

Abstract

Direct administration of drugs to the lungs has been used for millennia as a major treatment for a number of diseases. Origin of the inhalation therapies can be found 2000 B.C in India, where people were used to smoke *Atropa belladonna* leaves to suppress cough.

In 1986, researchers at Genentech Inc. (San Francisco, USA) discovered that the hormone of the human growth was naturally absorbed into systemic circulation of rats after its instillation into their lungs. Thus, the development of new inhalation medicines for both local and systemic administration raised a growing interest of academic and industrial researchers in the last 30 years. The first breakthrough for the treatment of a chronic systemic disease *via* inhalation was the inhaled insulin (Exubera®, Pfizer, New York, USA), available in USA from 2006 to 2007, then withdrawn from the market for economic reasons. The idea that serious diseases, such as diabetes, could be treated by pulmonary administration was going to be abandoned, until FDA (2014) decided to approve a new form of inhalable insulin, Afrezza® (Sanofi and MannKind, USA), obtained by a synergy of an innovative inhaler device Dreamboat™ and the Technosphere® technology.

Although pulmonary route is currently being exploited in ways never imagined before, local pulmonary drug delivery remains the preferred route for the administration of drugs to treat lung diseases, including tuberculosis, asthma, COPD and Cystic Fibrosis (CF).

A drug administered by the pulmonary route directly targets the airways with minimized systemic side effects, rapid pharmacologic response and reduction in the required dose. Traditional inhalers, namely MDIs (Metered Dose Inhalers), incorporate a propellant into the formulation, which provides the energy for aerosolization upon actuation. The MDIs major drawback is the need that patient must well coordinate both inhalation and actuation. Solvent- and propellant-free DPIs (dry powder inhaler) are breath-actuated, hence removing the coordination requirement above. Moreover, it has to be noted that an inhaler must 1) allow powder dispersion upon inhalation at reasonable flow rates, 2) have flow-rate-independent performances. As the dry powder formulation and the device have to be intrinsically linked to obtain a unique inhalation product, DPI is considered one of the most complex pharmaceutical product.

It is well known that a good deposition into the lung requires particles with an aerodynamic diameter in the range 1 to 5 μm . Different technologies are available to successfully produce inhalation medicines with desirable characteristic, including particle shape, size, adhesiveness, morphology and roughness.

However, no standardized methods and correlated regulatory requirements are available to predict the fate of particles after the lung deposition. Hence, the old concept of pulmonary drug delivery, which states that “efficient aerosol generation and particle deposition in the lung are the main and only challenges for effective inhalation therapy”, is no longer valid (Ruge *et al.* 2013).

The lack of standardized methods for the dissolution testing hinders a complete knowledge of the processes occurring after particles deposition in the respiratory tract. The Biopharmaceutics Classification System (BCS) established by Amidon and co-workers (Amidon *et al.* 1995) for the gastrointestinal absorption, predicting the *in vivo* pharmacokinetics of the drugs, is not transferable to pulmonary case. Lung administration requires an *ad hoc* study taking into account the lung specific biology (metabolism, clearance, mucus and surfactant) as well as the characteristics of formulation and solubility of drugs, as those parameters affect the pulmonary bioavailability.

Moreover, in some pathologies, such as cystic fibrosis (CF), the presence of a thick viscid mucus may reduce the efficacy of the inhalation therapy. Thus, the study of drug–mucus interaction is a crucial step in CF to check the ability of the drug to penetrate and distribute through airways surface fluids.

In the last decade, the Research Group in Pharmaceutical Technology of the University of Salerno has been involved in developing new dry powders for inhalation. Currently, the Group has active projects in this area addressing topics such as development of DPIs containing antibiotic, anti-inflammatory and antioxidant drugs. In this frame, the aim of the present PhD project was to design inhalable powder-based formulations that could improve the treatment of pulmonary diseases, mainly cystic fibrosis. Then, the first step of the project was to formulate in a respirable form the Ketoprofene lysine salt, a nonsteroidal anti-inflammatory drug (NSAID) using the well-known Mini Spray Drying and the innovative Nano Spray Drying technology, and to evaluate limits and strengths of these different techniques. Moreover, the research focused on *in vitro* assays to evaluate the aerodynamic behavior through the respiratory system of the produced powder, using the *monodose* DPI as device for the powder aerosolization.

In the second part of the project, an *in vitro* method based on Franz-type diffusion equipment was proposed to predict the fate of drugs after deposition and to study drug dissolution/permeation processes. Moreover, to better mimic the pulmonary environment, permeation properties of the drug were evaluated through artificial and/or native CF mucus layer. To this purpose, a mucus model was prepared taking in account physico-chemical composition and rheological behavior of CF bronchial sputum.

The final part of the project was performed at the Woolcock Institute of Medical Research in Sydney, under the supervision of Professors Daniela Traini and Paul M. Young. The research was aimed to study permeation processes of several antibiotics across Calu-3 cell line to obtain key information for the future formulation of inhaled products.

Specific objectives of the project were:

- *i)* design and development of Dry Powder Inhalers containing Ketoprofene lysine salt micronized powders by Mini and Nano spray drying production; *ii)* optimization of the aerodynamic characteristics of the powders, through the use of selected and safe excipients (amino acids) able to improve the powder flow properties and dispersion which, in turn, may increase lung deposition of the drugs (SECTION A).
- *i)* optimization and development of a model of Cystic Fibrosis artificial mucus for the permeation experiments; *ii)* rheological characterization of Cystic Fibrosis mucus patients; *iii)* permeation studies of developed formulations through both artificial and native CF mucus (SECTION B).
- investigation of the correlation between physico-chemical properties of different antibiotics, such as molecular weight, solubility, LogP and calculated permeability and their transport across Calu-3 cell line (SECTION C).