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Review Article

In vitro dissolution testing methods for inhaled drugs

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ABSTRACT

Mimicking the lung environment has always been a challenge with regards to dissolution testing of inhaled drugs from dry powder inhalers (DPIs). The aim of this review is to critically appraise the literature currently available on the in vitro test methods for dissolution of orally inhaled drug particulates. Reasons for the lack of standardised testing methods are discussed. Currently, there is not one test that fully represents the situation that occurs in the lungs in vivo, and this is the reason for the lack of a dissolution test recommendation by the pharmacopoeia. The importance of dose collection as a prerequisite to dissolution testing is also discussed using the Andersen cascade impactor as an example. Moreover, a study was carried out to determine the most robust method for testing the dissolution of fluticasone. Three different testing methods were used, i.e., the Transwell system, the paddle-over-disk method and DissolvIt. The results of this study determined that the paddle-over-disk method had the fastest dissolution rate. However, the data showed that there was a lack of similarity between all three tests. This lack of similarity between dissolution methods contributes to the reason why there is no standardised recommended dissolution method listed in the pharmacopoeia. Whilst the paddle-over-disk method yielded the fastest dissolution rate, it does not mean that it is reflective of in vivo dissolution.

Keywords: Pulmonary drug delivery, particle dissolution, inhalation biopharmaceutics

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INTRODUCTION

In recent years, the pulmonary route has become an increasingly favourable route for the delivery of medication to treat local pulmonary conditions (e.g., asthma, chronic obstructive pulmonary disease) and systemic diseases (e.g., diabetes mellitus). Reasons for the latter include, the highly perfused nature of the lungs in addition to the large surface area (>100 m^2) and ultra-thin epithelium (0.2 – 0.7 µm) of the alveoli (Agu et al., 2001). Despite these advantages, drug delivery via the pulmonary route still poses some challenges for pharmaceutical scientists.

Drug administration via the pulmonary route is non-invasive and allows for the absorption of macromolecules and small drug molecules. The lungs provide an increase in drug permeability than that of the gastrointestinal route. Drugs administered via this route also avoid the process of first-pass metabolism by the liver (Patton & Byron, 2007).

The speed of absorption via the pulmonary route is faster than any other non-invasive route of drug administration. This allows for drug absorption in seconds, and therefore, can be very useful in treating symptoms that require a rapid response (Patton et al., 2004).

The human lungs are extremely complex in nature, and can be subdivided into the conducting zone and the respiratory zone. Whilst the lungs are an invaluable route for drug administration, innate clearance mechanisms can sometimes limit their benefit. The epithelium within the conducting zone is lined with cilia and mucus designed to clear particles that are present here via mucociliary clearance (Henning et al., 2010). Thus, pulmonary drug dissolution is influenced by a range of factors including inhalation of the drug particles, deposition, absorption, mucociliary clearance (MCC) and phagocytosis by macrophages (Figure 1).

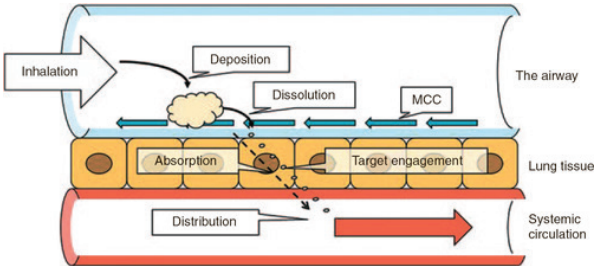

Figure 1. Schematic diagram of drug deposition, dissolution, mucociliary clearance (MCC), absorption and distribution within the lung (from Bäckmann et al., 2014).

The dissolution properties of a drug are a critical determinant of its bioavailability and therapeutic efficacy. They represent the process by which a solute disintegrates in a solvent to yield a solution. Dissolution testing is routinely used in testing solid oral dosage forms. It serves as a promising tool for predicting the release and dissolution of drugs *in vivo*. It can also be used to obtain a waiver for *in vitro* bioavailability studies in the Biopharmaceutics Classification System (BCS) (Klein, 2019). The US Food and Drug Administration (FDA) have set out a guidance for the dissolution testing of solid oral dosage forms. This guidance includes the standardised dissolution testing methods that must be used (basket method USP 1 and paddle method USP 2), the conditions that must be maintained during testing and the acceptance criteria that is required (US FDA, 2018). However, such guidance does not yet exist for orally inhaled drug products.

When a drug is inhaled orally from a dry powder inhaler (DPI), it is deposited onto the epithelium of the lung and must dissolve into the lung fluid before it can exert its action. The process is vital and acts as the rate-limiting step for absorption into systemic circulation as well as a prerequisite for local action (Rohrschneider et al., 2015).

The dissolution behaviour of a drug can be assessed using a number of different *in vitro* dissolution tests. Such tests can give us an indication of how this process occurs *in vivo*. However, to date, there is no standardised *in vitro* dissolution test for assessing the dissolution behaviour of orally inhaled drug products (US FDA, 2018). This lack of predictive *in vitro* dissolution tests makes it harder to determine the dissolution behaviour that an orally inhaled drug is likely to possess *in vivo* (Floroiu et al., 2018). According to the European Medicines Agency (EMA), there are a number of pharmaceutical development studies that a drug must initially undergo. These include aerodynamic particle size distribution testing, physical characterisation testing and testing of the rate at which the drug is delivered. Dissolution testing, however, is not a requirement set out by the EMA or the US FDA (EMA, 2006).

This lack of standardised dissolution testing makes it difficult to analyse the fate of drug particles in the lung after they are deposited. Limitations in the testing methods and biological clearance is the main reason for this. In this review, we aim to first outline the barriers which prevent the development of a reliable dissolution testing method for orally inhaled drug particles. We then review the importance of dose collection as a prerequisite to dissolution testing. Next, the benefits and limitations of the various methods used to carry out dissolution tests on orally inhaled drug particles are assessed, and finally, the results of a short study which was carried to compare the different dissolution profiles of fluticasone propionate are discussed.

STATE OF THE ART

Although, there is no pharmacopeial method for dissolution testing of orally inhaled drugs, there are still a wide variety of methods that have been developed for this purpose. This section of the paper aims to discuss these methods as well as discussing the factors that influence drug dissolution in the lungs.

Barriers to dissolution testing of orally inhaled drug particles

There are many limitations and barriers that occur when trying to simulate a dissolution test for orally inhaled particles. Some of these limitations relate to physiological conditions in the body that are extremely difficult to replicate *in vitro*. Others relate to pitfalls in the testing apparatus that prevent them from

being reflective of conditions *in vivo*. This lack of *in vitro-in vivo* correlations (IVIVC) means that *in vitro* testing is not fully predictive for the outcome of pharmacokinetic studies for orally inhaled formulations and there is, therefore, a need for the assessment of drug dissolution using *in vivo* testing techniques (Fröhlich, 2019).

Mimicking the lung environment in still a major limitation to the development of a reliable dissolution testing method for orally inhaled drugs (Radivojev et al., 2019). Mucociliary clearance of drug particles from the lung to the oral cavity and clearance by phagocytosis are parameters that occur *in vivo* but cannot be represented *in vitro*. These clearance mechanisms can also act as a barrier to the therapeutic effectiveness of the drug (Labiris & Dolovich, 2003). Mucus is secreted on top of the epithelium by goblet cells and submucosal glands. Cilia present in the trachea extend to the terminal bronchioles where they are in contact with the mucus. This thick mucus acts as a physical and chemical barrier to drug particles. When insoluble drug particles get trapped in the mucus, they are moved to the oropharyngeal region by mucociliary clearance and cough (Figure 2). These particles are mixed with saliva and are swallowed. This mechanism means that not all of the administered drug is dissolved and absorbed (Patton et al., 2010). Mucociliary clearance is a natural biological response and is one that may be difficult to replicate *in vivo*. Therefore, an *in vitro* dissolution test that does not take into account these clearance mechanisms cannot accurately reflect the conditions that occur *in vivo*.

Another clearance mechanism in the lungs is phagocytosis by alveolar macrophages in more peripheral regions. Once particles are internalised by phagocytosis, they under degradation by lysosomal enzymes. The extent of this mechanism is dependent on the number of particles deposited and the number of alveolar macrophages. This clearance mechanism affects dissolution and makes it more difficult for a robust *in vitro* dissolution test to be developed (from Ruge et al., 2013).

Another limitation to dissolution testing of orally inhaled drugs is the lack of sink conditions that occur in the lung. Sink conditions must be maintained during standard dissolution testing to maximise biological relevance (Radivojev et al., 2019). According to the European Pharmacopoeia, sink conditions represent a volume of solvent or dissolution media that should be at least 3-10 times the volume present in the drugs saturated solution. The lungs have a large surface area (>100 m^2), however, the volume of liquid in the respiratory tract of a healthy human is between 10-20 ml. These figures vary in those suffering with pulmonary diseases (Floroiu et al., 2018). However, most dissolution apparatus use non-physiological volumes of dissolution ranging between 60-1000 ml in order to maintain sink conditions (May et al., 2015). These conditions are not reflective of conditions *in vivo* and contribute to the difficulty in developing a robust dissolution test for inhaled drugs. In recent years, this has been improved by the use of Transwell inserts which only require a small amount of dissolution media (May et al., 2015).

Agitation and stirring of the dissolution media are commonly seen in dissolution testing of orally inhaled drugs. The rate of stirring increases as the volume of the dissolution vessel decreases, i.e., smaller vessels need faster stirring rates (Radivojev

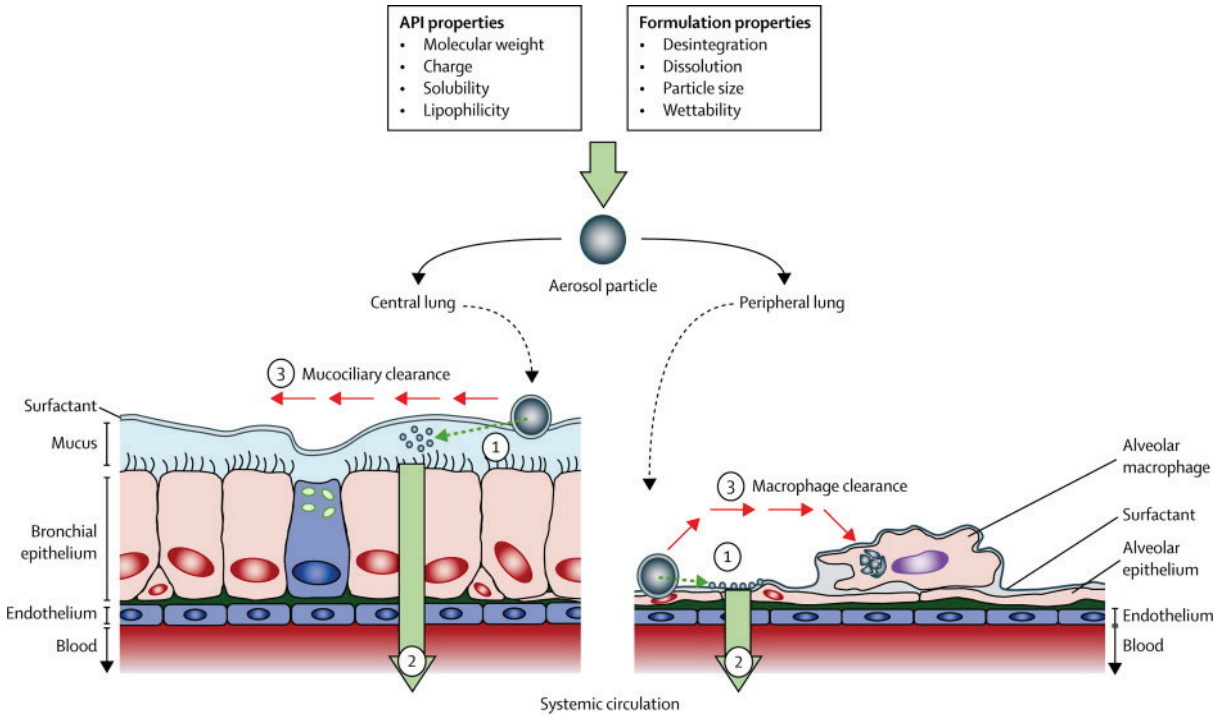

Figure 2. Biological clearance mechanisms of drug particles after inhalation. Another clearance mechanism in the lungs is phagocytosis by alveolar macrophages in more peripheral regions. Once particles are internalised by phagocytosis, they under degradation by lysosomal enzymes. The extent of this mechanism is dependent on the number of particles deposited and the number of alveolar macrophages. This clearance mechanism affects dissolution and makes it more difficult for a robust *in vitro* dissolution test to be developed (from Ruge et al., 2013).

et al., 2019). Stirring increases dissolution rates. However, the fluid in the lungs is not subjected to mixing forces. Therefore, the use of agitation *in vitro* is not reflective of the physiological conditions that occur *in vivo* (Shaji & Shaikh, 2016). The combined use of large vessels and agitation causes on overestimation of the amount of dissolution that is likely to occur *in vivo*.

In order to mimic *in vivo* conditions, the simulated lung fluid (SLF) used in the tests must be almost identical to the lung lining fluid. However, it is difficult to create an exact copy of SLF that contains the authentic mucus and the correct proteins present in the lung (Riley et al., 2012). The simulated fluid also needs be stable and easy to reproduce. Sometimes, different fluids may be needed to reflect the variations in the composition of the lining fluid along the respiratory system (Radivojev et al., 2019). However, it is difficult to produce SLF due to their complex formulations. They have been proven to be unsuitable for pH dependent drugs due to their low buffering capacity. For one dissolution test, the SLF caused an increase in pH from 7.4 to 8.8 over a 24-hour period (Floroiu et al., 2018).

Surfactants or lung surfactant preparations, for example, Survanta® is sometimes added to the dissolution media especially when analysing drugs with poor soluble drugs. The addition of these surfactants can increase the dissolution rate, solubility and wettability. One surfactant used is dipalmitoyl phosphatidylcholine (DPPC) which is present as the most abundant surfactant in the lungs. However, preparation of this surfactant is variable and time consuming, and so, synthetic surfactants are often used as an alternative (Riley et al., 2012).

Effect of aerodynamic particle size distribution (APSD) on dissolution

There are three main types of pulmonary drug delivery systems available to patients. These are: dry powder inhalers (DPIs), pressurised metered dose inhalers (pMDIs) and nebulisers. In DPI's, the drug particles are often bound to lactose carrier particles. This mix of particles are deagglomerated and the carrier particles are impacted on the walls of the upper airways while the drug particles move down into the lung. DPIs are generally the most favourable pulmonary delivery system as they are breath actuated and easier to use

(Kwon et al., 2020). Aerodynamic particle size is a crucial factor that effects drug dissolution. In order for a drug particle to reach the lungs, it must be small enough to avoid retention in the mouth but big enough to avoid being exhaled back into the environment. Aerosols with a mass median aerodynamic diameter (MMAD) of >10 µm are usually impacted in the oropharyngeal region by inertial impaction. Aerosols <1 µm remain suspended in the air and are therefore, moved out of the respiratory tract upon further exhalation (Shaji & Shaikh, 2016). Thus, the ideal particle size at which aerosols can be deposited in the lung and undergo dissolution is at a mass mean aerodynamic diameter of 1-5 µm (Labiris & Dolovich, 2003).

Examples of *in vitro* particle collection techniques accepted by the Food and Drug Administration (FDA) include the eight stage Andersen Cascade Impactor (ACI) at 60 L/min, the seven stage Next Generation Impactor (NGI) or the four stage Multi-stage Liquid Impinger (MSLI) at 60 L/min (Fröhlich, 2019). This usually represents the first step involved in *in vitro* dissolution testing and involves the collection of the appropriately sized drug particles on a membrane which can then be coupled to a chosen dissolution setup (Tay et al., 2018)

The ACI is an instrument consisting of eight stages. The apparatus is pressure sealed and a vacuum is applied throughout the system. There is a collection plate between each stage. There is a nozzle between each stage which narrows as you move down the impactor (Figure 3). If the particle diameter is too large, the particle will not continue to move in the airstream through the impactor, and will instead be impacted on the collection plate (Andersen, 1958).

The aerosolised dose will be present on the plate at the bottom of the chamber and can then be used for dissolution testing. Often, a polyvinylidene difluoride (PVDF) membrane filter is placed on the last collection plate in order to aid transfer of the aerosolised particles into the dissolution apparatus (Riley et al., 2012).

The next generation impactor (NGI) is another method of classifying aerosolised particles based on their size. It consists of seven stages with removable impaction cups that allow for assay (Yoshida et al., 2017). The impactor operates at an inlet flow rate between 30 and 100 L/min. The apparatus also contains a micro-orifice collector (MOC) which is used to capture extremely small particles which can then be coupled to the dissolution apparatus (Marple et al., 2003). Whilst the dose collec-

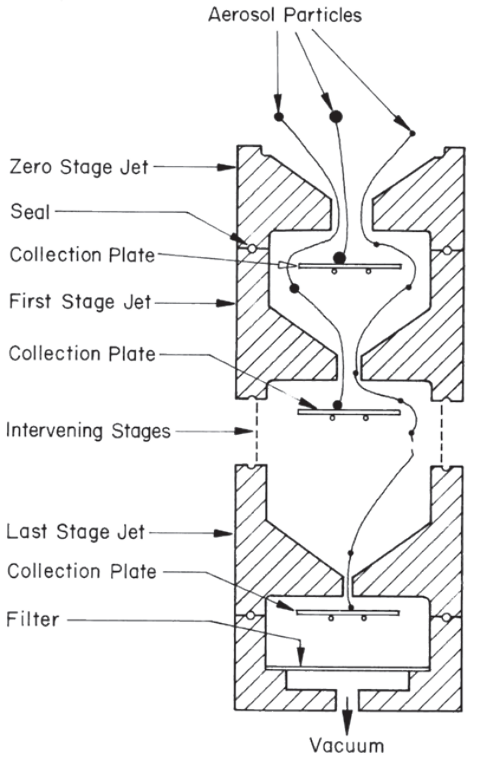

Figure 3. Schematic of an Andersen Cascade Impactor (ACI) (from USP)

tion step can be avoided and dissolution testing can be carried out using the active pharmaceutical product (API), performing tests on the aerosolised particles is the most accurate way to reflect the conditions that occur *in vivo*.

Dissolution testing methods for orally inhaled drug particles

After particle collection of the aerosolised drug using the ACI, dissolution testing can occur. The drug present on the membrane can then be transferred directly into the dissolution apparatus. Here, the drug will be released through the membrane and dissolution can be assessed (Frenning et al., 2020). There are different techniques used to carry out dissolutiontesting. Each technique has its own advantages and limitations.

USP 2 Paddle Apparatus

The Paddle Apparatus is the most common *in vitro* dissolution technique that has been used ever since it was founded in 1978 (Dokoumetzidis & Macheras, 2006). This method is easy to set up and can be used to the analyse dissolution behaviour of a range of different dosage forms. The basic apparatus consists of a semi-hemispherical vessel containing the dissolution medium at a volume up to 1000 ml. The vessel is immersed in a water bath, and inside the vessel, there is a paddle which agitates the system (Deepika et al., 2018). A common adaptation involves the placement of the membrane filter from the ACI into the dissolution vessel. The membrane is usually sandwiched with another membrane before it is placed in the vessel. This method is known as the "paddle over disk" method. The main advantage of this adaptation is that it allows different types of particle collection filters to be used in the dissolution vessel. However, the filter may act as a hinderance to wetting and can increase diffusion layer thickness (Riley et al., 2012). The large volumes of dissolution media in the vessel and the presence of agitation means that this technique, although robust, is not reflective of the conditions that occur in the lungs.

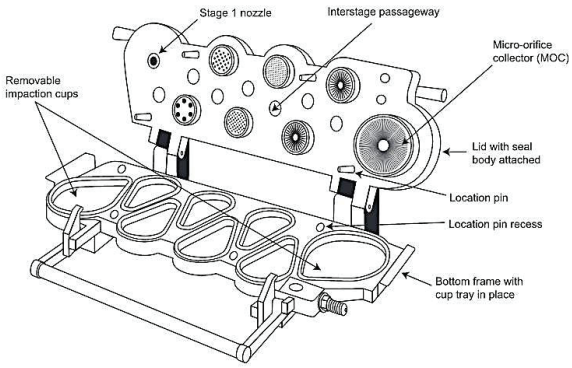

Figure 4. Schematic of a Next Generation Impactor (from Marple et al., 2003).

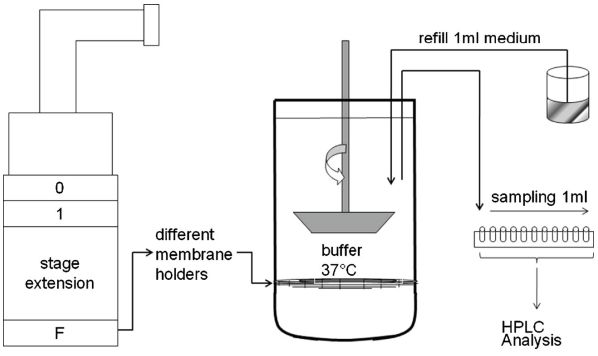

Figure 5. Schematic of an Andersen Cascade Impactor (ACI) coupled to Paddle Over Disk apparatus (from May et al., 2014).

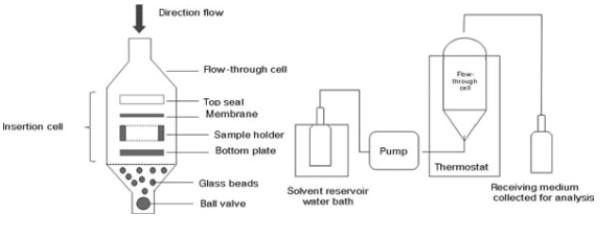

Figure 6. Schematic of an open flow through cell system (from Olejnik et al., 2012).

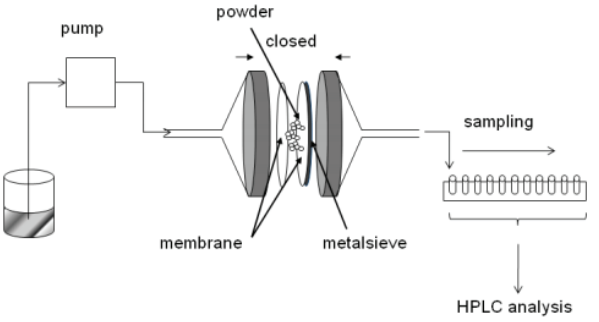

Figure 7. Schematic drawing of the modified flow through cell (from May et al., 2012).

USP 4 Flow Through Cell Apparatus

The flow through cell dissolution method is an analytical method that has been used for decades and was implemented into the European Pharmacopoeia in 2007 (29). It assesses dissolution based on the flow of the medium containing the drug through a cell. The medium is pumped through the flow through cell using a pulsating piston pump with a flow rate between 4 and 16 ml min^{-1} (McDonnell et al., 2018). The bottom of the cell contains small glass beads approximately 1 mm in diameter (Singh & Aboul-Enein, 2006). The cell mainly operates as an open system whereby fresh medium is continuously piped through the cell. The alternative is a close system where the medium is recycled through the cell (Fotaki & Reppas, 2005). This method is often used as the preferred method for dissolution testing of poorly soluble drugs (Eaton et al., 2012).

This technique has been modified by Boehringer Ingelheim to a flow through cell which features the particle collection membrane filter taken straight from the ACI. This modification occurred in order to ensure sink conditions, uniform flow and homogenous wetting (May et al., 2012). The filter is covered with another membrane filter and is placed into the cell where it is held in place using a filter holder. The dissolution medium is pumped through the cell by a HPLC pump and the fractions are collected. Whilst this technique is beneficial in terms of keeping the system homogenous, the high velocity of the

dissolution medium does not represent the behaviour of lung fluid *in vivo*. This system is also sensitive to entrapped air (Floroiu et al., 2018).

Franz Diffusion Cell

The Franz diffusion cell is a membrane type dissolution method that was first conducted by Thomas J. Franz in 1975. The original set up of the apparatus was used to test the permeability of a membrane, however, this is modified to allow for dissolution testing. This modification consists of dissolution media in a vessel with a volume of up to 1 litre to allow sink conditions. The membrane with the aerosolised particles from the ACI can be taken directly and placed on top of the vessel in a membrane holder (Radivojev et al., 2019). The system is agitated with a stirring bar that is located within the vessel. The system is heated to physiological temperature and condensed drops fall onto the particles on the membrane and stimulate dissolution. Samples are taken and the solvent removed is replaced with fresh dissolution medium in order to maintain a constant volume (May et al., 2012) This method is favourable as it takes into account the air liquid interface that is present in the lungs. However, there are still conditions that occur which do not represent *in vivo* dissolution such as the presence of agitation. It can also be challenging to distinguish between diffusion effects through the membrane and the dissolution rate (Riley et al., 2012).

Transwell® System

The Transwell system is another membrane type dissolution method, however, unlike the Franz cell there is no agitation present in the Transwell apparatus (Riley et al., 2012). The Transwell system only requires a small volume of dissolution medium which is more reflect of biological conditions present in the lung (Velaga et al., 2018). After aerosol deposition in the ACI, the membrane containing the required drug particles is removed and placed facing down onto the semi-permeable polyester membrane of the Transwell insert. One point 4 millimetres of the dissolution media is poured over the membrane to initiate the dissolution process. Aliquot samples of half a millimetre are taken and this is replenished with fresh dissolution medium in order to maintain the initial volume (Arora et al., 2010). The system must be maintained at a temperature of 37°C and a relative humidity of 100% (Floroiu et al. 2018). In order to determine the influence of mucus on dissolution, porcine tracheal mucus can be coated onto the Transwell insert (Cingolani et al., 2019). This method is robust; however, it does not take into account the sink conditions that occur in the lungs. In order to mimic the sink conditions that occur *in vivo*, a high diffusion coefficient and low retention must be present (Riley et al., 2012).

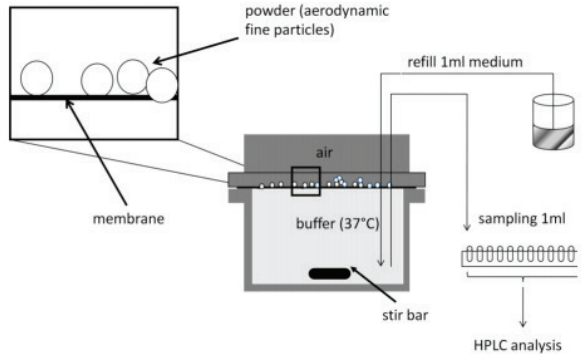

Figure 8. Schematic diagram of the modified Franz diffusion cell (from May et al., 2012).

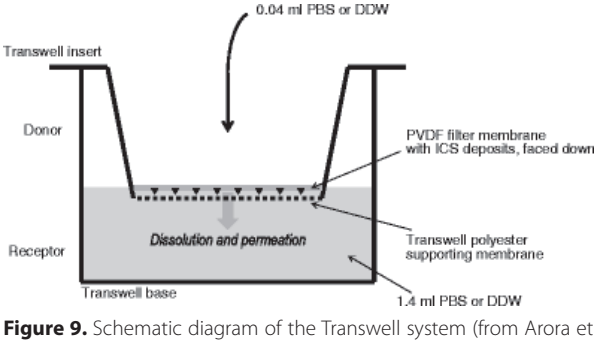

Figure 9. Schematic diagram of the Transwell system (from Arora et al., 2010).

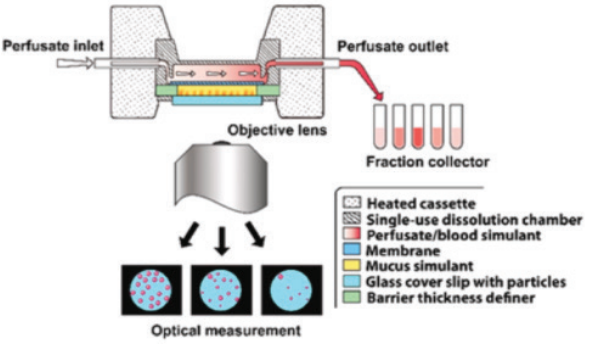

Figure 10. Schematic diagram of the DissolvIt dissolution apparatus (from Gerde et al., 2021).

DissolvIt Apparatus

This is a stimulation tool for dissolution and absorption testing of inhaled dry powders. Simulated blood acts as the dissolution medium and it is pumped through the system so that it flows across a membrane that is in contact with the aerosolised particles (Floroiu et al. 2018). These conditions mimic the air-blood barrier that exists in the upper airways. However, it must be noted that the air-blood barrier in the DissolvIt system is of greater thickness than the epithelium in the deep lung, which may increase the retention time *in vitro* (Floroiu et al. 2018). The particles are dissolved in a mucus stimulant and present on a glass cover slip. Over the mucus is a polycarbonate membrane which represents the basal membrane in the respiratory tract. As the simulated blood passes over the membrane, it absorbs the dissolved constituents of the particles and can then be analysed using mass spectroscopy. DissolvIt is capable of producing pharmacokinetic profiles of fluticasone propionate that resemble that in the rat lung. This shows that it may be useful for *in vivo in vitro* correlations in orally inhaled drugs dissolution testing (Börjel et al., 2015). Whilst this system looks promising, unfortunately, there is not a lot of reported data on its performance in dissolution testing (Hassoun et al., 2019).

A comparison of different dissolution testing methods using fluticasone propionate (FP) as an example

This study aimed to compare the dissolution profiles of FP using three different dissolution methods. The methods analysed include the Transwell system with a 0.4 µm polyester membrane, the paddle-over-disk method and the DissolvIt system.

MATERIALS AND METHODS

This study looked at the results obtained from dissolution testing of FP that have already been carried out. Various tests of the different dissolution testing methods were analysed. For the purpose of this study only the tests that used FP as the drug were used. This allows for comparison of the results obtained from each of the studies.

The Transwell system dissolution test, as described by Rohrschneider *et al.,* involved the use of a Flixotide DPI inhaler containing 100 µg of FP per actuation. The inhaler was actuated five times into an Andersen cascade impactor. The aerosolised dose present on the filter paper at the last stage was then transferred directly into the Transwell system. The Transwell system consisted of a 6-well plate and 1.5 mL of dissolution media (0.5% SDS in PBS). The dissolution test was initiated by pouring 0.1 mL of dissolution media over the filter paper. Samples were taken at various time points and the removed volumes were replaced with fresh medium. The samples were analysed using HPLC analysis (Rohrschneider et al., 2015).

The paddle-over-disk method as described by Price *et al.* involved the use of a Flixotide DPI inhaler containing 100 µg of FP per actuation. The next generation impactor was used to collect the aerosolised dose. The collected dose was then transferred onto a 50 mm diameter stainless steel disk with a 74-mesh screen. This disk was placed within a vessel containing 300 mL of dissolution medium (0.2% SDS in PBS). The paddle was set to a speed of 75 rpm. Samples were taken at various time points and the removed volumes were replaced with fresh medium. The samples were analysed using HPLC analysis (Price et al., 2020).

The DissolvIt method, as described by Hassoun *et al.,* involved the use of a Flixotide DPI inhaler containing 50 µg of FP per actuation. The aerosolised dose was collected using the US Pharmacopoeia Induction Port No.1 which is a standardised simulation of the throat. The aerosolised particles were placed on a glass cover slip. The dissolution media (5.7 µL of Survanta) was applied to the polycarbonate membrane. Perfusate containing phosphate buffer and 4% w/v albumin was streamed over the membrane at a flow rate of 0.4 mL/min. Samples were taken at various time points and the removed volumes were replaced with fresh medium. The samples were analysed using LC-MS analysis (Hassoun et al., 2019).

The numerical data for each of the three tests was found by extrapolation of graphs from each study. The points were extrapolated at the same time points for each of the studies. These data points could then be graphed as connected scatterplots using Microsoft Excel and were also combined in order to allow the various results to be compared (Figure 11).

The three sets of numerical data could then be analysed and compared by performing a one-way ANOVA test. This allowed for comparison of the three different means. It also gave a p value and F value for significance which allowed the null hypothesis to be rejected. In order to assess where exactly the differences occur between the three groups, a post-hoc test was conducted using a Bonferroni correction. The Bonferroni correction value can be determined using the following formula:

$$\text{Bonferroni corrected p value} = \alpha/n$$

where α is 0.05 and n is the number of tests being compared.

The data was also compared by determining the similarity (f_2) and the difference (f_1) factor. Similarity and difference factors are often used to compare two or more dissolution profiles. The difference factor (f_1) represents the percentage difference between two dissolution profiles at each timepoint. It is calculated using the following equation:

$$f_1 = \left(\frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right) \times 100$$

where n is the number of time points, R_t is the mean dissolution value for the reference at time t, and T_t is the mean dissolution value for the test at the same time point. If f_1 is lower than 15 (0-15), there is no difference between the two dissolution profiles.

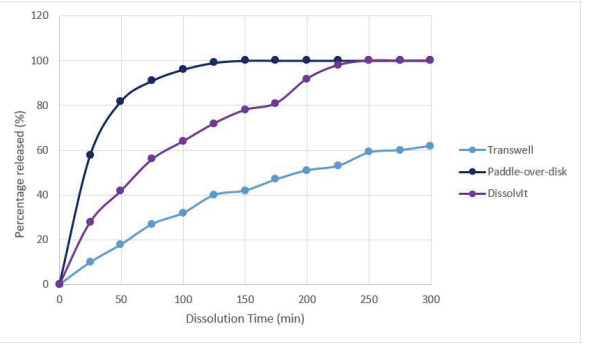

Figure 11. Comparison of fluticasone propionate dissolution profiles.

Table 1. Similarity and difference factor results.

	Transwell System vs. paddle-over-disk	Transwell System vs. DissolvIt	DissolvIt vs. paddle-over-disk
Similarity Factor (f_2)	13	23	30
Difference Factor (f_1)	144	88	23

The similarity factor measures the similarity that exists between two dissolution profiles at each timepoint. It can be measured using the following equation:

$$f_2 = 50 \times \log_{10}\left[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{n}(R_t-T_t)^2}{n}}}\right]$$

where n is the number of time points, R_t is the mean dissolution value for the reference at time t, and T_t is the mean dissolution value for the test at the same time point. An f_2 value of greater than 50 (50-100) indicates that two dissolution profiles are similar (Diaz et al., 2016).

Comparison of the three graphs show that the paddle-over-disk system has the fastest dissolution rate with all of the FP being dissolved by 150 min. DissolvIt also reaches 100% dissolution, however, at a slower rate than the paddle-over-disk set up. The Transwell system has the slowest dissolution rate with only 62% of the drug dissolved by the time the study was ceased at 300 min.

Calculation of dissolution similarity and difference factor:

The results for the similarity factor (f_2) calculation shows that all three dissolution profiles lack similarity. A f_2 value of greater than 50 indicates that the dissolution profiles of the two methods are similar. All of the results obtained are below 50, which indicates that the profiles are dissimilar.

The results for the difference factor (f_1) show that there is a great variation between the dissolution profiles. A f_1 of less than 15 indicates that there is no difference between the dissolution profiles. The results obtained are well above 15, thus indicating the variability that exists between the dissolution profiles.

The results of the ANOVA test show that there is a significant difference between the dissolution profiles obtained for the three different testing methods. For data to be statistically significant, it must yield a p-value of less than 0.05. The p-value in this study is 0.00024812. This result means that the null hypothesis must be rejected in favour of the alternative hypothesis.

Post hoc analysis

Bonferroni correction value: 0.05/3 = 0.0167

A post hoc was conducted to find out exactly where the differ-

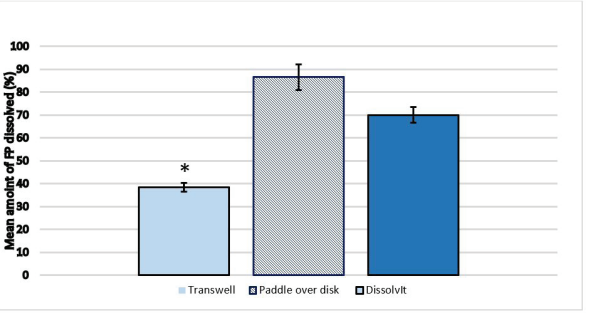

Figure 12. Comparison of the three mean FP dissolved with error bars representing the standard errors of means and an asterix (*) to show a difference in significance.

Table 2. ANOVA test results.

ANOVA: Single Factor					
SUMMARY					
Groups	**Count**	**Sum**	**Mean**	**Variance**	
Transwell	13	501	38.54	398.10	
Paddle over disk	13	1126	86.62	821.42	
DissolvIt	13	911	70.08	986.41	
ANOVA					
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**
Between Groups	15511.5	2	7755.7	10.5475901	0.00024812
Within Groups	26471.2	36	735.3		
Total	41982.7	38			

Table 3. Post hoc test results.

	P-value	**< 0.0167**	**Significant**
Transwell vs. Paddle Over Disk	0.0000455655	YES	YES
Transwell vs. DissolvIt	0.005429612	YES	YES
Paddle over disk vs. DissolvIt	0.173584067	NO	NO

ences noted from the ANOVA test lie. The p-value was determined using the Bonferroni correction. The results show the Transwell system is statistically different from both the paddle-over-disk and DissolvIt methods. Thus, the null hypothesis must be rejected. When comparing the paddle-over-disk to DissolvIt, the p-value obtained was greater than 0.0167. Thus, the null hypothesis can be accepted.

DISCUSSION

Whilst dissolution testing is a very important parameter for the quality control of solid dosage forms, there is currently no standardised dissolution testing method recommended for orally inhaled drugs (Floroiu et al., 2018). However, the importance of testing the rate of dissolution prior to drug absorption has been emphasised since the introduction of the Biopharmaceutics Classification System (BCS) (Rohrschneider et al., 2015).

Dissolution testing is highly beneficial in drug development and can often be used to obtain a biowaiver for *in vivo* bioequivalence studies (Ku, 2008). However, the lack of a fully validated dissolution method makes it difficult to produce an *in vitro - in vivo* correlation (IVIVC) for orally inhaled drugs (Fröhlich, 2019). The main reason for this is the lack of an *in vitro* dissolution testing method that accurately mimics the physiological conditions that occur *in vivo*. Each of the testing methods comes with its own limitations as discussed in this paper.

As well as limitations in the testing methods, there are also certain biological events that occur in the lung that make it difficult to develop a suitable dissolution test. The two main events being clearance by the mucociliary escalator and clearance by phagocytosis (Labiris & Dolovich, 2003). These clearance mechanisms do not occur *in vitro*. Many *in vitro* dissolution testing methods also feature non-sink conditions and agitation, both of which do not occur in the lungs. The presence of these conditions creates an overestimation of dissolution *in vitro,* and therefore, make it difficult to compare this to dissolution behaviours *in vivo*. The presence or absence of lung surfactant also affects *in vitro* dissolution. Ideally, dipalmitoyl phosphatidylcholine (DPPC) is the preferred surfactant as it is the same one that is present in the lungs. However, synthetic surfactants are often used as they are more reproducible (Riley et al., 2012).

A number of academics have previously carried out dissolution tests on orally inhaled drug particles. These studies analyse the dissolution profiles obtained when using the different methods individually (18), the influence of simulated lung fluid composition with or without surfactant (Kumar et al., 2017) and the effect that varying the membrane pore size has on dissolution (Frenning et al., 2020). Since data exists in these areas, it was decided that this study would look at the different methods previously tested, and compare them to each other to determine the most suitable method. Fluticasone propionate was chosen as the drug to study due to its prevalence in treating asthma and other respiratory conditions.

The results obtained from this study show a great deal of variability between the different methods. The results show that the paddle-over-disk method yielded the fastest dissolution rate (Figure 11). This method saw 99% of all fluticasone dissolved at 125 min. A separate study conducted by Velaga *et al.* similarly found that the paddle apparatus is advantageous for dissolution testing due to its discriminatory power and its reproducibility (Velaga et al., 2018). Despite this, it is hard to accept that this method is suitable for orally inhaled drugs. The paddle apparatus uses large volumes of dissolution media and stirring in order to maintain sink conditions (Floroiu et al., 2018). These conditions do not in any way reflect the *in vivo* dissolution process of orally inhaled drugs.

FP in the Transwell system and DissolvIt dissolves slower than it does in the paddle-over-disk apparatus (Figure 11). However, these methods are more reflective of the lung physiology due to the presence of membranes representing the air-liquid interface. The volumes (which appear to have a significant impact on the dissolution rate) of dissolution media are much lower, and thus reflect biorelevant conditions. However, these systems still have their own limitations such as the fact that diffusion acts as the rate limiting step which can make it difficult to distinguish between diffusion through the membrane and the drug dissolution rate (Riley et al., 2012).

The data obtained from the three different systems was then compared statistically by performing a one-way variance (ANOVA) test, where $p<0.05$ is significant. The results of this test produced a significant F and p value (Table 2). The p value obtained is less than 0.05 (i.e., 0.00024812), which means that the null hypothesis must be rejected. This indicates that there are differences that exist between the three dissolution profiles. This is expected as the conditions and the limitations vary between the different tests.

A post hoc test using the Bonferroni correction then had to be conducted to determine where exactly the difference lies between each of the systems. The results (Table 3) show the Transwell system is significantly different to the other two testing methods. The lack of similarity determined from these tests can be backed up with the results of the similarity (f_2) and difference (f_1) factor calculations (Table 1). All of the f_1 values above 15 indicate that there are major variations between the dissolution profiles. The f_2 values are also less than 50, which indicates that the dissolution profiles lack similarity. The results involving the Transwell system produced the greatest difference factor (f_1) values and the least similarity factor (f_2) values. This is expected as the post hoc test results show that the Transwell system is the one that is significantly different to the other testing methods.

Overall, the results show that despite the inaccuracy in reflecting the *in vivo* dissolution process, the paddle over disk apparatus appears to be the fastest and most discriminatory dissolution method. The Transwell system appeared to be the technique with the greatest amount of variability. It was also the slowest dissolution method, and had not reached completion by the time that the test was ceased at 300 min. However, a previous study noted that the use of Transwell inserts acted as a barrier to diffusion especially for poorly water-soluble inhaled corticosteroids. When the 0.4 µm Transwell polyester membrane was

switched for porcine mucus layers present on a glass microfiber with a pore size of 3.0 µm, there was a significant improvement in the dissolution rate (Alqahtani et al., 2020).

The Transwell insert used in this study was a polyester membrane with a pore of 0.4 µm. However, if the study is replicated using the same alteration as Alqahtani *et al*., more significant and comparable results may be seen.

The thickness of the particle/perfusate barrier could have also influenced the results obtained for the Transwell system and DissolvIt. The results for dissolution using DissolvIt showed that complete dissolution occurred, but at a slower rate than that of the paddle-over-disk apparatus. This slower dissolution rate is most likely attributed to the fact that the perfusate barrier has a thickness of 60 µm. This would cause retention of the drug particles, and thus, give rise to a slower dissolution rate. In the isolated perfused lung of a rat, this barrier has a thickness of 0.5-5 µm (Börjel et al., 21015). The paddle over disk system avoids this complication as the particles are in direct contact with the perfusate. As a result, retention of drug particles does not occur.

Taken together, the results show the degree of dissimilarity that exists between each of the systems. The paddle over disk method is the fastest and most reproducible, however, of the three, it least represents physiological conditions. DissolvIt acts as a good comparison to physiological conditions, however, it is limited by its overestimation of retention time as a result of a thick air-blood barrier (Floroiu et al., 2018). The Transwell system also serves as a good comparison, however, it is limited due to the varying membrane pore sizes and the lack of a concentration gradient that reflects *in vivo* conditions.

In order to be able to produce comparable dissolution tests, future areas of research need to be considered. These areas include:

- The use of lung cell lines in *in vitro* dissolution tests.
- The use of simulated lung fluid as the dissolution medium instead of phosphate buffered saline (PBS)
- The need for the development of *in vitro in vivo* correlation (IVIVC)
- The development of an artificial lung simulation where dissolution can be tested (Marques et al., 2011).

CONCLUSIONS

This paper aimed to critically review the available literature on dissolution testing of orally inhaled particles. It discussed the different testing methods available and touched on their benefits as well as their limitations. The methods were compared to each other and it was determined that the variability that exists between the methods makes them incomparable.

To achieve this aim, the dissolution profile of fluticasone propionate using different dissolution methods was determined and analysed. The methods studied were the Transwell system, the paddle-over-disk apparatus and the DissolvIt method. The results obtained showed us a lack of similarity between all three methods. This lack of comparability between dissolution methods contributes to the reason why there is no standard dissolution testing method set out by the pharmacopoeia.

In order for a standardised validated dissolution test to be developed for orally inhaled drug particles, certain parameters that accurately reflect *in vivo* conditions must be defined. These parameters are:

- Type of dissolution apparatus
- Composition and recommended volume of the dissolution medium
- Method of aerosolization and sample collection of particles
- Quantification of tested particles

This project also examined the method of aerosolised dose collection using the Andersen cascade impactor (ACI). It is essential that the particles are homogenous and of a mean aerodynamic diameter between 1 – 5 µm before dissolution commences. This is to ensure that the particles being tested are only those that would make it down into the lung and are capable of being dissolved here.

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