240 mesh sieve 10 times. A suitable size capsule was used to hold Paclitaxel DPI.

Particle morphology

Particle morphology of the Paclitaxel DPI was visually analyzed

using scanning electron microscopy (ZEISS GeminiSEM 500 Scanning Electron Microscope, Carl Zeiss Co., Ltd, Germany) at operating accelerating voltages of 3.0 kV. Samples were placed on carbon tape and then coated with 15 nm thickness of gold.

Particle size analysis

Particle size was determined by laser diffraction technique using a Mastersizer MS2000 (Malvern Instruments, Worcestershire, UK). Each analysis was performed in triplicate.

Angle of repose

The material was dropped freely through a funnel to create a pile roughly 5 cm in height (Tan et al., 2015). The angle of repose was calculated by inverting tangentially the ratio of height and radius of the pile. Each sample was performed in triplicate.

Density and Carr index

To determine tapped and bulk densities, samples were put into a 5 ml cylinder, and initial volume was recorded. Then samples were tapped until there was no obvious change in volume, and this volume was recorded. The tapped density is reported as $\rho_{\text{tapped}} = \text{weight of sample} / \text{final volume}$, and bulk density is $\rho_{\text{bulk}} = \text{weight of sample} / \text{initial volume}$. Carr index was calculated according to the following equation (Tan et al., 2015):

$$\text{Carr index} = (\rho_{\text{tapped}} - \rho_{\text{bulk}}) / \rho_{\text{tapped}} \times 100\%$$

Emitted dose

Emitted dose (ED%) was measured by a Twins stage impinge (TSI) in accordance with the Pharmacopoeia of People's Republic of China (ChP, 2015 edition, Part IV, appendix 0951). Simply, each capsule was suctioned by TSI at a $60 \pm 5 \text{ L} \cdot \text{min}^{-1}$ airflow rate for 10 s, 4 times. The initial weight of 10 drug-containing capsules is m_1 , and the weight of capsules after suction is m_2 . After the capsules were cleaned, the weight of empty capsules was recorded and is reported as m_3 . Emitted dose was calculated according to the following equation:

$$\text{ED}\% = \frac{m_1 - m_2}{m_1 - m_3} \times 100\%$$

Respirable fraction, RF%

Respirable fraction was measured using the same method as emitted dose outlined above. Briefly, 7 and 30 ml of distilled water were put into the first and the second receiving bottles, respectively. The drug content of the second receiving bottle was measured by High-performance liquid chromatography (HPLC), and RF was calculated using the ratio of drug content in the second receiving bottle to the expected dose.

In vitro drug release

A rotating-basket method was used for the *in vitro* drug release analysis. The method was as follows: samples were put in phosphate buffered saline (PBS) (pH 7.4) with 0.2% Tween 80 and

stirred at a speed of 100 rpm at 37°C. The release fluid was collected and analyzed at predetermined times (1, 2, 5, 10, 15, and 20 min). The concentration of PTX in the release solution was determined by HPLC (Hong et al., 2019). Samples were analyzed with a Shimadzu HPLC system (Kyoto, Japan), consisting of an LC-20AT pump and a SPD-20AV UV detector. A Hypersil ODS2 column (250 × 4.6 mm, 5 μm, Dalian Elite Analytical Instruments Co. Ltd., China) was used for the separation of analytes. The mobile phase for gradient elution consisted of methanol and water with a volume ratio of: 70: 30 (v/v). The flow rate was 1.0 ml/min and the detection wavelength was 227 nm.

RESULTS AND DISCUSSION

Scanning electron microscopy

Paclitaxel DPI was prepared by spraying paclitaxel solution directly onto inhalation 400 lactose. Representative scanning electron micrographs (SE) of inhalation 400 and Paclitaxel DPI are shown in Figure 1. Most of the particles appear as irregular blocks, and some of the particles appear spherical. The SE micrographs confirmed that the spraying of paclitaxel solution to Inhalation 400 had no impact on its morphology. Compared with Figure 1 (A) and (B), (C) and (D) show more small paclitaxel particles that are adsorbed onto the larger lactose particles.

The particle size distribution of the Inhalation 400 and Paclitaxel DPI were determined and are shown in Table 1. After DPI preparation, the particle size decreases, as noted with the median particle size (d_{50}) decreasing from 8.16 to 6.15 μm. This reduction in median is assumed to be contributed to the mixture of relatively smaller paclitaxel particles with the lactose particles, reducing the average particle measured.

The flowability of the DPI was suitable based on the data collected for the angle of repose and the calculated Carr index (Table 1). Each angle of repose was smaller than 45°, showing that the mobility of the particles is sufficient to meet the requirements for production and use of DPIs. Each Carr index was below 0.25, which is considered favorable for flowability. ED% and RF% were measured and are shown in Table 1. The data showed that the ED% was greater than 90%, meaning the majority of the powder was able to be removed from the capsule after inhalation. RF% was greater than 20%, which means the drug can effectively reach the lungs. Because the lactose carrier is quite small, it is able to be carried with the air flow into the lungs, and at the same time, paclitaxel can desorb from lactose carrier.

Paclitaxel is insoluble in water, which means it will dissolve slowly after application, resulting in low bioavailability. Therefore, rotating-basket method was employed for the *in vitro* paclitaxel release analysis (Figure 2). It can be noted that at 5 min, there is greater than 95% release of paclitaxel from the DPI, while the physical mixture releases only about 70%. This is likely because the DPI is produced by spraying paclitaxel onto

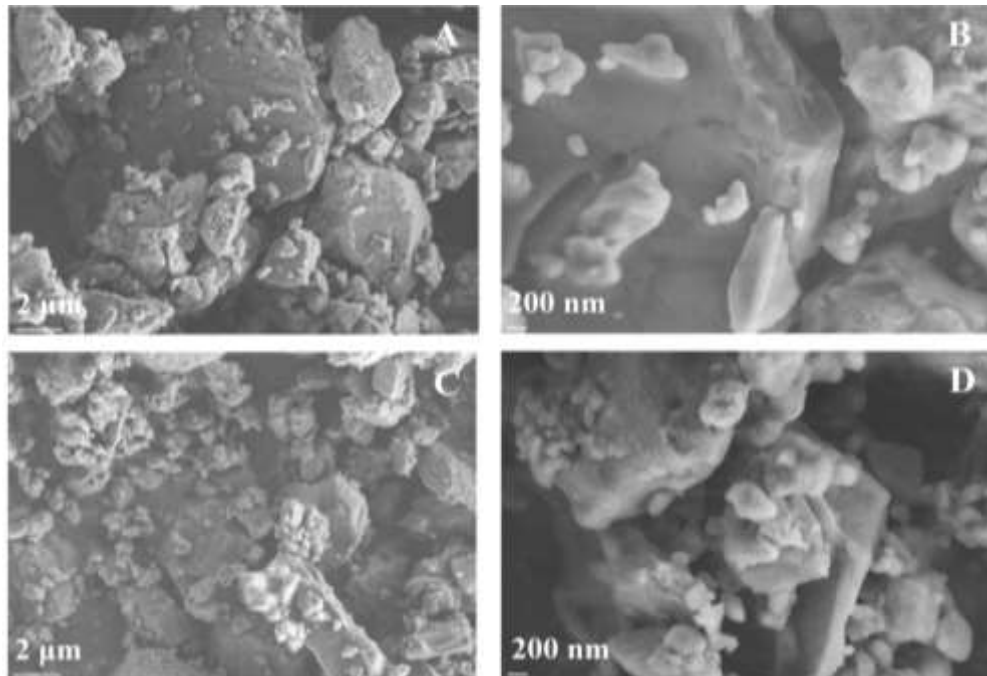


Figure 1. SE micrographs of Inhalation 400 (A), (B), Paclitaxel DPI (C), (D).

Table 1. Particle size, angle of repose, bulk and tapped density, Carr index, ED%, and RF% of Inhalation 400 and Paclitaxel DPI (n=3).

Parameter	Size			Angle of repose	Carr index	ED%	RF%
	D (0.1)	D (0.5)	D (0.9)				
Inhalation 400	1.23±0.05	8.16±0.21	28.10±0.25	40.54±1.23°	0.23± 0.04	-	-
Paclitaxel DPI	1.01±0.03	6.15±0.15	20.90±0.22	41.78 ±1.18°	0.24± 0.06	94.7%	27.4±2.25%

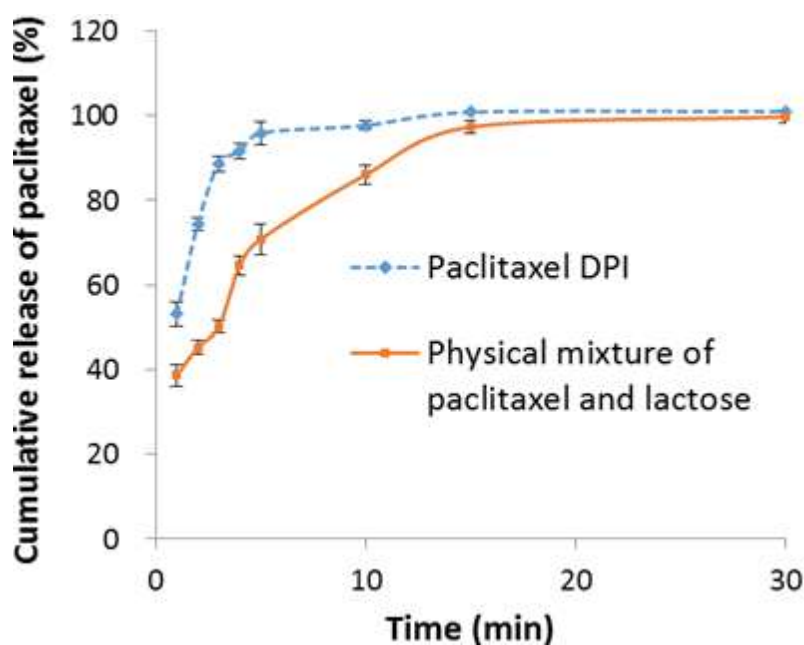


Figure 2. Cumulative release curve of paclitaxel (%).

lactose, resulting in a smaller size and higher dispersity of paclitaxel, thus allowing a faster dissolution rate. As such, the DPI will have a higher bioavailability, which can contribute to greater drug efficacy.

Conclusion

Chemotherapy is still the standard method for the treatment of lung cancer. However, due to side effects and toxicities, researchers have put much consideration into the administration route of chemotherapeutic drugs for lung cancer treatment. The development of dry powder for inhalation has been beneficial for accomplishing direct drug delivery to lung tumors. However, there are numerous obstacles to utilizing this administration route. Thus, we developed a novel preparation method for dry powder inhalation to circumvent these challenges associated with dry powder fabrication. Using atomization to spray antitumor drugs, like paclitaxel, the drugs can be highly dispersed onto carrier lactose. This improved solubility, and increased bioavailability for the treatment on lung cancer. This method of fabrication for powder aerosol preparation holds great promise in improving the delivery of treatment, and potentially the lives of those affected by lung cancer. This method certainly warrants further research for expounding on drug loading characterizations, as well as *in vivo* evaluations for safety and efficacy.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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