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Development of a standardized dissolution test method for inhaled pharmaceutical formulations

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ABSTRACT

The aim of this research was to investigate a potential standardized test method to characterize the dissolution properties of formulations intended for pulmonary delivery. A commercially available dissolution tester was adapted to be used as a testing apparatus by incorporation of a membrane containing cassette. The cassette was designed to enclose previously air-classified formulations, so that they could be uniformly tested in the dissolution apparatus. The influence of particle size, amount of drug loading, and the composition of a simulated lung fluid (SLF) dissolution media on the dissolution rate were studied. Dissolution rate was significantly affected by the uniformity of drug loading, and particle size. Diffusion coefficients, estimated using the Higuchi model, showed an increase from 2.28 to 9.60 $\times$ 10^{-7} cm^2/h as the particle size decreased. Addition of DPPC (0.02%, w/v) to the SLF dissolution media resulted also resulted in an increase in the diffusion coefficient value. This study demonstrated that the developed method was reproducible and may be used to evaluate the dissolution properties of pharmaceutical inhalation products following their aerodynamic particle classification.

1. Introduction

Dissolution testing is an extremely well established and standardized test in all the Pharmacopoeias for evaluating solid and semi-solid dosage forms. Dissolution testing allows one to examine the dissolution behavior of pharmaceutical dosage forms *in vitro* in order to differentiate formulation types and perhaps give an estimate of a dissolution behavior *in vivo*. Dissolution testing is routinely used in quality control (QC) studies such as batch-to-batch consistency, stability and detection of manufacturing deviations. While there are many standardized dissolution test methods for solid dosage forms such as tablets and capsules, there is no applicable method to estimate the dissolution behavior of inhaled active ingredients, although many dissolution methods for testing aerosols have been attempted (Grey et al., 2008). Designing a standardized method applicable to the lung is not an easy task, as the lung has very unique features which are difficult to replicate *in vitro*, such as the extremely small amount of aqueous fluid and lung surfactant (Patton, 1996; Grey et al., 2008). Additionally, it would require that the collection of the API fraction from the entire formulations before dissolution tests, as many inhalation products contain carrier materials. However, several experimental difficulties exist on dose collecting due to very fine and electrostatic powder characteristics (Grey et al., 2008).

Commercial dissolution systems have been widely used to study the dissolution of aerosols. Dry powders have been directly dispersed into an apparatus II dissolution tester (Asada et al., 2004), or placed directly into a modified basket to prevent drug particles from escaping directly into to the dissolution media (Jaspart et al., 2007; Learoyd et al., 2008). However, dry powders intended for pulmonary delivery are hard to disperse homogeneously into the vessel/basket, and dispersed particles stick on the vessel wall and/or paddle/basket during such dissolution tests. In addition, floating powders may be inadvertently collected during the sampling procedure. In an attempt to make up for some of the shortfalls of this type of testing using commercial dissolution systems, several custom built dissolution apparatuses have been investigated. Davies and Feddah (2003) modified a flow-through cell by direct incorporation of HPLC pump. In this method the aerosol particles, obtained using impaction, were collected onto a glass pre-filter for dissolution studies. In another study using a horizontal diffusion cell, manufactured powders were dispersed onto the hydrated membrane and the dissolution rate was estimated by observing their diffusion rate (Cook et al., 2005). In addition to these methods, twin-stage impinger (TSI) (McConville et al., 2000), dissolution cell (Ansoborlo et al., 1998; Sdraulig et al., 2008) and shaking incubator (Ungaro et al., 2006; Kwon et al., 2007) apparatuses were also modified to conduct *in vitro* dissolution studies for dry powders. Although, these approaches do in some way make up for the drawbacks indicated above for a commercial dissolution appara-

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tus, problems are still evident such as the impact of the amount of drug loading, adequate particle dispersion, and the uniformity of particle size distribution. Therefore, no single *in vitro* test system has yet emerged as the ideal choice for performing dissolution measurements for inhalation formulations.

The aim of this paper is to describe a potential standardized test method to characterize the dissolution properties of the multitude of formulation types available for pulmonary delivery. Micronized hydrocortisone (HC) was selected as a model drug to conduct *in vitro* dissolution on dry powders that were aerodynamically classified using the next generation impactor (NGI).

2. Materials and methods

2.1. Materials

Micronized hydrocortisone (HC) (USP grade) and size 3 empty gelatin capsules were purchased from the Spectrum Chemical Co. (Gardena, CA). Dipalmitoyl phosphatidylcholine (DPPC) was purchased from Avanti Polar Lipid, Inc. (Alabaster, AL). InhaLac®70 was donated by Meggle (Wasserburg, Germany). Polycarbonate (PC) membranes of 0.05 and 1 μm were purchased from Whatman (Florham Park, NJ). Cellulose acetate (CA) dialysis membranes of molecular weight cut-off (MWCO) 3500, 12,000 were purchased from Spectrum®Laboratories INC. (Rancho Dominguez, CA). An Aerolizer® dry powder inhaler device was donated by Schering-Plough (Kenilworth, NJ). Histology cassettes were purchased from VWR International, LLC (Suwanee, GA). Methanol (HPLC grade) and all other reagents were purchased from the Sigma Chemical Co. (St. Louis, MO).

2.2. Solubility studies

The solubility of HC was determined in: simulated lung fluid (SLF) and modified simulated lung fluid (mSLF), which contained 0.02% (w/v) DPPC. Samples of HC (100 mg) were added to 20 mL SLF, or 20 mL mSLF and shaken for 24 h at 37 °C. Samples (1 mL) were taken from each vial at 1, 2, 4, 8, 12 and 24 h intervals and filtered with 0.45 μm syringe filter. All the samples were analyzed by an 8453 UV/VIS spectrophotometer (Agilent, Santa Clara, CA) at 248 nm, the resultant equilibrium sample concentration was used to calculate the saturated solubility concentration.

2.3. Dissolution apparatus

A commercial dissolution tester, Hanson SR8-plus dissolution test station (Hanson Co., Chatsworth, CA), was employed to conduct the dissolution study of micronized HC particles. A schematic diagram of modifications to the dissolution apparatus is shown in Fig. 1. The two main components of the dissolution setup include: Component (A) the dissolution test station and component (B) a membrane cassette. (A) The dissolution station consisted of (i) mini-paddles (for 150 mL vessel), (ii) dissolution vessels (150 mL glass vessel round-bottom), (iii) a water bath for temperature control, and (iv) a sampling probe with 10 μm filter tip. The membrane cassette (B) was customized and consisted of a histology cassette frame, and two pieces of selected membrane sandwiched inside the frame (acting a powder holding device).

2.4. Dose collection

2.4.1. Aerodynamic particle separation

Particle separation of both bulk HC, or HC mixed with carrier lactose was conducted using a modified next generation impactor (NGI) (MSP Co. Shoreview, MN). Fig. 3(A) shows assembled NGI dose-plates. The NGI was fitted with a PC membrane at the base of each dose-plate. This membrane was placed beneath wax paper (which had been pre-trimmed to match the plate shape). A rectangular uniformly sized hole was pre-cut (2 cm × 2.5 cm) in the center of the trimmed wax paper that exposed the PC membrane situated beneath it. Micronized HC (2 g) was mixed with 30 g InhaLac® 70 and 150 mg of the mixed powder was filled into #3 empty gelatin capsules. Bulk HC (50 mg) was also filled into #3 empty gelatin capsules. Capsules were placed into the Aerolizer® (Schering-Plough, Kenilworth, NJ) and the device was primed according to the manufacturer's guidelines. Each of the HC containing capsules was dispersed into the NGI through the USP induction port at the flow rate 60 L/min, for 15 s per each actuation. The flow rate was measured by a Model DMF 2000 flow meter (MSP Co. Shoreview, MN).

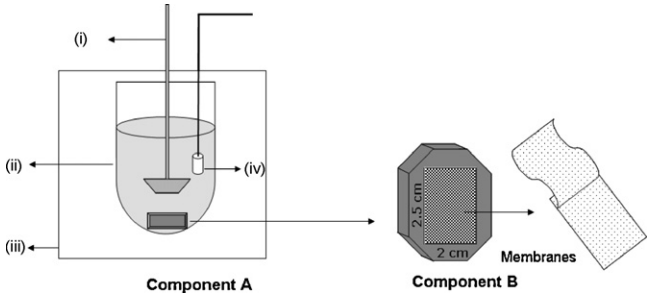

Fig. 1. Schematic diagram of the dissolution apparatus. Component A: dissolution station (i) mini-paddles (for 150 mL vessel), (ii) dissolution vessels (150 mL glass vessel round-bottom), (iii) water bath for temperature control, and (iv) sampling probe with 10 μm filter tip. Component B: membrane cassette.

2.4.2. Scanning electron microscopy (SEM)

Micronized bulk HC, and HC following particle separation by impingement were observed using a LEO 1530 SEM (Zeiss/LEO, Oberkochen, Germany). In this procedure each PC membrane containing separated HC particles was removed from the dose-plate of NGI and cut into a small segment (suitable for mounting on a pin plate SEM stub). The cut membrane segments from each dose-plate were mounted separately onto the stubs using double-sided copper tape, before sputter coating with silver for 40 s under vacuum at 30 mTorr. Additionally, bulk HC powder was mounted and coated as described above. The SEM was operated at high vacuum with accelerating voltage 5 kV and specimen working distance 4 mm.

2.5. Dissolution testing

2.5.1. Dissolution media

Simulated lung fluid (SLF), and modified SLF (mSLF) containing 0.02% (w/v) of dipalmitoylphosphatidylcholine (DPPC) were used for dissolution study. The SLF was first developed by Moss (1979), and was described as being close to that of actual lung fluid in ionic composition and pH. The mSLF differs from the SLF by adding 200 μg/mL DPPC to 100 mL of SLF solution. Both SLF and mSLF have been used in previous published *in vitro* dissolution studies due to their similarity in composition to actual lung fluid (Ansoborlo et al., 1998; Davies and Feddah, 2003; Sdraulig et al., 2008).

To prepare the mSLF, DPPC (200 mg) was weighed into a 500 mL round bottom flask and dissolved in a 40 mL chloroform/methanol (1:1) mixture. The solvent was evaporated by rotary evaporation (Buchi Corporation, New Castle, DE). The dry thin film was dehydrated with 200 mL distilled water at 55 °C and rotated for 2 h. The warm suspension was placed into a Branson 5500 sonic bath (Branson ultrasonics, Denbury, CT) and sonicated at 55 °C for 1 h. The concentrated DPPC solution was stored at 4 °C diluted with SLF before use.

2.5.2. Dissolution testing of bulk HC particles

Using the apparatus setup described above the dissolution medium was equilibrated at 37 °C and the paddle speed was set to 50 rpm. Both bulk micronized HC and dispersed HC onto a membrane was tested. Firstly, bulk micronized HC (3 mg) was carefully weighed, and dispersed into each of 3 dissolution vessels containing 100 mL of SLF. Secondly, 3 mg HC was weighed separately onto a membrane (either PC or CA dialysis membranes). A second presoaked membrane with SLF of the same type was placed on top of the first membrane containing the powder; this was then sandwiched together and fitted into a modified histology cassette. Each cassette was placed into a small volume dissolution vessel containing 100 mL of SLF (sink conditions were given all the test samples). Samples were manually withdrawn from the dissolution vessel at 0.5, 1, 1.5, 2, 3, 4, and 6 h to be analyzed by HPLC. The cumulative amount of dissolved HC was determined from the sum total of HC released.

2.5.3. Dissolution testing of aerodynamically separated HC particles

To aerodynamically classify carrier-free HC and carrier-mediated HC, capsules containing either formulation were fired into the NGI at the flow rate 60 L/min as previously described. The NGI plates were assembled as above (with PC membranes and wax paper impaction templates). Following actuation for a given formulation the wax paper impaction template was removed from each dose-plate, and a PC membrane (pre-soaked with the appropriate dissolution media) was placed onto the top of each plate membrane (that now contained impinged HC particles). The sandwiched membrane enclosed into the cassette as above, was then placed into a dissolution vessel (100 mL, 37 °C, and 50 rpm, sink conditions were given all the test samples). The release rate of HC in either SLF and mSLF media was evaluated. Samples were withdrawn manually at the following time points: 10, 20, 40, 60, 90, 120 min (SLF), and 5, 15, 30, 45, 60, 90 min (mSLF). All samples were analyzed by HPLC. The residual amount of HC on the membranes was determined after the test by washing with 5 mL mobile phase prior to analysing using the validated HPLC method. The total amount of HC initially loaded between two membranes was back calculated using the sum of cumulated amount of HC, plus the remaining quantity of HC on each of the membranes.

2.6. Analysis of HC in SLF, and mSLF

Collected samples in SLF and mSLF were analyzed by using a HPLC system (Waters Breeze, Waters Co., Milford, MA) with UV detection. The system consisted of a 717 plus autosampler, 2487 dual wavelength detector, 1525 binary pump, and 1500 column heater. Dissolution samples of HC in SLF or mSLF were filtered using 0.45 μm PTFE syringe filter before injection. Chromatography was performed using a Capcell Pak C18 5 μm 4.6 mm × 250 mm column (The Shiseido Fine Chemicals, Tokyo, Japan) and a SecurityGuard™ guard column (Phenomenex®, Torrance, CA). The mobile phase which consisted of methanol, water, and phosphate buffer solution pH 7.4 in a ratio of 10:10:0.4 respectively, was eluted at a flow rate of 1.0 mL/min and the UV detector was set to a wavelength 220 nm (Barichello et al., 2006). The column temperature was maintained at 40 °C and the volume of each sample injected was 20 μL (Barichello et al., 2006).

2.7. Mathematical modeling

A diffusion coefficient for each dissolution study was determined using computer fitted data. The least square method was performed using MatLab (The Mathworks Inc., Natick, MA). The multidimensional unconstrained non-linear minimization (Nelder-Mead) method was used as an algorithm.

2.8. Statistical analysis

Data were expressed as the mean plus/minus standard deviation (SD). The statistical differences of release rates were studied by analysis of variance (ANOVA) using Jump 7.0 software (SAS Institute Inc., Cary, NC). Dissolution rate differences between, each NGI dose-plate containing different median particle sizes, were compared at several time points using a one-way ANOVA. To identify the statistically significant differences between groups, Tukey–Kramer test was used. α value of 0.05 was applied to denote statistical significance. SD values were compared to assess consistency of release. p-Values of less than 0.05 were considered as statistically significant.

3. Results

3.1. Solubility

The saturated solubilities of HC in mSLF and SLF were 461 and 345 μg/mL, respectively. As indicated in the results, the addition of DPPC to SLF increased the solubility of HC. These results are in good agreement with the solubilizing effect of lung surfactant, previously reported in the literature by several groups (Wiedmann et al., 2000; Pham and Wiedmann, 2001).

3.2. Membrane cassette optimization

A membrane cassette was newly designed for conducting the dissolution studies of dispersed particles (Fig. 1). To select a suitable membrane as the diffusion layer of dissolved drug, dissolution studies of model drug, HC, with three different membranes were conducted. In a comparison study between PC and CA dialysis membranes, the PC membrane demonstrated faster release of HC than that of other membranes as shown in Fig. 2. An additional advantage was that the sandwiched PC membranes did not facilitate the formation of entrapped air between membranes, and visually demonstrated a tight seal due to their thin and rigid structure within the membrane cassette. In contrast, entrapped air was

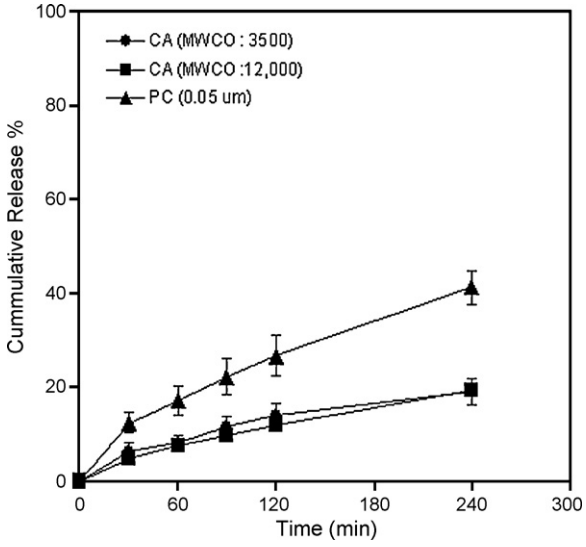

Fig. 2. Release profiles of HC powders placed into the three different types of membrane cassette per unit area (CA membrane: MWCO 3500 and 12,000, PC membrane: 0.05 μm pore size). The error bars indicate the standard deviation of three tests.

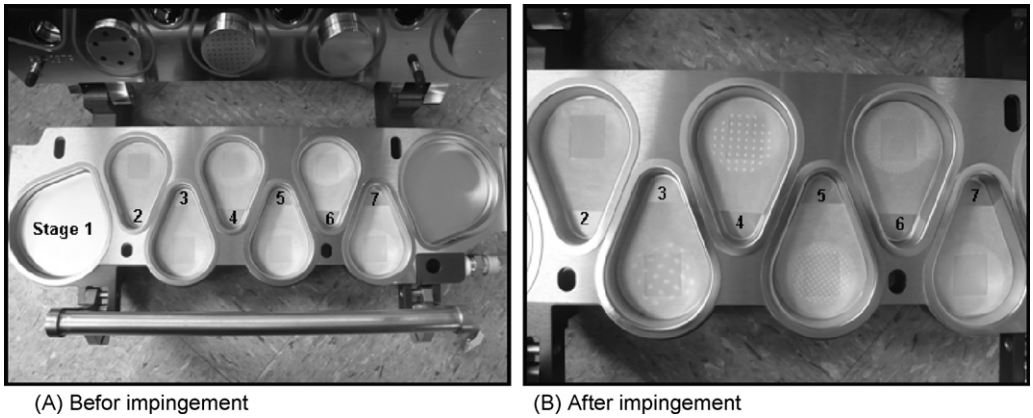

Fig. 3. Modified next generation impactor (NGI). (A) NGI setup before impingement and (B) resulting impingement.

readily seen between sandwiched CA dialysis membranes during dissolution.

3.3. Aerodynamic particle separation of HC particles

HC aerosol particles were collected using the NGI (impaction plates before and after device actuation impingements are shown in Fig. 3). In the figure, the wax paper impaction template can be clearly seen. Separated particles accumulated on the wax paper and exposed area of the PC membranes (under the template) on each dose-plate. The dispersion state of particles collected on each dose-plate was observed by SEM and is shown in Fig. 4. The SEM images show that bulk HC particles consist of irregularly shaped crystals ranging <1–5 μm. On the other hand, aerodynamically separated particles on each dose-plate displayed a very homogenous dispersion. The sizes of collected particles on dose-plates 3, 5, and 6 could be separated into ranges of approximately 3, 1, and <1 μm, respectively. Dose-plate cut-off particle sizes calibrated from the NGI are reported to be 4.46, 2.82, 1.66, 0.94, 0.55, 0.34 μm at an inlet flow rate 60 L/min for dose-plates 2–7, respectively (Marple et al., 2003a,b).

3.4. Dissolution

3.4.1. Dissolution testing of bulk HC particles

The dissolution behavior of micronized bulk HC was compared using two different dissolution methods: dissolution setup with a membrane cassette and without a membrane cassette. In this study, the impact of powder loading into the dissolution vessels on the test reproducibility and the dissolution of HC powders were investigated. The dissolution profiles of dispersed HC powders without a membrane cassette into the dissolution vessel was much more variable than that of the same powder enclosed inside the membrane cassette. However, there were no significant differences in

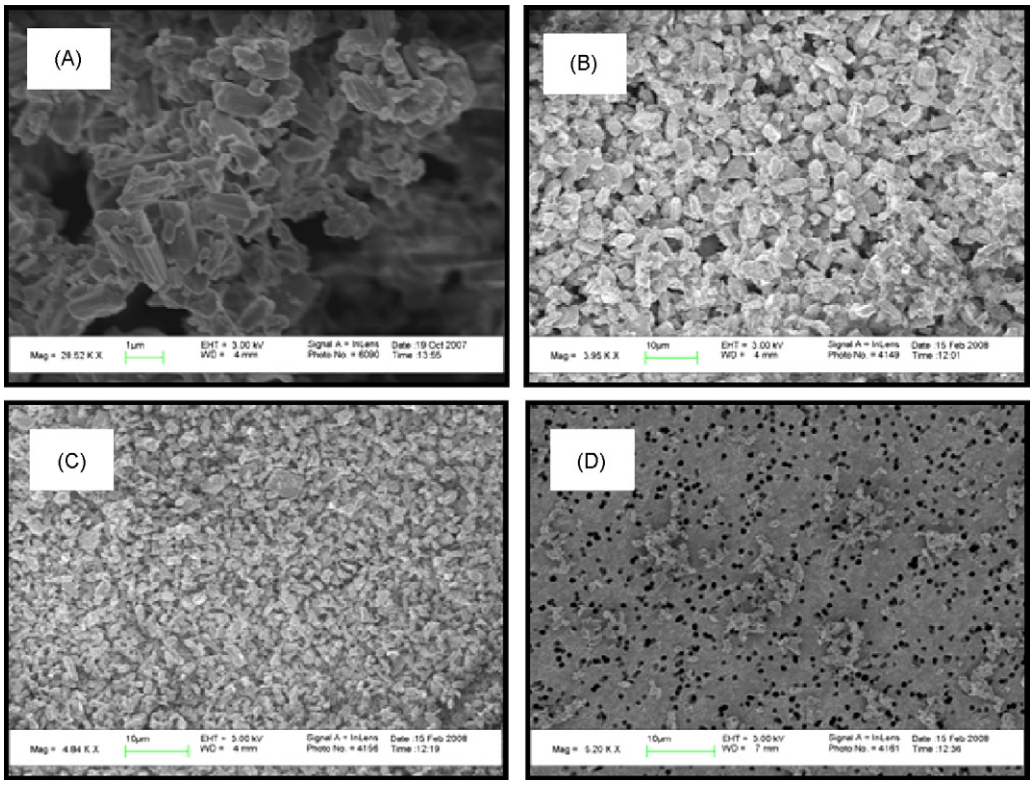

Fig. 4. Scanning electron microscope (SEM) images of non-separated bulk HC powders (A), of aerodynamically separated HC into the dose-plates 3 (B), 5 (C), and 6 (D).

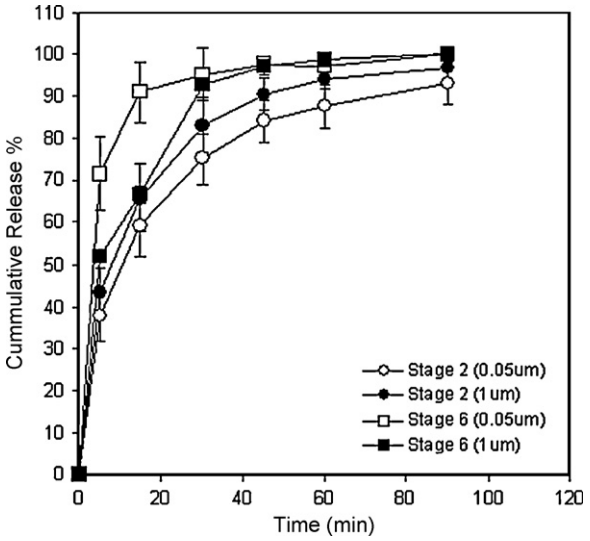

Fig. 5. Release profiles of HC from two membrane cassettes having different pore size on the surface in mSLF for dose-plates 2, and 6. HC separated from the lactose carrier. The error bars indicate the standard deviation of three tests.

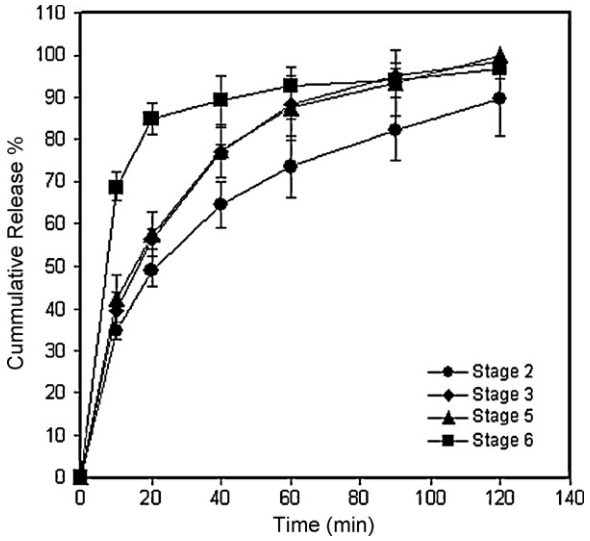

Fig. 6. Release profiles of HC separated from the lactose carrier in SLF for dose-plates 2, 3, 5, and 6. The error bars indicate the standard deviation of three tests.

the total dissolved amount of HC between the two methods. This result suggests that the membrane cassettes used in this study have no significant influence on the diffusion of dissolved drug, or diffusion of dissolution media into the cassette.

3.4.2. Dissolution testing of aerodynamically separated HC particles

3.4.2.1. Influence of membrane pore size. The influence of membrane pore size on the drug release profile was studied. PC membranes having different pore sizes (0.05 and 1 μm) were tested. Fig. 5 shows the release profiles of HC enclosed in two different membrane cassettes. It appears that the release profiles of HC particles accumulated in dose-plate 2 are not significantly different between the membranes. The PC membrane having pore size of 1 μm hindered initial drug release of HC particles accumulated in dose-plate 6 which have a median particle size of 0.55 μm. There is greater than 20% differences in drug release rate during the first 40 min.

3.4.2.2. Influence of particle size and drug loading. The influence of powder presentation inside the membrane cassette on the dissolution was studied. The total amount of loaded HC, by mass, following aerodynamic separation for dissolution studies is summarized in Table 1. Fig. 6 shows release profiles of HC for each NGI dose-plate. When the formulation contains a lactose carrier, the accumulated amount of HC on each dose-plate appeared to increase, as the NGI dose-plate number decreases (demonstrating the apparent efficiency of the carrier at preventing particle aggregation prior to deposition). The results demonstrate that the release rate of HC increases with decreasing particle size and amount of loading in accordance with the Noyes–Whitney equation for dissolution (as the exposed surface area is increased per unit mass

Table 1
Loaded amount of HC into the membrane cassette for each stage. HC powders were loaded by aerodynamic separation of carrier-free HC and carrier-mediated HC.

	Carrier-free HC (mg)			Carrier-mediated HC (mg)		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
Stage 2	2.97	0	3.02	0.76	0.60	0.80
Stage 3	4.33	5.14	6.12	0.37	0.46	0.59
Stage 5	2.31	6.10	1.57	0.32	0.36	0.29
Stage 6	0.53	2.70	0.76	0.05	0.07	0.05

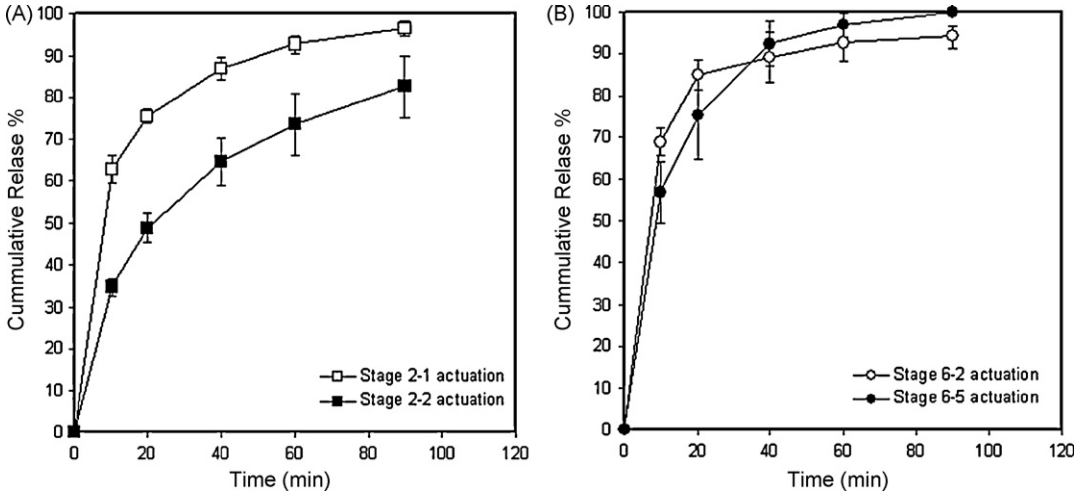

Fig. 7. Release profiles of HC from the cassette having different drug loading in SLF for dose-plates 2 (A) and 6 (B). HC separated from the lactose carrier. The error bars indicate the standard deviation of three tests.

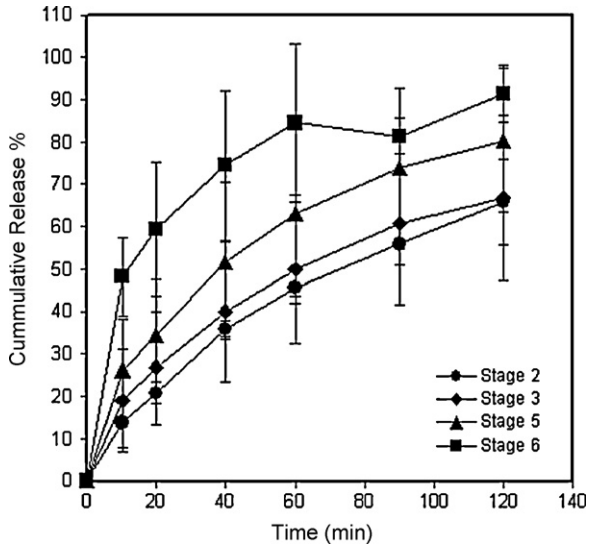

Fig. 8. Release profiles of HC separated from the carrier-free HC in SLF for dose-plates 2, 3, 5, and 6. The error bars indicate the standard deviation of three tests.

of drug deposited). The degree of significance of these two factors on the release rate was further confirmed by the following studies. Multiple actuations were conducted to obtain different drug loadings, followed by dissolution testing. For each actuation, an average dose of 300 μg was collected on dose-plate 2, and 70 μg of HC powders were accumulated on the dose-plate 6. As shown in Fig. 7, the dissolution rates of smaller particles are less affected by amount of loading drug compared to that of bigger particles.

As expected for the carrier-free HC, amount of loading is much more variable between actuations of the Aeroilizer® compared to the carrier-mediated HC powders as shown in Table 1. The release profiles shown in Fig. 8 reflect this non-uniform drug loading very well. A large degree of variability due to the irregular powder deposition is observed.

3.4.2.3. Influence of dissolution media. Fig. 9 shows the release profiles of HC for dose-plates 2 and 6 in SLF or mSLF. The release rates of HC increased with the addition of 0.02% DPPC, which was expected given the results from the solubility study that demonstrated increased saturation solubility in the media containing

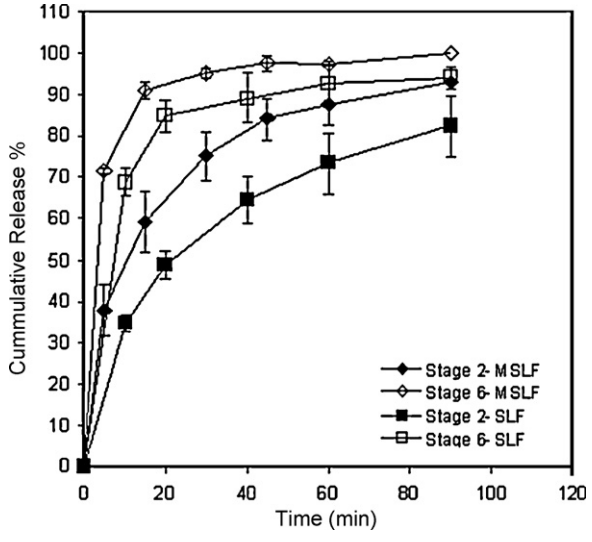

Fig. 9. Release profiles of HC separated from the lactose carrier in SLF and mSLF for dose-plates 2 and 6. The error bars indicate the standard deviation of three tests.

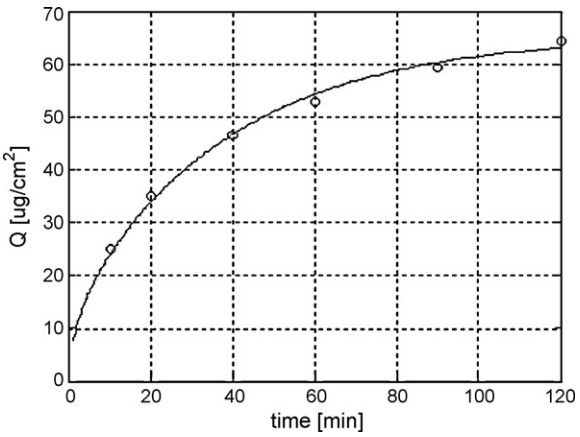

Fig. 10. Release profiles of HC (open circle) from the membrane cassette. Line shows a theoretical curve fit by Higuchi equation.

DPPC. The media containing DPPC may increase the wettability of hydrophobic drugs, and prevent aggregation to allow an enhanced dissolution rate (Wiedmann et al., 2000; Pham and Wiedmann, 2001).

3.5. Diffusivity from the membrane cassette

To verify the agreement between experimentally obtained results and a present mathematical diffusion model, the release profiles of HC particles were fitted to the Higuchi model (Eq. (1)), using the least square method, as shown in Fig. 10. According to the Higuchi model (Higuchi, 1962), the cumulative amount of drug released from the cassette per unit area, Q (μg/cm^2), under sink condition is as follows:

$$Q = hC_0 \left[1 - \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \times \exp\left(-\frac{D(2m-1)^2 \pi^2 t}{4h^2} \right) \right] \quad (1)$$

where h = thickness of PC membrane, C_0 = initial loading concentration of drug, D = diffusion coefficient of drug, t = time after application and m = integer (from 0 to ∞).

Using this fit from Eq. (1), C_0 and D could be calculated from the data (Table 2). An important parameter, the volume of media (V) inside the cassette could be calculated from obtained the C_0 and from the experimental data. As shown in Table 2, overall volume of media between two membranes was constant, approximately 6 μL, suggesting that the test condition inside the cassette are very reproducible. C_0 increased with increasing HC mass (M) in the membrane cassette. The diffusion constant (D) also increased with decreasing particle size, decreasing C_0, or the addition of 0.02% DPPC. The D values, therefore, suggest that DPPC helps the migration of HC molecule from inside the cassette into the dissolution vessel.

Table 2
Estimated parameters calculated by data from release experiments. Standard deviation was calculated on three different tests.

	M (mg ± SD)	C_0 (μg/mL)	V (μL)	D (cm^2/h)
Stage 2-SLF	0.72 ± 0.11	10.9×10^4	6.62	2.28×10^{-7}
Stage 3-SLF	0.47 ± 0.11	7.90×10^4	5.96	2.70×10^{-7}
Stage 5-SLF	0.32 ± 0.04	5.22×10^4	6.15	3.15×10^{-7}
Stage 6-SLF	0.06 ± 0.01	0.86×10^4	6.41	9.60×10^{-7}
Stage 2-mSLF	0.62 ± 0.05	9.54×10^4	6.55	4.86×10^{-7}
Stage 6-mSLF	0.06 ± 0.02	1.46×10^4	6.20	18.9×10^{-7}

3.6. Statistical analysis

The HC mixed with carrier lactose shows more consistent release rates compared to that of carrier-free HC. Overall the relative standard deviation (% RSD) values were <10 in the case of carrier-mediated HC and >30 for carrier-free HC. The release rate differences between the NGI dose-plates were much clearer with the carrier-mediated HC particles ($p < 0.05$) than that of carrier-free HC ($p > 0.05$). For the carrier-mediated HC, the dissolution rate of the particles accumulated on dose-plate 6 was significantly different from that of all other groups for initial 40 min by Tukey–Kramer test. The differences between groups became less defined as dissolution reached 100% release. The influence of DPPC (0.02%, w/v) on the release rate was not statistically significant, although DPPC helps with the solubilization of the drug.

4. Discussion

4.1. Dissolution apparatus

A novel dissolution method for inhaled pharmaceutical formulations was developed, which comprised a commercial dissolution system, and a membrane cassette as a formulation holding device. The mechanism of this dissolution method can be explained by dissolution–diffusion controlled drug release from the membrane cassette. During the dissolution process using the membrane cassette, the dispersed drug sandwiched between two membranes undergoes dissolution as dissolution media migrates through the pores on the membrane surface and the dissolved drug then releases out to the reservoir by diffusion.

A PC membrane was selected as a diffusion layer of the cassette. As shown in Fig. 2, the PC membrane demonstrated almost a twofold increase in drug release rate than CA dialysis membranes over the test period. This difference can be explained by the differences in physical structure of the two membranes. PC membranes are thin (approximately 6 μm) but still relatively robust for their handling requirements. Additionally, they do not swell and have a well-defined uniform pore size (Rzepka and Neidhart, 2000; Marre and Palmeri, 2001), consisting homogeneous 0.05 μm cylindrical pores on the surface, to allow free diffusion of dissolved drug and dissolution media. These uniform characteristics are important for the purposes of this dissolution study because they can affect diffusion properties of dissolved drug. In contrast to PC membranes, although CA membranes have been successfully applied in various dialysis studies (Kesting, 1985), many drawbacks have been reported such as reduction of permeability induced by protein adsorption on the membrane surface, thick membrane structure, and swelling (Arthanareeswaran et al., 2004; Cohen-Atiya et al., 2007).

The PC membrane surface of the cassette constitutes a perfect sink for the released drug. Dissolution media is able to rapidly diffuse into the cassette and reach the drug inside through the numerous pores, subsequently allowing dissolved (released) drug to be immediately removed from the exposed membrane face under suitable hydrodynamic conditions. In comparison study between two different dissolution setups (with or without a membrane cassette), no significant difference was found in the total dissolved amount of HC between the two methods, but reproducibility is greatly affected. The differences in test reproducibility may suggest that dissolution properties of the dispersed particles varies considerably depending on their degree of aggregation, and may have a more profound meaning when one considers the electrostatic properties of inhalable formulations. As previously mentioned, fine dry powders are hard to disperse homogeneously, they may stick on the dissolution vessel wall or paddle, and they may also agglomerate. Conversely, powders enclosed within the membrane cassette demonstrated a very consistent dissolution rates. This is due mainly to the uniform diffusion area of membrane cassette (10 cm^2), presented to the dissolution media, and the uniformity of the membrane barrier preventing premature particle escape (and a subsequent change in the surface area of drug presented to the media).

4.2. Dissolution studies

It was confirmed that the PC membrane with a pore size of 0.05 μm provided a perfect sink condition for the aerodynamically separated HC particles inside the cassette. As shown in Fig. 5, the obtained release profiles from two membrane cassettes are almost the same for the big particles (stage 2). However, PC membranes with larger pores often act as a diffusion barrier for small particles with similar particle size distribution to the membrane pore size. The enclosed particles are homogeneously separated thus, are easily released into the reservoir in the undissolved form and may clog the membrane pores in the process of escaping.

The dissolution profiles of inhalation formulations were influenced by powder presentation inside the cassette. As shown in Fig. 3(B), the dispersed powders form a powder bed on each dose-plate and the thickness of the powder bed is dependent on the amount drug loaded on each membrane. A larger amount of drug loaded on each dose-plate corresponds to a thicker powder bed deposited on the membrane (which would possibly lead to particle bounce with older types of vertical cascade impaction designs). Given that a thicker powder layer is deposited on the PC membrane then it follows that there will be more dissolution/diffusion activity required to release all of the drugs from the inner space of the membrane cassette. The size distributions of drug particles which constitute a powder bed also have effect on the dissolution rate. Bigger particles loaded inside the cassette require more energy input to be completely dissolved and released out because the portion of the drug that remains undissolved inside the membrane cassette acts as reservoir, allowing drug to dissolve in accordance with the Noyes–Whitney equation (Cabrera et al., 2006). Thus, the release rate of drug could be attributed to both particle size and amount of loading. The extent of significance of these two factors on the release rate can be explained from the data presented in Fig. 7. The results demonstrated that the release rates of the powder bed consisting of smaller particles were less influenced by the amount of loaded drug on the membrane due to their ability to wet and dissolve quickly (as the exposed surface area is increased per unit mass of drug). It indicates that the amount of loading may determine wetting powders and particle size could determine dissolution rate of wetted particles inside the cassette.

This dissolution test setup may be applied to the quality control studies for inhalation products, where the performance of carrier-based delivery and the effect of electrostatic interaction of powders are critical for lung deposition and disease management. As shown in Fig. 8, the dissolution profiles of aerodynamically dispersed particles reflect a powder dispersion status. For the carrier-free HC powder, a large degree of variability in test reproducibility was found due to the non-uniform drug loading, which can be directly attributed to the propensity of powder wetting within the membrane cassette. Additionally, this method can be used to examine the dissolution behaviors of inhalation dosage forms with similar particle distribution by selecting drug particles accumulated in same dose-plate. It can be expected that dissolution of particles having similar size distribution may provide more discriminative capability to the result in formulation variables.

The dissolution media containing DPPC (mSLF) can be used to predict the solubility and solubilization process of inhalation formulations which have very low aqueous solubility. DPPC increases

the wettability of hydrophobic drugs, and prevent aggregation to allow an enhanced dissolution rate (Wiedmann et al., 2000; Pham and Wiedmann, 2001) as shown in Fig. 9. These results are in good agreement with the solubilizing effect of lung surfactant, previously reported in the literature by several groups (Wiedmann et al., 2000; Pham and Wiedmann, 2001).

4.3. Mathematical modeling

Higuchi model was applied to calculate several unknown factors, such as D, C_0 and V. The Higuchi model is known to be a very simplified mathematical model to evaluate the release behaviors of matrix systems (Cabrera et al., 2006). In particular, the Higuchi equation well describes the release kinetics of models based on a moving dissolution front that proceeds inwards with time separating a region of coexisting solid and dissolved drug from a region of completely dissolved drug. From the Higuchi equation (Eq. (1)), D values for all the experimental release data were calculated (Table 2). The calculated D values are in good agreement with experimentally obtained release data. D values increased with decreasing particle size, decreasing C_0, or the addition of 0.02% DPPC. The D values, therefore, suggest that DPPC helps the migration of dissolved HC molecules from inside the cassette into the dissolution vessel. Additionally, it indicates that non-uniform drug loading, and non-uniform size distributions (which are directly related to the device or aerosol performance) could contribute to diffusion rate of drug from the membrane cassette. Generally, beyond saturated solubility, the amount of initially loaded drug inside cassette does not affect its release, if drug particles are evenly dispersed in the whole cassette (Hayashi et al., 1997). However, in the cassette, sandwiched powders between two membranes form a layer so that the dissolution of drug gradually progresses inward layer by layer, until all solid drug particles have been wetted and dissolved. Consequently, the diffusion rate of molecules is governed by the thickness of the drug layer, and the solubilization rate of that drug layer.

5. Conclusion

A new dissolution testing prototype apparatus for evaluating the *in vitro* dissolution behavior of inhalation formulations was designed. The dissolution rate of drug can be successfully estimated by analyzing the amount of drug released from the prototype membrane cassette. This dissolution method may be used to quality control study for various dry powder inhalers, in particular, the *in vitro* dissolution profiles of drug may provide an estimate of its dispersion characteristics which directly related to the device or aerosol performances.

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