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Abstract: In vitro dissolution testing is routinely used in the development of pharmaceutical products. Whilst the dissolution testing methods are well established and standardized for oral dosage forms, i.e. tablets and capsules, there are no pharmacopoeia methods or regulatory requirements for testing the dissolution of orally inhaled powders. Despite this, a wide variety of dissolution testing methods for orally inhaled powders has been developed and their bio-relevance has been evaluated.
The review provides an overview of the in vitro dissolution methodologies for dry inhalation products, with particular emphasis on dry powder inhaler, where the dissolution behavior of the respirable particles can have a role on duration and absorption of the drug. Dissolution mechanisms of respirable particles as well as kinetic models have been presented. A more recent bio-relevant dissolution set-ups and media for studying inhalation biopharmaceutics were also reviewed. In addition, factors affecting interplay between dissolution and absorption of deposited particles in the context of biopharmaceutical considerations of inhalation products were examined.

Cover Letter

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Dr. Martin Brandl
Editor in Chief
European Journal of Pharmaceutical Sciences

Parma, August 28th, 2017

Object: Submission of the review manuscript to the Special Issue "Siminhale" of European Journal of Pharmaceutical Sciences

Dear Dr. Brandl,
on behalf of all co-authors I am pleased to submit to your attention the revised version of the manuscript (Ms. No. EJPS-D-17-00819) entitled "*Dry powder inhalers: an overview of the in vitro dissolution methodologies and their correlation with the biopharmaceutical aspects of the drug products*", authored by S. Velaga, J. Djuris, S. Cvijic, S. Rozou, P. Russo, G. Colombo, A. Rossi.
We would like to thank the positive comments of the two reviewers and all the proposed changes have been addressed to improve the manuscript.

The review provides an overview of the *in vitro* dissolution methodologies for inhalation products, with particular emphasis on dry powder inhaler, to assess the dissolution behavior of the respirable particles. Factors affecting interplay between dissolution and absorption of deposited particles in the context of biopharmaceutical considerations of inhalation products will be also examined.
I state that no conflict of interest exists concerning the manuscript and the manuscript has not been published elsewhere and is not under simultaneous consideration by any other journal. Furthermore, the publication of this work is approved by all authors and tacitly or explicitly by the responsible authorities where the research work was carried out. If accepted, the manuscript will not be published elsewhere in the same form without the written consent of the copyright-holder.
I look forward to having our manuscript reconsidered for publication in the Special Issue "SimInhale" of the European Journal of Pharmaceutical Sciences.

Yours sincerely

Alessandra Rossi

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RESPONSES TO THE REVIEWERS

Reviewer #1

GENERAL RESPONSES

This review pertaining to the in vitro dissolution of dry powder inhalers is a good one. It uses a good range of literature and it provides some nice summary tables.

Answer. The Authors are grateful for the very positive feedback of the manuscript.

SPECIFIC QUERIES AND ANSWERS

Q1. A specific section should be inserted to review the effects of solid-state properties on dissolution and choice of dissolution methods

A1. A text related to the effects of solid state properties of drug on dissolution has been inserted in the manuscript.

Q2. A section should be added to review the use of dissolution of powders without performing deposition studies and the dissolution of powders that have been through a deposition process. These two types of dissolution experiment are very different and this has not been appropriately highlighted in the current document.

A2. We agree with the reviewer's comment. We think that the different types of dissolution methods are described in sections 3 and 5.

Q3. The sources of the equations on page 12, 13 and 14 should be made clear, did the authors derive them or are they taken from previous work?

A3. We appreciate the suggestion. The sources of the equations have been clarified in the revised manuscript.

Q4. The original references for the equations on pages 25 and 26 should be inserted to give the reader access to the literature that supports these models as they are only suitable in a given set of scenarios which is indicated in the original documents.

A4. As suggested, the original references were added.

Q5. The formatting of table 2 should be modified to make it easier to read, at present it is difficult to read across the lines because the information is condensed into a small space.

A5. For the clarity of information, we have modified the table. Still, we kept all the data, because we believe this is important for the review. We hope that the modified form complies with the reviewer expectations.

Q6. It would be good to add some critique of the dissolution models used to analyze the data as it is not clear what practical difference in dissolution rate indices the different models would actually make.

A6. A text was added in section 3.4.

Reviewer #2

GENERAL RESPONSES

This is a very interesting and well written review. The topic is clearly explained and of first importance when developing dry powder for inhalation.

Answer. The Authors thank the reviewer for the very positive feedback of the manuscript.

SPECIFIC QUERIES AND ANSWERS

Q1. In the graphical abstract: It should be Mucociliary clearance and not "muciciliary"

A1. We have modified the graphical abstract and Figure 2 accordingly.

Q2. In the table 1: there are very few DPI to treat pulmonary infection. Maybe the authors could add the colobreathe (CMS) product from Forest Laboratories.

A2. We thank the reviewer for the suggestion and the product was added in Table 1.

Q3. In figure 1: Maybe the authors should add the drug molecular weight as parameter influencing the drug permeability

A3. The reviewers' suggestion has been accepted and Figure 1 was modified.

Q4. Line 146 : Is the volume of 10-30 mL for the mucus layer (gel) only or it also includes the aqueous layer (sol) where are the ciliated cells?

A3. The reviewer made a good point. Since mucus is the major part of the lung lining fluid, some authors state 10-30 ml as the volume of mucus (Hastedt, 2014). However, this is also stated as the volume of lung lining fluid in the conducting airways (Hastedt et al, 2016; Frohlich et al, Measurements of Deposition, Lung Surface Area and Lung Fluid for

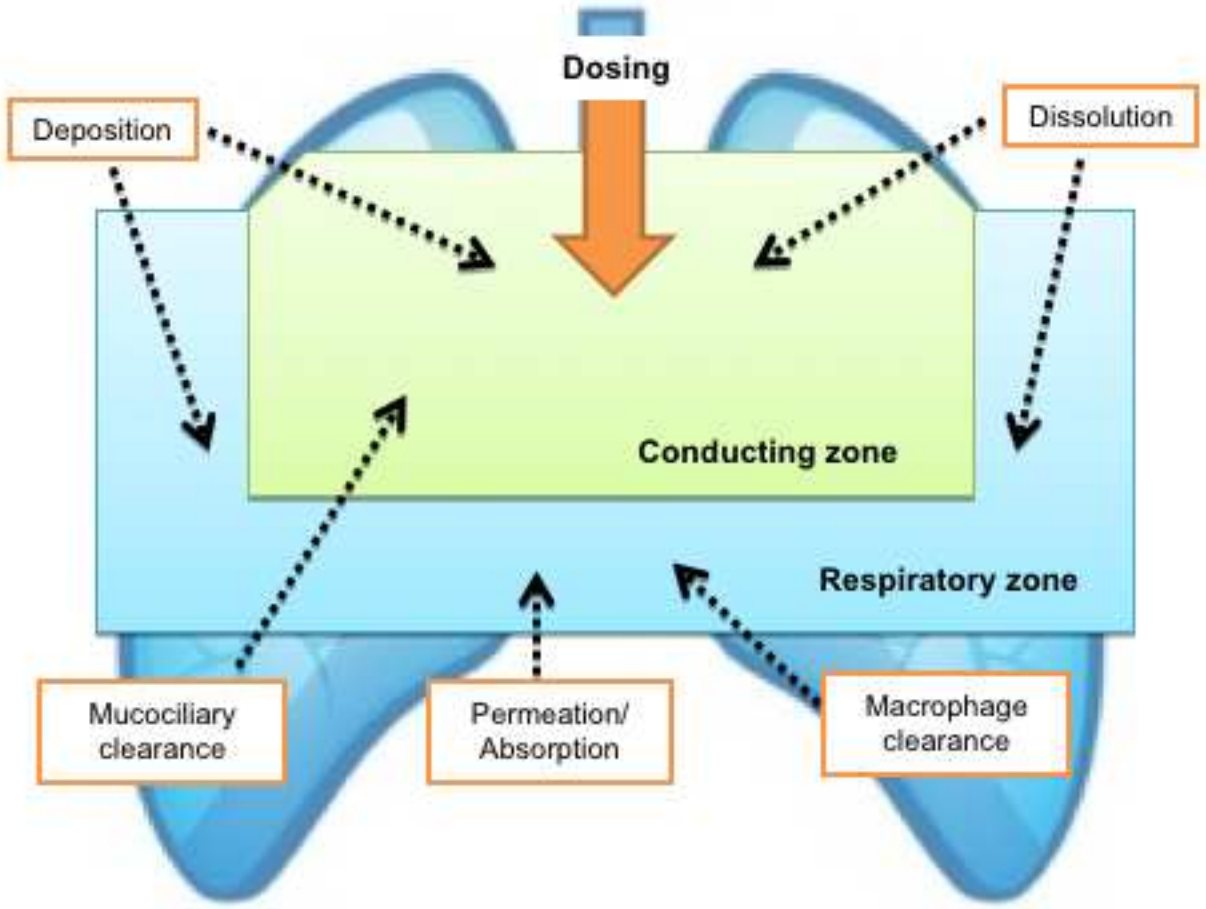
Simulation of Inhaled Compounds, Front. Pharmacol.7:181; 2016). Also, literature data regarding the volume of LLF differ depending on the source and method of determination, and according to Frochlich et al, 2016, there is no optimal method to determine the volume of the LLF. Determination of the precise volume of the mucus layer would be even more challenging.

Therefore, we have modified the text and stated 10-30 ml as the volume of the lung lining fluid.

Q.4. Line 461: the numbering is wrong (should be 3.2)

A4. The number of section 3.2 was corrected.

*Graphical Abstract (for review)



Dry powder inhalers: an overview of the *in vitro* dissolution methodologies and their correlation with the biopharmaceutical aspects of the drug products

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Abstract

In vitro dissolution testing is routinely used in the development of pharmaceutical products. Whilst the dissolution testing methods are well established and standardized for oral dosage forms, i.e. tablets and capsules, there are no pharmacopoeia methods or regulatory requirements for testing the dissolution of orally inhaled powders. Despite this, a wide variety of dissolution testing methods for orally inhaled powders has been developed and their bio-relevance has been evaluated.

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Keywords:

pulmonary delivery, dry powder inhalation, solubility, dissolution methods, biopharmaceutical classification

Abbreviations:

API- Active pharmaceutical ingredient

ACI – Andersen cascade impactor

ALF – alveolar lung fluid

BCS – Biopharmaceutics Classification System

iBCS – Biopharmaceutics Classification System for inhalation products

CFC – Chlorofluorocarbons

COPD – Chronic obstructive pulmonary disease

DPI – Dry powder for inhalation or Dry powder inhaler

DPPC – dipalmitoylphosphatidylcholine

EMA – European Medical Agency

FDA – Food and Drugs Administration

FPF – Fine particle fraction

HFA – Hydrofluoroalkanes

IVIVC – In vitro-in vivo correlation

NGI – Next generation impactor

OIDP – Orally inhaled drug product

QbD – Quality by Design

PBS – phosphate buffer solution

SDS – Sodium lauryl sulfate

SLF – simulated lung fluid

TPGS – D-α-tocopherol polyethylene glycol 1000 succinate

USP – United States Pharmacopoeia

1. Introduction

Today, lungs are considered a common route for the administration of therapeutics not only for the treatment of local pulmonary diseases like asthma, COPD, bronchiectasis, lung infections, but also to achieve systemic effect (e.g. insulin in diabetes).

Dry powder inhalers (DPIs) become quite popular devices for pulmonary drug administration. The reasons for popularity are that these devices are easy to handle and patients comply better than with metered dose inhalers (MDIs); moreover, they afford higher stability of the product since the drug is in the solid state. Though systemic drug delivery applications are emerging, DPIs have mainly been used for the treatment of local inflammation or infections in the lungs (e.g. asthma, COPD and cystic fibrosis infections) (Virchov, 2005; Usmani et al., 2005; Demoly et al., 2014). For an effective and safe inhalation therapy, a DPI must reproducibly deliver an adequate fine particle dose (FPD) to the site of action (receptor, infection, absorption site) in the respiratory tract (Demoly et al., 2014). The inhaler design and powder formulation are major determinants in meeting those requisites. Also, correct use of the inhaler and adherence to therapy is important. In general, API powder with aerodynamic particle size < 3 µm shows high FPF and peripheral lung deposition (Corradi et al., 2014).

Currently, marketed DPIs are either pre-metered (unit-dose in cartridges or capsule) or device metered (multiple doses stored in a device reservoir), both are breath activated. Table 1 reports a non-exhaustive list of the DPI products commercially available in US and EU market (Berkenfeld et al., 2015; Muralidharan et al., 2015). As for the DPI formulation, two strategies have been generally employed: (i) micronized drug adhered to coarse carrier particles (often lactose monohydrate) by

ordered mixing (adhesive mixtures) or (ii) carrier free formulation where the drug is spheronized into loose aggregates (de Boer et al., 2012). Aerodynamic size of formulated particles affects predominantly their deposition, and is a function of the drug-carrier agglomerate size, density and shape characteristics (Riley et al., 2012). The drug dissolution process is dependent not only on the deposition site but also on the physicochemical characteristics of the particles.

In the last decades, great attention has been devoted to establish a dissolution method that can appropriately characterize the *in vitro* behavior of particles from DPI (Davies and Feddah, 2003; Son and McConville, 2009; May et al., 2012 and 2014; Riley et al., 2012, Forbes et al., 2015).

Table 1

Examples of DPI drug products available on US* and/or EU# market.

Drug Product	Drug	Indication	Device type	Company
Tudorza® Pressair®*	Aclidinium bromide	COPD	Multi dose (reservoir)	Forest Pharmaceuticals Inc./Almirall
Foster NEXThaler#	Beclomethasone dipropionate/formoterol fumarate	Asthma/COPD	Multi dose (reservoir)	Chiesi
Pulmicort Flexhaler*	Budesonide	Asthma	Multi dose (reservoir)	Astra Zeneca
Colobreathe® Turbospin*	Colistimethate sodium	Cystic fibrosis infection	Single dose (capsule)	Forest Laboratories
Flovent Diskus*	Fluticasone propionate	Asthma	Multi dose premetered	GSK
Foradil Aerolizer*	Formoterol fumarate	Asthma/COPD	Single dose (capsule)	Novartis
Afrezza#*	Insulin humane	Diabetes	Single dose (cartridge)	Sanofi Aventis
Adasuve#*	Loxapine	Schizophrenia/bipolar disorder	Single dose	Teva
Asmanex Twisthaler*	Mometasone furoate	Asthma	Multi dose	Schering

			(reservoir)	
Buventol Easyhaler[#]	Salbutamol sulphate	Asthma/COPD	Multi dose (reservoir)	Orion
Serevent Diskus*	Salmeterol xinofoate	Asthma/COPD	Multi dose premetered	GSK
Seretide Diskus[#]	Fluticasone propionate/ Salmeterol xinofoate	Asthma/COPD	Multi dose premetered	GSK
Advair Diskus*	Fluticasone propionate/ Salmeterol xinofoate	Asthma/COPD	Multi dose premetered	GSK
Spiriva Handihaler*	Tiotropium bromide	COPD	Single dose (capsule)	Boehringer Ingelheim
Toby Podhaler[#]*	Tobramycin	Cystic fibrosis infection	Single dose (capsule)	Novartis
Relenza Diskhaler*	Zanamivir	Influenza	Multi dose (blister)	GSK

Traditionally, dissolution testing has been used as a valuable tool for: (i) formulation development, and (ii) bioequivalence investigations. However, currently there is no official *in vitro* drug release compendia method for aerosol products. It's not an easy task to reproduce *in vitro* the lung conditions. However, the dissolution can be useful for establishing differences related to the inclusion of different excipient in the formulation (Buttini et al., 2014).

This review presents a comprehensive overview of published research on the DPIs dissolution methodologies, with the intent to highlight the emerging need for dry powder dissolution methods. We will also discuss biopharmaceutical considerations for inhalation powders to provide an evidence of the importance of the interplay between particle deposition, dissolution, absorption and clearance.

2. Biopharmaceutical considerations for inhalation products

Biopharmaceutical characterization of inhaled medicines is rather challenging, as a number of factors influences the bioperformance of the final product (Fig. 1). Distinctiveness of lungs anatomy and physiology is one of the key determinants of inhaled drugs biopharmaceutical properties. Human lungs can roughly be divided into two functionally diverse zones: conducting zone that comprises trachea, bronchi, bronchioles and terminal bronchioles, and respiratory zone, that consists of respiratory bronchioles, alveolar ducts and alveolar sacs.

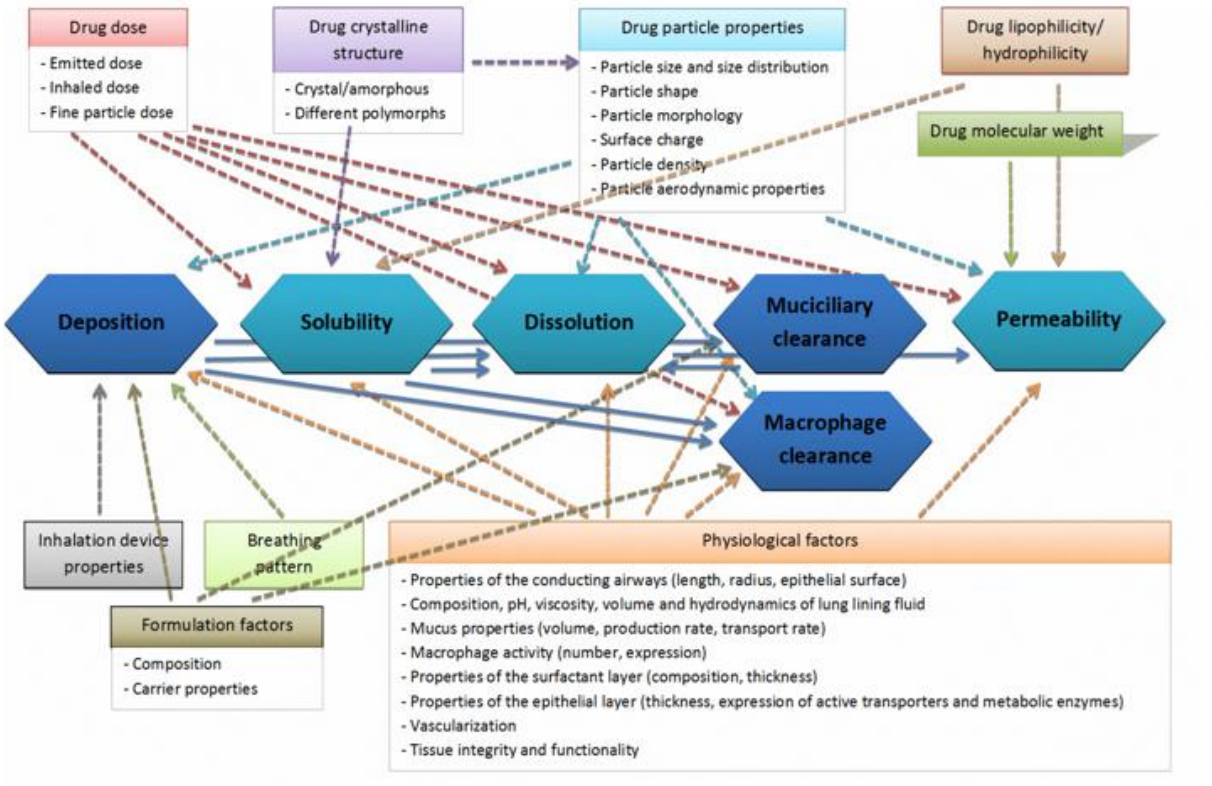

Fig. 1. Complex interplay among the factors affecting the key biopharmaceutical properties of inhaled drugs.

144 The dominant fluid in central conducting parts of lungs is the mucus layer covering
145 the apical surface of epithelial cells. This is the major part of the lung lining fluid
146 which has an approximate volume of 10-30 ml. Mucus thickness is around 3-15 μm,
147 with lower values in distal airways (Hastedt, 2014). Due to high viscosity of this layer,
148 drug particles are “trapped” and cleared by mucociliary escalator (or mucociliary
149 clearance) or diffused through it to reach the epithelium cells (Yang et al., 2008),
150 where only a small portion of inhaled particles is absorbed (Byron and Patton, 1994).
151 Physicochemical properties of the particles and physiological characteristics of the
152 gel on the site of deposition affect the diffusion across (muco-penetration/muco-
153 adhesion phenomena) (Sigurdsson et al., 2013; Smart, 2005). Pharmacokinetic
154 studies have demonstrated that for slowly dissolving drugs, a significant portion of the
155 deposited drug will be removed from the upper parts of the lung by the mucociliary
156 clearance and swallowed (Hochhaus et al., 2015).
157 Alveolar epithelium is composed of the monolayer of type I and type II cells, which
158 are the sites of the pulmonary absorption and secretion of the lung surfactant,
159 respectively (Patton and Byron, 2007). Alveolar fluid acts as a physical protection
160 against inhaled particles, but it also works as a solvent for various mediators of the
161 lung function, including lung surfactant molecules, cytokines, etc. (Marques et al.,
162 2011). Lung surfactant is a lipoprotein complex composed of phospholipids
163 (predominantly DPPC), proteins, neutral lipids (cholesterol) and traces of other
164 substances. This layer is much thinner in comparison to mucus (~0.07 μm), with an
165 estimated volume of approximately 7-20 ml (Hastedt et al., 2016), 36 ml (Fronius et
166 al., 2012), 50 ml (Clark et al., 2006) or 10-20 ml per 100 m^2 of the lung surface area
167 available for deposition (Gray et al., 2008). The presence of surfactant in the alveolar
168 fluid can promote the solubility of the drug, and consequently the dissolution of poorly

water-soluble drugs. In addition, pulmonary surfactant has good spreading capabilities, facilitating transport and preventing adhesion of inhaled particles. It also helps drug diffusion through the air-liquid interface.

Particles deposited in the alveolar region are exposed to alveolar macrophage clearance, endocytosis or other clearance mechanisms (Patton, 1996; Nel et al., 2006). The main role of macrophages is to remove insoluble or slow dissolving particles from the lung surfaces by phagocytic uptake (Geiser, 2010; Forbes et al., 2014). Altered particle properties (size, shape, surface charge, rugosity) may influence the fate of the drug, and therefore particle engineering techniques can be used to manipulate drug uptake.

The amount of inhaled dose, available for local action or systemic absorption, also depends upon regional particle deposition. This phenomenon is influenced by a number of factors, including physical properties of the inhaled particles (particle size, density and shape), lung geometry, breathing pattern and ventilation (Schulz et al., 2000).

The influence of drug and formulation properties on the bioperformance of inhaled drugs should be considered in conjunction with the physiological conditions and specific phenomena that happen *in vivo*, as mentioned above. A simplified scheme of the lung compartments illustrating the interplay between particle deposition, dissolution, absorption and clearance is presented in Figure 2.

The overall concentration of a drug in the lung can vary from a few µg/ml to several mg/ml, depending on the dosage. Moreover, due to regional variations in liquid volume, and specific particle deposition pattern of inhalation product, there may be extreme variations in drug concentration between lung compartments.

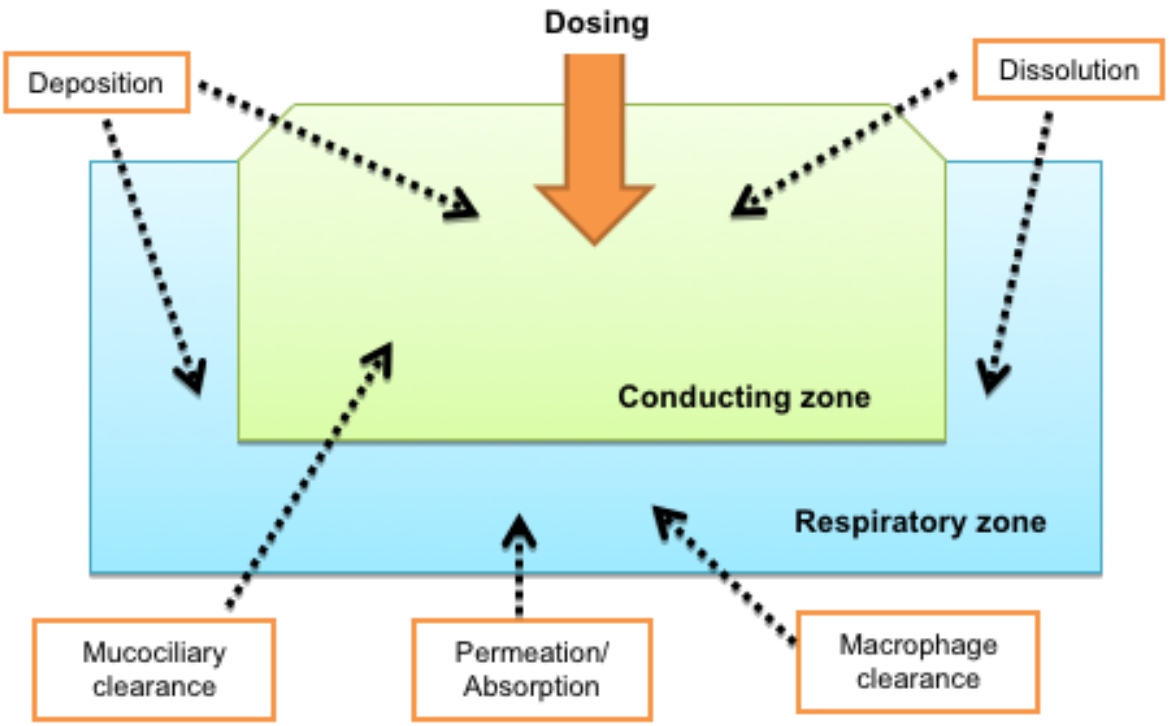

Fig. 2. Graphical representation of the interplay among particle deposition, dissolution, absorption and clearance in the pulmonary tract.

As pointed out by Hastedt et al. (2016), inhaled drugs with fast dissolution rate and absorption will shortly enter into the bloodstream, and this behavior could make them good candidates for systemic action. On the other hand, prolonged dissolution and slow absorption can increase drug residence time in the airways and favor local therapeutic effect, depending on the deposition site and drug's ability to escape physiological defense mechanisms. These considerations highlight the importance of defining functional relationship between drugs' biopharmaceutical properties and their performance in the lungs, by the implementation of the biopharmaceutics classification system for inhalation products (iBCS). Such an approach may facilitate engineering of drug particles with desired properties (Hastedt et al., 2016).

The first proposal for the iBCS was given by Eixarch et al. (2010). This subject was further elaborated in the meeting report from the AAPS/FDA/USP workshop (Hastedt et al., 2016).

A reasonable strategy for the development of iBCS would be to start with the basic postulates of BCS for oral drugs (Amidon et al., 1995), and then refurnish the system to accommodate the performance of inhaled drugs. Notable differences exist between oral and pulmonary drug delivery, and these differences need to be reflected in the design of iBCS. Orally administered drugs with favorable properties fall into BCS class I (highly soluble, highly permeable). However, in case of pulmonary drug delivery and iBCS, a drug is usually intended to act locally, and the systemic absorption should be minimized, meaning that drugs with poor permeability and/or slow dissolution are preferred. On the other hand, inhaled drugs intended for systemic action should possess similar biopharmaceutical properties as highly absorbed oral drugs, having fast dissolution and high permeability. In addition to the assessment of factors affecting drug bio-performance in the lung, it should be noted that a certain amount of the inhaled dose will be swallowed and absorbed through the gastrointestinal tract. This fact might not be relevant for iBCS considerations, but must be taken into account in the prediction of bioavailability of inhaled drugs.

In the view of iBCS, drug aqueous solubility should be considered in conjunction with the regionally deposited dose. It has been estimated that approximately 50% of the delivered dose reaches the peripheral region (Hastedt et al., 2016). This value depends upon formulation factors, dosing device characteristics, lung geometry and ventilation. Tolman et al. (2010) found out that even if the poor aqueous solubility of drug does not uniformly affect the pharmacokinetic profiles of inhaled particles, the physico-chemical properties of the formulation and its solubility can influence drug

absorption from the lungs. For example, nanosized drugs usually show improved saturation solubility and dissolution rate in comparison to larger particle sizes (Khadka et al., 2014).

The Dose number (D_0) for the inhaled drugs is site-specific (due to variations in the regional deposited dose and volume of lining fluid). D_0 in the respiratory zone can be calculated using the standard BCS equation (Amidon et al., 1995):

$$D_0 = \frac{M_0}{V \times C_s} \quad \text{(Eq. 1)}$$

where M_0 is the drug dose, V is the volume of fluid (approximately 250 ml) and C_s is drug solubility. This equation can be modified to comply with pulmonary drug delivery:

$$D_0 = \frac{M_a}{V \times C_s} = \frac{M_0/2}{V \times C_s} \quad \text{(Eq. 2)}$$

where M_0 is the inhaled dose, M_a is drug dose reaching alveoli (roughly 50% of the inhaled dose (Hastedt et al., 2016), although this portion can vary significantly), V is the volume of alveolar fluid (approximately 30 ml), and C_s is the drug solubility at neutral pH (lung lining fluids are aqueous media with nearly neutral pH 6-7). Hastedt et al. (2016) illustrated the relationship between drug dose in the conducting airways and minimum solubility required for dose dissolution, assuming 10-30 ml of lung fluid volume, and demonstrated that most of the currently marketed inhalation drugs are not solubility- nor dissolution-limited.

Dissolution of inhaled particles is another key step that needs to be considered in iBCS. Drug dissolution is a pre-step to the concomitant absorption or uptake via epithelial cells in the pulmonary tract. Dissolution rate affects the drug pulmonary residence time and consequently the pulmonary target (Rohrschneider et al., 2015).

Several factors can affect pulmonary drug dissolution, including drug dose, solubility, particle size, drug deposition pattern, volume, viscosity, and lung fluids hydrodynamics. It was observed that the aerodynamic drug particle size influence drug dissolution (Arora et al., 2010). Furthermore, it was also identified that the deposited mass influences dissolution rate, depending on the undissolved drug particles (Arora et al., 2010; Mees et al., 2011). Moreover, the dissolution of the individual particles and of the entire powder blend may be different (Balducci et, 2015).

Among the various factors affecting the solubility, solid state properties play an important role. Different polymorphic forms, amorphous, solvate and co-crystals can be exploited to improve drug's solubility. In general, the solubility and thus the dissolution of metastable solid forms is higher than the thermodynamically stable form due to differences in crystal lattice energies (Hancock and Parks, 2000). In fact, the high energy forms can create supersaturation in the surrounding lung fluid, promoting the conversion to a stable form. Amorphous beclomethasone dipropionate particles have been reported to recrystallize in contact with the bronchial fluid in vitro (Freiwald et al., 2005).

Dissolution of inhaled drugs can be described by a BCS parameter, the dissolution number (D_n) (Amidon et al., 1995):

$$D_n = \frac{t_{res} \times 3DC_s}{\rho \times r_0^2} \qquad \text{(Eq. 3)}$$

where t_{res} represents the mean residence time (in the case of pulmonary drug delivery, this parameter corresponds to the mean lung residence time), D is the drug

diffusion coefficient, ρ is the drug particle density, and r_0 is the initial mean drug particle radius.

If we consider pulmonary vs. oral drug delivery, it is evident that decreased particle size (often less than 3 μm) and density (specially engineered particles, e.g. via spray/freeze-drying) significantly enhance dissolution of inhaled drugs, and we can expect higher D_n values. Changes in drug solubility can further promote or hinder drug dissolution, depending on the desired (local or systemic) effect. In general, drug dissolution will be retarded if a drug is poorly soluble (e.g. some glucocorticoids) or if highly doses are administered (e.g. some anti-infective drugs). The freely soluble drugs like salbutamol sulphate (250 mg/ml) will be absorbed from the lung almost completely. On the other hand, the absorption of insoluble or sparingly soluble drugs like fluticasone propionate and beclomethasone dipropionate (~ 0.1 μg/ml) is affected by the regional deposition and lung clearance mechanisms. For highly soluble compounds, the dissolution is not considered to impact the lung clearance rate and no or small differences in pharmacokinetics are expected for different formulations. Poorly soluble and slowly absorbed compounds showed poor correlation between the total lung dose and systemic pharmacokinetics (Olsson and Bäckman, 2014).

As for the other factors in Eq. 2, low drug diffusivity in mucus-rich viscous lung fluids can be an interfering factor for drug dissolution, while lung residence time is drug and/or formulation-specific and depends upon the concomitant physiological processes (e.g., drug AM clearance rate and extent).

As already mentioned, the goal of the inhalation therapy should determine the desired rate of drug dissolution. Slow drug dissolution increases lung residence time and favors local effects, but accumulation phenomena should be considered,

especially in the case of high delivered doses. Fast dissolution is prerequisite for rapid therapeutic onset of systemically acting drugs.

A drug that escapes both mucociliary and alveolar macrophage clearance can pass into the epithelial cell or through the epithelia to the systemic circulation. Therefore, another step controlling the absorption rate of inhaled medicines is drug permeability through lung mucosal tissues.

In BCS, drug absorption is described by the absorption number (A_n) (Amidon et al., 1995):

$$A_n = t_{res} \times k_a \qquad \text{(Eq. 4)}$$

where k_a is the absorption rate constant, which is directly proportional to drug permeability and absorption surface area. However, the calculation of this parameter in terms of iBCS might be difficult, since k_α values for pulmonary absorption are time-dependent and also depend on the site of absorption. In addition, other transportation media, e.g. protein transporters in the lung membrane, indicate that some inhaled drugs are absorbed via active mechanisms (Gumbleton et al., 2011). It has also been reported that larger molecules, such as immunoglobulins, might be absorbed through receptor-mediated transcytosis (Spiekermann et al., 2002).

Eixarch et al. (2010) demonstrated large differences between lung and gastrointestinal drug permeability values, besides significant differences between drug permeability in the upper and lower pulmonary compartments. The same authors provided an overview of the available cellular *in vitro* models for the prediction of pulmonary drug permeability, indicating that Calu-3 cells (as a model of bronchial epithelium) and porcine alveolar epithelial primary cells can be promising tools to assess pulmonary drug permeability. However, more data (both *in vivo* and *in*

vitro) are needed to investigate the possible correlation/relationship between results from cell cultures and human lung permeability values. Also, additional studies are needed in order to derive a cut-off value between highly and poorly permeable drugs within iBCS.

Overall, basic premises and equations established within BCS for oral drugs, with certain modifications, can be used to describe biopharmaceutical properties of the inhaled drugs. However, in order to set up class boundaries regarding drug dissolution rate and lung permeability for iBCS classification, we need more data from human clinical trials, animal experiments and biorelevant *in vitro* studies.

Another annotation regarding iBCS is that favorable drug biopharmaceutical properties are related to the therapeutic goal of the inhalation therapy.

In addition to iBCS considerations, recent trends in drug product biopharmaceutical assessment point out the advantages of *in silico* modelling and simulation (M&S) tools for the prediction of drug *in vivo* performance. These tools offer a distinctive opportunity to mechanistically interpret the influence of the underlying processes on drug absorption and disposition, and understand the complex interplay between drug properties, formulation factors and human physiology characteristics on drug pharmacokinetic profile (Borghardt et al., 2015; Wu et al., 2013). In recent years, several software tools for physiologically-based pharmacokinetic (PBPK) modelling of inhaled drug absorption (e.g. GastroPlus™ Nasal–Pulmonary Drug Delivery Additional Dosage Routes Module, PulmoSim™) have been introduced (Borghardt et al., 2015). The review of pulmonary PBPK models provides in-depth information about the current status.

A novelty has been introduced with the development of an *in vitro* model, named DissolvIt®, that simulates the dissolution and absorption of drugs from inhaled dry

powders (Gerde et al., 2017). Budesonide and fluticasone propionate were used as model drugs. DPIs were aerosolized with PreciseInhale® aerosol generator and the collected particles on cover slips were put in contact with simulated mucus in the DissolvIt® system. This method also permits to mimic the pharmacokinetic data.

3. Dissolution methodologies for DPIs

3.1 Dissolution method set-ups

Davies and Feddah (2003) were the firsts to introduce an *in vitro* method for assessment of dissolution properties of DPIs. Their apparatus was based on the flow-through principle and was set up by modifying the USP Dissolution Apparatus 4. The aerosolized particles were collected at the connection point of the USP induction port with the inlet part of the Andersen Cascade Impactor (ACI), in order to get representative samples for the dissolution studies. In the following years, other methods for *in vitro* dissolution testing of powders for inhalation (more specifically controlled release microparticles) were evaluated by Salama et al. (2008), including the modified USP apparatus 2, modified flow-through cell (according to Davies and Feddah (2003) and Franz-type diffusion cell. They concluded that, due to the lack of differentiation between formulations for USP Apparatus 2 and 4, diffusion controlled set-up (modified Franz cell) was more appropriate for the evaluation of controlled release DPIs.

Son et al. (2010) reported on the optimization of the dissolution method for DPIs based on the Apparatus 2, modified by adding a membrane holder on top of the deposited particles. Particles were collected in the accordingly modified cups through aerodynamic separation using the Next Generation Impactor (NGI). Authors emphasized the potential for application of this method in the quality control of

developed OIDPs. May et al. (2012) have also compared different dissolution techniques for *in vitro* testing of DPIs, including the Apparatus 2 with the membrane holder, modified flow-through cell and Franz diffusion cell. It was concluded that the paddle apparatus (Apparatus 2) with the membrane holder has the best discriminatory power, with optimal reproducibility, for differentiating between different forms of the same substance and also in case of substances having close solubility values.

However, since the lung fluid is limited in volume, and is much more stationary in comparison to GIT fluids, the above listed methods may not be reflective of the actual *in vivo* dissolution process of inhaled particles. In order to overcome the issues related to the use of non-physiologically large amounts of dissolution media, the aerosol particles in the 2.1 – 3.3 μm aerodynamic diameter range, collected onto a filter, were inserted in a Transwell® system containing small amount of stationary dissolution medium (Arora et al., 2010). Membrane-based Transwell® inserts provide an air interface to the sample and only a small amount of dissolution medium, assuring more biorelevant conditions in comparison to other methods (May et al., 2015). In this work, detailed account of the influence of various factors, like dose collection technique, membrane type, additional dissolution medium, stirring, on the drug dissolution using Transwell® inserts was provided.

Maretti et al. (2016) investigated the rifampicin release profile from solid lipid nanoparticles by using dialysis membrane for the *in vitro* dissolution method in sink conditions that could estimate the drug release from the nanoparticles when in contact with the lung lining fluid. 30 ml of Simulated Lung Fluid at pH 7.4, under gently magnetic stirring, at a temperature of 37 °C was used to reproduce stagnant lung conditions.

Table 2 lists experimental set-ups for dissolution studies of OIDPs reported in the literature. These *in vitro* dissolution studies differed in sample preparation, dissolution apparatus, media, etc., then it is rather impossible to make comparisons among them. However, although this review is limited to the dissolution behavior of DPI, it is not possible not to mention methods that were developed for MDIs, as it can be observed in Table 2.

Table 2

Experimental conditions for some dissolution studies of OIDPs reported in the literature.

Dissolution apparatus (system)	Drug / Formulation or commercial product	Collection of samples	Dissolution medium	Reference
Modified USP apparatus 2	Albuterol/Ventolin® HFA Budesonide/Pulmicort® Flexhaler®	modified NGI containing a dissolution cup	SLF, PBS pH 7.4, PBS with DPPC or polysorbate 80	Son et al., 2010
	Budesonide/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012
	Disodium cromoglycate/ polyvinyl alcohol microparticles	microparticles were manually sprinkled on the cellulose filter membrane	PBS pH 7.4	Salama et al., 2008
	Fenoterol/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012
	Isoniazid/poly-ε-caprolactone microparticles	microparticles were dispersed in PBS and filled in the pre-treated dialysis membrane and sealed with clips	SLF pH 7.4, ALF pH 4.5	Parikh and Dalwadi, 2014
	Itraconazole/mannitol+TPGS microparticles	modified NGI containing a dissolution cup with a removable insert placed on stage 3	0.063 M HCl solution with 0.3 % of SLS	Duret et al., 2012
Modified USP paddle over disc method	Clarithromycin and tobramycine/ co-spray dried nanoparticles	modified NGI containing a dissolution cup with a removable insert placed on stage 3	PBS pH 7.4	Pilcer et al., 2013
USP apparatus 1	Salbutamol acetonide/ glyceryl behenate solid lipid microparticles	powder samples were wrapped up in glass fiber filters	PBS pH 7.4	Jaspart et al., 2007
	Dapsone/chitosan microparticles	powder samples were filled in the gelatin capsules no. 0	PBS pH 7.4	Ortiz et al. 2015
(Modified) flow-through cell	Budesonide/Pulmicort® Turbuhaler®	connection point of the USP induction port with the inlet part of the ACI	Water, SLF, modified SLF (with DPPC)	Davies and Feddah, 2003

	Disodium cromoglycate/ polyvinyl alcohol microparticles	microparticles were manually sprinkled on the cellulose filter membrane	PBS pH 7.4	Salama et al., 2008
	Fenoterol/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012
	Fluticasone propionate/ Flixotide® Accuhaler®	connection point of the USP induction port with the inlet part of the ACI	Water, SLF, modified SLF (with DPPC)	Davies and Feddah, 2003
	Triamcinolone acetonide/ Azmacort®			
(Modified) Franz diffusion cell	Bovine serum albumin, terbutaline sulfate, diprophylline/ zinc-alginate microparticles	microparticles were manually sprinkled on the regenerated cellulose filter membrane	PBS pH 7.4, modified SLF	Möbus et al., 2012
	Beclomethasone dipropionate Qvar®/ Sanasthmax	twin stage impinger	PBS pH7.4, 0.1% SDS	Grainger et al, 2012
	Budesonide/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012
	Disodium cromoglycate/ polyvinyl alcohol microparticles	microparticles were manually sprinkled on the cellulose filter membrane	PBS pH 7.4	Salama et al., 2008
	Fenoterol/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012
	Pyrazinamide, rifampicin, isoniazid/co-spray dried particles	nitrocellulose membrane was placed on stage 3 of an NGI	SLF pH 7.4	Chan et al., 2013
	Salbutamol/micronized powders of salbutamol base and sulfate form, Ventolin®	twin stage impinger was used to deposit particles on the Transwell® polyester membranes	Hanks balanced salt solution, SLF with 0.02 % DPPC	Haghi et al., 2012
	Salbutamol/solid lipid microparticles	samples were manually sprinkled on the membrane	PBS pH 7.4	Scalia et al., 2012
	Salmeterol xinafoate/blends with lactose	samples were manually sprinkled on the filter	PBS pH 7.4	Balducci et al., 2015
(Modified) Transwell® system	Beclometahasone dipropionate/ Vanceril® Qvar®	stages 2 and 4 of 8-stage ACI	PBS pH 7.4 distilled deionized water	Arora et al., 2010
	Budesonide/Pulmicort® Turbuhaler®			
	Budesonide/micronized particles	abbreviated ACI with a stage extension	PBS pH 7.4	May et al., 2015
	Budesonide/Symbicort®	filter papers placed on stage 4 of the ACI or NGI	PBS with 0.5 % SDS	Rohrschneider et al., 2015
	Ciclesonide/Alvesco®			
	Flunisolide/Aerobid®	stages 2 and 4 of 8-stage ACI	PBS pH 7.4 distilled deionized water	Arora et al., 2010

	Fluticasone propionate/Flovent® Diskus®			
	Triamcinolone acetonide/ Azmacort®			
	Fluticasone propionate/ Flixotide®	filter papers placed on stage 4 of the ACI or NGI	PBS with 0.5 % SDS	Rohrschneider et al., 2015
Dialysis bag	Rifampicin, rifabutin/ chitosan microparticles	Microparticles were placed in dialysis bag which was suspended in a stoppered tube	SLF pH 7.4	Pai et al., 2015
	Rifampicin/ freeze-fried microparticles			Maretti et al., 2016
	Voriconazole/Polylactide large porous particles	Samples were manually dispersed in the dialysis bag	PBS pH 7.4 with 0.1 % polysorbate 80	Arora et al., 2015

It can be summarized that the development of an *in vitro* dissolution method for selected OIDP requires to define:

- dissolution apparatus type (various modifications of compendial apparatuses)
- dissolution medium (composition, volume)
- introduction of sample in the dissolution apparatus and sample collection
- quantification and fitting

3.2 Selection of dissolution apparatus type

Different types of powder material have been investigated including raw API, micronized API, formulated DPIs including microparticles for inhalation, commercial products, aerosolized particles of respirable size range, etc., as listed in Table 2. Dissolution set-ups (apparatus types and various modifications) may, in general, be divided into two distinct groups: systems that incorporate high fluid volumes (50 ml – 1000 ml) subjected to influence of hydrodynamic factors (such as stirring or flow of the medium), and systems that rely on small medium volumes and absence of agitation. The first group includes paddle apparatus and flow through cells

(compendial and modified), whereas the second group is representative of diffusion controlled systems, such as Franz diffusion cell and Transwell® inserts.

Collection of aerosolized particles is usually carried out by inserting filters or membranes in twin-stage impingers, at the induction port or on the appropriate stages (generally on stage 4) of ACIs (8-stage or abbreviated ACIs, with examples of stage extension inclusion) (examples are listed in Table 2). As filter papers, regenerated cellulose membrane filters, cellulose acetate membrane filters, glass microfiber filters and polyvinylidene difluoride (PVDF) are some of the materials used (Davies and Feddah, 2003; Arora et al., 2010; May et al., 2012, Rohrschneider et al., 2015). Homogeneous and non-agglomerated particle distribution is essential for *in vitro* testing of OIDPs dissolution (May et al., 2015). In order to collect amounts of dispersed particles sufficient for quantification in dissolution studies, sometimes several activations of the inhalation device are required. When greater amounts of given formulation are collected, slower dissolution rates might be observed, probably due to *in-situ* formation of agglomerates on the filter during the collection of the appropriate dose (Mees et al., 2011). In the case of NGI, special cups for the collection of particles have been introduced, which, covered with a membrane secured in place with an appropriate holder, are transferred for dissolution testing (Son et al., 2010).

Systems such as Transwell® inserts or Franz cells have membranes that separate the donor and acceptor compartments, providing diffusion of dissolved drug (Balducci et al., 2015; Rohrschneider et al., 2015). Semipermeable membranes mimic the air-liquid interface of the epithelial lung wall (May et al., 2012). The flow through cell, on the other hand, is not diffusion controlled but flow rate controlled system.

Due to the lower amount of dissolution media, Transwell® inserts could provide more biorelevant conditions in comparison to the Franz diffusion cell. Transwell® inserts are available in a range of diameters, membrane types and pore sizes; with the smaller pore size (0.1 – 0.4 μm) polycarbonate and polyester membranes being primarily used for the drug transport studies (Transwell® Permeable Supports, 2003). Multi-culture systems comprising various types of epithelial cells and macrophages are used as more advanced models for Transwell® inserts (de Souza Carvalho et al., 2014, Nahar et al., 2013). Other membranes that were used for *in vitro* studies of OIDPs dissolution include regenerated cellulose and Isopore® polycarbonate (May et al., 2015).

A drawback in the application of Franz-type diffusion cells and Transwell® inserts is the fact that the amount of the drug released into the donor compartment is limited by the process of diffusion through the membrane. Rohrschneider et al. (2015) realized that only modified systems, incorporating faster equilibrating membranes, resulted in the dissolution and not the diffusion being the rate limiting step for the drug transfer from donor to acceptor compartment. Instead of the original 0.4 μm Transwell® polycarbonate membrane, authors have placed only microfiber filters with collected aerosolized particles in the Transwell® insert that was further modified by thermo-formation of notches at the insert base. May et al. (2015) further demonstrated that there was an interaction between the polycarbonate and polyester membranes and the substances used for dissolution testing. On the other hand, regenerated cellulose and Isopore® polycarbonate membranes were more appropriate. Also, an improvement of the dissolution process was reproducibility achieved with the introduction of stirrer (a spacer was put in the Transwell® setup in order to lift the inserts and allow addition of stirring bars). It was also demonstrated that, if an

477 additional dissolution medium was added on the membrane to aid the contact
478 between the drug particles and fluid, greater variability in dissolution process was
479 observed due to the substance-dependence of the process (May et al., 2015).
480 Therefore, prior to set-up the dissolution test, it is necessary to investigate the
481 potential drug–membrane interactions through investigation of the permeability of the
482 selected membrane for both original and dissolved drug. There are also reports on
483 use of dialysis membranes for *in vitro* dissolution studies of OIDPs (Arora et al.,
484 2015; Pai et al., 2015, Maretti et al., 2016).
485
486 ***3.3 Dissolution media***
487 Another important issue for proper set-up of an *in vitro* dissolution test for OIDPs is
488 the selection of the dissolution medium. As for the quantity of the dissolution medium,
489 it has to be sufficient to assure the sink conditions, which is often feasible due to the
490 low doses of pulmonary administered drugs. However, the bio-relevance of the sink
491 conditions might be questionable due to the limited amount of the lung fluid (approx.
492 10-20 ml/100 m^2 (Son et al., 2010). Furthermore, occurrence of the non-sink
493 conditions in the deep lung has been suspected (Sakagami and Arora Lakhani,
494 2012). Published studies demonstrate that researchers have used various dissolution
495 media, ranging from water, acidic solutions and phosphate buffers to more bio-
496 relevant simulated lung fluids, with or without addition of surfactants or complexing
497 agents such as cyclodextrins, as presented in Table 3. Simulated lung fluids are
498 being recognized as the most discriminative and bio-relevant media for dissolution
499 studies of DPIs due to the complex ionic composition (Möbus et al., 2012). Addition
500 of surfactants to the SLF further mimics the natural environment in the lung fluids,
501 with DPPC being the preferred selection of surface active agent, preferably for low

soluble drugs; the preparation of such dissolution media is time consuming due to the risk of micelle formation, and most importantly, they lack of buffering capacity and clogging of the membrane pores (Son et al., 2011). In some of the referenced studies, sodium lauryl sulfate and polysorbate 80 were also used as surfactants, allowing more affordable and convenient testing. Rohrschneider et al. (2015) reported that the presence of a surfactant (e.g. 0.5 % SDS) is essential to obtain the rank order of dissolution rates that is in agreement with the absorption rates of the selected drugs obtained in human pharmacokinetic studies. Marques et al. (2011) have compiled details on the composition and preparation of various simulated lung fluids.

On the other hand, an example is provided where the dissolution of poorly soluble drug itraconazole, from solid dispersions for pulmonary application, was performed in 0.063 N HCl (pH 1.2) and 0.3% sodium lauryl sulfate in order to obtain sink conditions (Duret et al., 2012). In the same study, PBS pH 7.2 was used with the addition of 0.02 % w/v of DPPC, since the authors have noticed that pH of SLF increases rapidly after preparation, due to its poor buffering power. Parikh and Dalwadi (2014) have used one of modifications of the original SLF, a Gamble's solution (with pH adjusted to 7.4) and alveolar lung fluid (ALF) with pH 4.5. Gamble's solution represents the interstitial fluid, present deeply within the lung, whereas ALF is analogous to the fluid with which inhaled particles would come in contact after phagocytosis by alveolar macrophages (Marques et al., 2011). Drug dissolution and permeation in simulated mucus and in sputum obtained from cystic fibrosis patients was studied by Russo et al. and by Stigliani et al., which are of specific importance for patients with cystic fibrosis (Russo et al., 2013; Stigliani et al., 2016).

Table 3

Compositions of the physiological lung fluid, simulated lung fluid (SLF), modified SLF and the applied SLF in mEq/L (adapted from Kalkwarf, 1983; Davies and Feddah, 2003).

Ions	Physiological	SLF	Modified SLF with 0.02 % DPPC	Applied SLF
Calcium, Ca^{2+}	5	5	5	-
Magnesium, Mg^{2+}	2	2	2	2
Potassium, K^{+}	4	4	4	4
Sodium, Na^{+}	145	145	145	150
Total cations	156	156	156	156
Bicarbonate, HCO_3^{-}	31	31	31	31
Chloride, Cl^{-}	114	114	114	115
Citrate, $C_6H_5O_7^{3-}$	-	1	1	-
Acetate, $C_2H_3O_2^{-}$	7	7	7	7
Phosphate, HPO_4^{2-}	2	2	2	2
Sulfate, SO_4^{2-}	1	1	1	1
Protein	1	-	-	-
DPPC	-	-	200 mg	-
Total anions	156	156	156	156
pH	7.3 – 7.4	7.3 – 7.4	7.3 – 7.4	7.3 – 8.7

Recent study, conducted using modified Transwell® method with a glass microfiber filter as the dissolution membrane and SDS in the dissolution media, revealed that the size distribution of fluticasone propionate particles influenced dissolution rates significantly (Kippax et al., 2016).

3.4 Modeling of DPI dissolution rates

The selection of an appropriate model to describe the dissolution data might be challenging, as OIDPs are poly-disperse systems and application of statistical or mathematical techniques, used traditionally in oral solid forms, is not yet established. Model dependent or independent methods aim to interpret dissolution data or compare different dissolution profiles, but in most cases, results are based on

543 assumptions taken from the knowledge of other solid forms. This underlies possible
544 misinterpretations or distortion of the experimental errors.
545 Interpretation of release mechanism (e.g. dissolution or diffusion) for OIDPs depends
546 on the drug properties such as solubility and affinity towards membrane (if used) and
547 various aspects of the dissolution set-up. Therefore, fitting of dissolution profiles to
548 different models must be interpreted in the context of the dissolution set-up: if the
549 diffusion controlled set-ups are used, such as Franz cell system, then good fit with
550 Higuchi model is to be expected (Salama et al., 2008). However, it might be useful to
551 apply model-dependent methods, such as fitting to the Weibull equation, in order to
552 compare different release profiles, but this has been argued (Riley et al., 2012).
553 Model-independent methods, such as similarity and difference factors, f_2 and f_1
554 values, are often calculated for comparison of OIDPs release profiles (May et al.,
555 2015; Salama et al., 2008; Riley et al.,2012), but their statistical power to discriminate
556 between formulations could be more refined if they are calculated for each particle
557 size range.
558 *In vitro*-based mean dissolution times (MDTs) may be an indicator for the *in vivo* lung
559 absorption rates of slowly-dissolving lipophilic corticosteroids, e.g., FP, ciclesonide
560 and budesonide (May et al., 2012; Rohrschneider et al., 2015). MDT is a model-
561 independent parameter and can easily be compared to non-compartmental
562 pharmacokinetic parameters, such as the mean absorption time (MAT)
563 (Rohrschneider et al., 2015). However, it should be kept in mind that MDT is not
564 meaningful if the plateau of the dissolution profile is not reached (May et al., 2015).
565 May et al. (2014) have developed a mechanistic model for inhaled API particles
566 release rate based on the modified version of the Noyes-Whitney dissolution model
567 i.e. Nernst-Brunner equation (Dokoumetzidis and Macheras, 2006):

$$\frac{dm}{dt} = \frac{DS}{h}(c_s - c_t) \quad \text{(Eq. 5)}$$

where m is the mass of solid material at time t, S is the surface area of the particles, D the diffusion coefficient of the substance in the solvent, h is the diffusion boundary layer thickness, c_s is the saturation solubility of drug and c_t is the concentration of the drug in the solution at time t.

Diffusion coefficient D was calculated by applying the Hayduk-Laudie equation (Haydak and Laudie, 1974; Sheng et al., 2008):

$$D = \frac{13.26\times10^{-5}}{\eta_{water}^{1.4}\times V_M^{0.589}} \quad \text{(Eq. 6)}$$

where, η_{water} is the dynamic viscosity of water at 37 °C and V_M is the Van-der-Waals volume. There is a consensus that below a critical particle size the diffusion layer of a spherical particle can be approximated by the particle radius, where the critical particle radius is assumed to be 30 µm (Hinz and Johnson, 1989). The modeling of the dissolution layer of aerosolized particles is based on the following assumptions: sink conditions, spherical particles, well-stirred medium, isotropic dissolution, saturated solution at the surface of the particle/interface, constant diffusion coefficient along the diffusion layer and no impact of stirred medium on the dissolution due to the membrane (May et al., 2014). In order to take account of different particle size fractions, collected at the different ACI stages, the following sum was calculated (Hintz and Johnson, 1989; Okazaki et al., 2008):

$$\frac{dX_{sum}(t)}{dt} = \sum_{e=1}^{n}\frac{dX_e(t)}{dt} = \sum_{e=1}^{n}\frac{DS_e(t)}{h_e t}(c_s - \frac{X_d}{V}) \quad \text{(Eq. 7)}$$

where $X_{sum}(t)$ is the total amount of undissolved drug at time t, $X_e(t)$ is the amount of undissolved drug in a particle size group e, S_e is the surface area of each particle size fraction, and h_e is the thickness of the diffusion layer, which depends on the particle radius r_e. Due to irregular particle shape, for the determination of the particle

surface area the aerodynamic diameter must be converted in the geometric diameter, incorporating the cubical particle shape factor for correction. The FPD on the membrane, the particle shape, the diffusion layer thickness, the solubility and the particle size distribution were also varied for evaluating possible influencing factors (May et al., 2014).

Sadler et al. (2011) developed an *in vitro* model based on the deposition of salmeterol xinafoate particles on Calu-3 respiratory epithelial cells to study their dissolution and absorption.

4. Regulatory considerations and potentials for DPIs dissolution testing

Official statements from the regulators regarding the potential for the application of dissolution test as an aid in formulation development, quality control tool or for the bioperformance assessment of DPIs is rather scarce. EMA (European Medicines Agency) and FDA (Food and Drug Administration) guidelines on the quality of inhalation products do not provide any suggestions regarding dissolution testing of DPIs. The list of proposed tests for the quality assessment of DPIs include: appearance, assay, moisture content, mean delivered dose, delivered dose uniformity, fine particle mass, particle size distribution of emitted dose and microbiological limits (FDA, 1998; EMA, 2006).

Current approach by regulatory authorities (EMA, FDA), in bioequivalence testing of orally inhaled powders, is a step-wise procedure including 1) *in vitro* characterization, 2) pharmacokinetics and, if necessary, 3) pharmacodynamics, i.e., clinical studies (Hochhaus et al., 2015). *In vitro* testing is predominantly based on determination of aerodynamic particle size distributions (by cascade impactors) using bio-relevant batches. This *in vitro* data may be accepted as a surrogate for *in vivo* bioequivalence

617 studies, even though an *in vitro* – *in vivo* relationship (IVIVR) has not been
618 established to date. There are examples of good correlation between aerodynamic
619 properties of the particles (e.g. delivered dose and FPF) and pharmacokinetic
620 outcomes (Reisner et al., 2014; Horhota et al., 2015). However, discrepancies that
621 arouse between *in vitro* and pharmacokinetic studies suggested that the latter are
622 more sensitive to differences in DPI formulations than cascade impactor studies.
623 Therefore, additional *in vitro* tests, such as dissolution studies (especially in the case
624 of poorly soluble APIs), might be necessary for establishment of a proper IVIVC
625 (Hochhaus et al., 2015). EMA has issued a guideline (EMA, 2009) on the
626 requirements for demonstration of therapeutic equivalence between the inhaled
627 products for use in the treatment of asthma and COPD. It was recognized that
628 bioequivalence can be demonstrated through selected *in vitro* tests, if dissolution
629 properties of the active substance lie between the reference and test product
630 (amongst other requirements). Some regulatory authorities recommend combination
631 of *in vitro* tests, including cascade impactor studies and determination of the
632 dissolution rates in physiologically relevant dissolution media, in combination with
633 pharmacokinetic studies to demonstrate pulmonary bioequivalence (Mendes Lima
634 Santos et al., 2014). Moreover, apart from the potential for the bioperformance
635 assessment of DPIs, dissolution studies enable to differentiate among orally inhaled
636 formulations and to set criteria for compliance. Furthermore, it was recognized that
637 dissolution testing was valuable as quality control tool, for discrimination between
638 formulations with similar aerodynamic but different release properties (Forbes et al.,
639 2015). Also, dissolution testing may provide better understanding of inhalation drug
640 delivery and guide/support formulation development. This could be important in the
641 context of QbD driven pharmaceutical development with the potential for coupling

642 dissolution testing with computational fluid dynamics (CFD) and physiologically-
643 based pharmacokinetic (PBPK) modeling.
644
645 **5. *In vitro – in vivo relationships***
646 As stated earlier, regulatory authorities (FDA, EMA) currently recommend
647 pharmacokinetic studies in healthy volunteers, to assess the pulmonary deposition
648 (bio-performance) of orally inhaled drugs (Hochhaus et al., 2015). However, recent
649 discussions introduced the idea that *in vitro* data might be used to waive *in vivo*
650 studies (Garcia-Arieta et al., 2014). A relationship between dissolution rate and
651 appearance of drug in plasma has been reported (Grainger et al., 2012). Convolution
652 and deconvolution can be applied to evaluate drug release and absorption, assuming
653 linear pharmacokinetics. In order to develop a bio-relevant dissolution test for DPIs, it
654 should be taken into account the physiological factors influencing dissolution *in vivo*,
655 including the composition and viscosity of the airway lining fluid, permeability of the
656 airway epithelium and the rate of particle clearance, all of which vary between
657 different regions of the lung. Optimization of *in vitro* dissolution methods for OIDPs,
658 using membranes with increased permeability and dissolution media with added
659 surfactants represents a good starting point to further evaluate *in vitro - in vivo*
660 (cor)relations (Rohrschneider et al., 2015). Furthermore, coupling of dissolution and
661 permeation studies could also be beneficial in terms of increased bio-relevancy.
662 Haghi et al. (2012) investigated the deposition, dissolution and transport of
663 salbutamol (base and sulfate form) inhalation powders using the Calu-3 interface cell
664 culture model and Franz diffusion cell, while Sadler et al. (2011) did it, as mentioned
665 before, for salmeterol xinafoate powders using Calu-3 respiratory epithelial cells and
666 a cascade centripeter impactor.

In order to realistically mimic deposition of aerosolized particles onto the lung surface and subsequent released drug uptake, several methods were developed, in which ACI was coupled with cultures of Calu-3 bronchial cells (Haghi et al., 2014; Ong et al., 2015; Meindl et al., 2015). It was mentioned that the modification of standard API plate with Snapwell® cell culture inserts did not affect deposition of aerosolized particles (Ong et al., 2015). This study evidenced that drug absorption from different inhaled formulation devices was not equivalent depending on their physical chemical properties upon aerosolization. Then, these findings once again were indicative of the necessity to develop *in vitro* dissolution methodologies for OIDPs, since dissolution of drug particles might be the limiting step for the rate and amount of drug absorption.

6. *Conclusions and future perspectives*

In vitro dissolution testing for solid oral dosage forms is well stablished and the data are widely used in the formulation development as well as quality control. Dissolution data are also used to study the effect of formulation change and/or support the claims of bioequivalence of generic solid oral products. However, in the case of orally inhaled products, the efficiency of DPI is linked to fine particle fraction without giving much attention to other factors. In fact, currently there are no regulatory requirements or standardized methods for dissolution testing of inhalation products. However, there is a significant interest and need in developing dissolution technologies for OIPs that can guide particle engineering and formulation to tailor release properties of particles for local as well as systemic drug delivery and for quality control testing. In this review, we attempted to summarize the comprehensive research on dissolution of inhaled powders.

692 The dissolution methods mainly differed in apparatus setup and dissolution medium.
693 Compared to the first in vitro dissolution studies, that used apparatus approved for
694 the characterization of oral formulations, the researchers focused their attention on
695 systems that better mimic the lung environment and particle's deposition.
696 Given the variety of inhalation therapeutic goals (systemic or local action), along with
697 emerging particle engineering techniques and formulation strategies, special
698 attention should be paid to the biopharmaceutical aspects of pulmonary drug
699 delivery. A thorough biopharmaceutical characterization of the inhaled drugs in terms
700 of drug solubility, dissolution and pulmonary permeability should be an integral part of
701 a sound formulation development strategy.
702 Determination of the key factors that influence drug bio-performance in the lungs is
703 one of the priorities in the pharmaceutical development of the inhaled products, and
704 therefore the introduction of the iBCS would facilitate the selection of drug candidates
705 and identification of the critical quality attributes of the inhalation products. Still, at this
706 moment, even a tentative iBCS would only be a rough estimate, since there are
707 multiple factors that influence the behavior of the inhaled drugs, and the importance
708 of these factors has yet to be determined.
709 The fact that more lipophilic drugs pass through the lungs rapidly is in contrast with
710 the basic postulate of BCS for oral products that poor water solubility is a limiting
711 factor for drug absorption. As discussed by Patton et al. (2004), more hydrophilic
712 drugs pass through the lungs much slower, most likely through aqueous pores in the
713 intercellular tight junctions. Ionized (generally water soluble) molecules have lower
714 absorption rate, because of the interactions with lipids and proteins that surround the
715 aqueous pores, whereas absorption can become even lower with increased

molecular weight of the drug. Such findings imply that iBCS solubility classification criterion might be expressed as lipid solubility.

Furthermore, different regions in the respiratory tract have different wall thickness, composition and mechanisms of defense, so dissolution and absorption can differ depending on the deposition site.

All these factors could be considered when designing appropriate *in vitro* dissolution and permeation tests for the inhalation drugs. Even if a drug is not dissolved adequately in aqueous layer, there are mechanisms that facilitate drug transportation through the cellular membrane, and interpretation of the *in vitro* data need to be taken with caution.

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Figure legends

Fig. 1. Complex interplay among the factors affecting the key biopharmaceutical properties of inhaled drugs.

Fig. 2. Graphical representation of the interplay among particle deposition, dissolution, absorption and clearance in the pulmonary tract.

Figure 1

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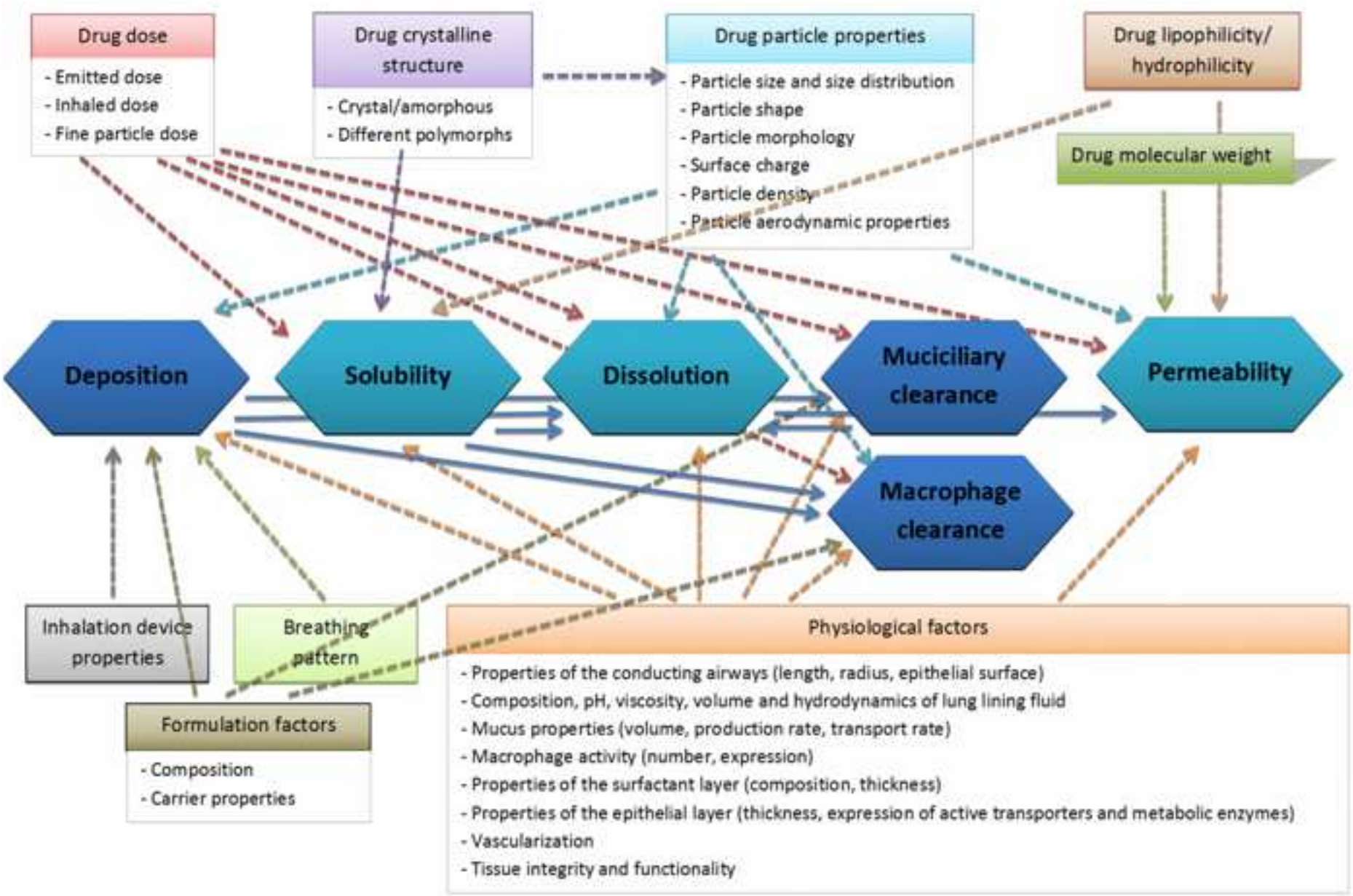

Figure 2

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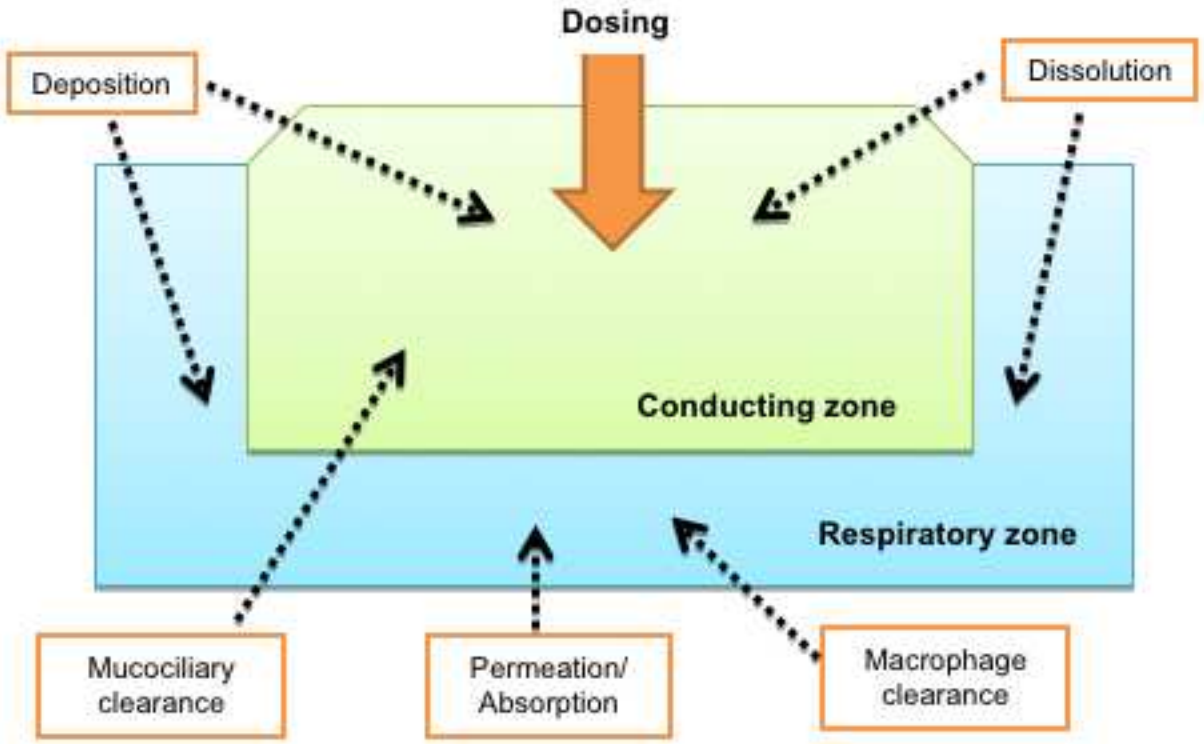

Table(s)

Table 1. Examples of DPI drug products available on US* and/or EU# market.

Drug Product	Drug	Indication	Device type	Company
Tudorza® Pressair®*	Aclidinium bromide	COPD	Multi dose (reservoir)	Forest Pharmaceuticals Inc./Almirall
Foster NEXThaler#	Beclomethasone dipropionate/formoterol fumarate	Asthma/COPD	Multi dose (reservoir)	Chiesi
Pulmicort Flexhaler*	Budesonide	Asthma	Multi dose (reservoir)	Astra Zeneca
Colobreathe® Turbospin*	Colistimethate sodium	Cystic fibrosis infection	Single dose (capsule)	Forest Laboratories
Flovent Diskus*	Fluticasone propionate	Asthma	Multi dose premetered	GSK
Foradil Aerolizer*	Formoterol fumarate	Asthma/COPD	Single dose (capsule)	Novartis
Afrezza#*	Insulin humane	Diabetes	Single dose (cartridge)	Sanofi Aventis
Adasuve#*	Loxapine	Schizophrenia/bipolar disorder	Single dose	Teva
Asmanex Twisthaler*	Mometasone furoate	Asthma	Multi dose (reservoir)	Schering
Buventol Easyhaler#	Salbutamol sulphate	Asthma/COPD	Multi dose (reservoir)	Orion
Serevent Diskus*	Salmeterol xinofoate	Asthma/COPD	Multi dose premetered	GSK
Seretide Diskus#	Fluticasone propionate/ Salmeterol xinofoate	Asthma/COPD	Multi dose premetered	GSK
Advair Diskus*	Fluticasone propionate/ Salmeterol xinofoate	Asthma/COPD	Multi dose premetered	GSK
Spiriva Handihaler*	Tiotropium bromide	COPD	Single dose (capsule)	Boehringer Ingelheim
Toby Podhaler#*	Tobramycin	Cystic fibrosis infection	Single dose (capsule)	Novartis
Relenza Diskhaler*	Zanamivir	Influenza	Multi dose (blister)	GSK

Table 2. Experimental conditions for some dissolution studies of OIDPs reported in the literature

Dissolution apparatus (system)	Drug / Formulation or commercial product	Collection of samples	Dissolution medium	Reference
Modified USP apparatus 2	Albuterol/Ventolin® HFA Budesonide/Pulmicort® Flexhaler®	modified NGI containing a dissolution cup	SLF, PBS pH 7.4, PBS with DPPC or polysorbate 80	Son et al., 2010
	Budesonide/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012
	Disodium cromoglycate/ polyvinyl alcohol microparticles	microparticles were manually sprinkled on the cellulose filter membrane	PBS pH 7.4	Salama et al., 2008
	Fenoterol/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012
	Isoniazid/poly-ε-caprolactone microparticles	microparticles were dispersed in PBS and filled in the pre-treated dialysis membrane and sealed with clips	SLF pH 7.4, ALF pH 4.5	Parikh and Dalwadi, 2014
	Itraconazole/mannitol+TPGS microparticles	modified NGI containing a dissolution cup with a removable insert placed on stage 3	0.063 M HCl solution with 0.3 % of SLS	Duret et al., 2012
Modified USP paddle over disc method	Clarithromycin and tobramycine/ co-spray dried nanoparticles	modified NGI containing a dissolution cup with a removable insert placed on stage 3	PBS pH 7.4	Pilcer et al., 2013
USP apparatus 1	Salbutamol acetonide/ glyceryl behenate solid lipid microparticles	powder samples were wrapped up in glass fiber filters	PBS pH 7.4	Jaspart et al., 2007
	Dapsone/chitosan microparticles	powder samples were filled in the gelatin capsules no. 0	PBS pH 7.4	Ortiz et al. 2015
(Modified) flow-through cell	Budesonide/Pulmicort® Turbuhaler®	connection point of the USP induction port with the inlet part of the ACI	Water, SLF, modified SLF (with DPPC)	Davies and Feddah, 2003
	Disodium cromoglycate/ polyvinyl alcohol microparticles	microparticles were manually sprinkled on the cellulose filter membrane	PBS pH 7.4	Salama et al., 2008
	Fenoterol/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012
	Fluticasone propionate/ Flixotide® Accuhaler®	connection point of the USP induction port with the inlet part of the ACI	Water, SLF, modified SLF (with DPPC)	Davies and Feddah, 2003
	Triamcinolone acetonide/ Azmacort®			
(Modified) Franz diffusion cell	Bovine serum albumin, terbutaline sulfate, diprophylline/ zinc-alginate microparticles	microparticles were manually sprinkled on the regenerated cellulose filter membrane	PBS pH 7.4, modified SLF	Möbus et al., 2012
	Beclomethasone dipropionate Qvar®/ Sanasthmax	twin stage impinger	PBS pH7.4, 0.1% SDS	Grainger et al, 2012
	Budesonide/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012

	Disodium cromoglycate/ polyvinyl alcohol microparticles	microparticles were manually sprinkled on the cellulose filter membrane	PBS pH 7.4	Salama et al., 2008
	Fenoterol/micronized particles	regenerated cellulose membranes using abbreviated ACI	PBS pH 7.4	May et al., 2012
	Pyrazinamide, rifampicin, isoniazid/co-spray dried particles	nitrocellulose membrane was placed on stage 3 of an NGI	SLF pH 7.4	Chan et al., 2013
	Salbutamol/micronized powders of salbutamol base and sulfate form, Ventolin®	twin stage impinger was used to deposit particles on the Transwell® polyester membranes	Hanks balanced salt solution, SLF with 0.02 % DPPC	Haghi et al., 2012
	Salbutamol/solid lipid microparticles	samples were manually sprinkled on the membrane	PBS pH 7.4	Scalia et al., 2012
	Salmeterol xinafoate/blends with lactose	samples were manually sprinkled on the filter	PBS pH 7.4	Balducci et al., 2015
(Modified) Transwell® system	Beclometahasone dipropionate/ Vanceril® Qvar®	stages 2 and 4 of 8-stage ACI	PBS pH 7.4 distilled deionized water	Arora et al., 2010
	Budesonide/Pulmicort® Turbuhaler®			
	Budesonide/micronized particles	abbreviated ACI with a stage extension	PBS pH 7.4	May et al., 2015
	Budesonide/Symbicort®	filter papers placed on stage 4 of the ACI or NGI	PBS with 0.5 % SDS	Rohrschneider et al., 2015
	Ciclesonide/Alvesco®			
	Flunisolide/Aerobid®	stages 2 and 4 of 8-stage ACI	PBS pH 7.4 distilled deionized water	Arora et al., 2010
	Fluticasone propionate/Flovent® Diskus®			
	Triamcinolone acetonide/ Azmacort®			
	Fluticasone propionate/ Flixotide®	filter papers placed on stage 4 of the ACI or NGI	PBS with 0.5 % SDS	Rohrschneider et al., 2015
Dialysis bag	Rifampicin, rifabutin/ chitosan microparticles	Microparticles were placed in dialysis bag which was suspended in a stoppered tube	SLF pH 7.4	Pai et al., 2015
	Rifampicin/ freeze-fried microparticles			Maretti et al., 2016
	Voriconazole/Polylactide large porous particles	Samples were manually dispersed in the dialysis bag	PBS pH 7.4 with 0.1 % polysorbate 80	Arora et al., 2015

Table 3. Compositions of the physiological lung fluid, simulated lung fluid (SLF), modified SLF and the applied SLF in mEq/L (adapted from Kalkwarf, 1983; Davies and Feddah, 2003).

Ions	**Physiological**	**SLF**	**Modified SLF with 0.02 % DPPC**	**Applied SLF**
Calcium, Ca^{2+}	5	5	5	-
Magnesium, Mg^{2+}	2	2	2	2
Potassium, K^{+}	4	4	4	4
Sodium, Na^{+}	145	145	145	150
Total cations	156	156	156	156
Bicarbonate, HCO_3^{-}	31	31	31	31
Chloride, Cl^{-}	114	114	114	115
Citrate, $C_6H_5O_7^{3-}$	-	1	1	-
Acetate, $C_2H_3O_2^{-}$	7	7	7	7
Phosphate, HPO_4^{2-}	2	2	2	2
Sulfate, SO_4^{2-}	1	1	1	1
Protein	1	-	-	-
DPPC	-	-	200 mg	-
Total anions	156	156	156	156
pH	7.3 – 7.4	7.3 – 7.4	7.3 – 7.4	7.3 – 8.7