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International Journal of Pharmaceutics 255 (2003) 175–187
international journal of pharmaceutics
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A novel method for assessing dissolution of aerosol inhaler products

Neal M. Davies [a,*], Majid R. Feddah [b]

[a] *Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, P.O. Box 646534, Pullman, WA 99164-6534, USA*
[b] *Faculty of Pharmacy, University of Sydney, Sydney, NSW, Australia*

Received 14 May 2002; received in revised form 27 January 2003; accepted 28 January 2003

Abstract

Glucocorticoids administered by inhalation remain a first-line treatment of patients with asthma allergic rhinitis and advanced chronic obstructive pulmonary disease. Budesonide (BD), fluticasone propionate (FP) and triamcinolone acetonide (TA) have high hepatic first-pass inactivation of the swallowed fraction of the inhaled dose, whereas there is no first-pass metabolism in the lung. Hence, the lung bioavailability will determine the overall systemic absorption and the systemic bioactivity. Efficacy of inhaled agents in the respiratory tract depends on the site of deposition and physicochemical properties of the drug, which dictates rate of dissolution, absorption, metabolism and elimination. However, to date no official method exists for testing dissolution rates from inhalation aerosols. An in vitro flow through dissolution method may be useful to provide information on rate of release and determine formulation differences between products or in product development. After administration of three glucocorticoids into a cascade impactor they underwent dissolution in a flow through cell utilising water, simulated lung fluid (SLF) and modified SLF with L-α-phosphatidylcholine (DPPC) as a dissolution medium, at constant flow and temperature. Modified SLF significantly increased the dissolution rate compared with SLF alone. This novel technique appears to be a useful method of evaluating dissolution of these glucocorticoids and may also be applied to other respiratory products administered via aerosols.

Keywords: Glucocorticoid; Aerosol; Dissolution; Lung; Solubility

1. Introduction

Glucocorticoids administered by inhalation are effective and widely used as anti-inflammatory agents for treatment of patients with asthma allergic rhinitis and advanced chronic obstructive pulmonary disease. Budesonide (BD), triamcinolone acetonide (TA) and fluticasone propionate (FP) all have a high degree of hepatic first-pass inactivation of the swallowed fraction of the inhaled dose, whereas there is no evidence of first-pass metabolism of these drugs in the lung. (Brogden and Tavish, 1992; Falcoz et al., 1996). Hence, the lung bioavailability of glucocorticoids is likely to determine the overall systemic exposure. Efficacy of inhaled agents in the respiratory tract depends on the site of deposition and on the physicochemical properties of the drug. The latter dictates the rate of dissolution and subsequent systemic absorption, metabolism and elimination of the drug. If the drug particles are deposited at the upper airway, mucociliary clearance take place prior to dissolution and absorption. The mucocilary clearance rate of a normal

* Corresponding author. Tel.: +1-509-335-4754; fax: +1-509-335-5902.
E-mail address: ndavies@wsu.edu (N.M. Davies).

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doi:10.1016/S0378-5173(03)00091-7

person's lungs is about 1–2% per min, i.e. half-life is about 1–2 h. If the drug particles are deposited at the lower airways the drug would be cleared by macrophages via phagoyctosis. The clearance rate by macrophages, however, is significantly slower compared with mucociliary clearance. Insoluble particles in the alveoli can, therefore, reside for days before being completely removed by phagocytosis depending on particle size, shape and load (Daviskas et al., 1995).

Dissolution is defined as the process by which a solid substance enters into a solvent to yield a solution and is controlled by the affinity between the solid substance and the solvent. Dissolution testing is an official test in the Pharmacopoeias for evaluating solid and semi-solid dosage forms. Furthermore, dissolution testing allows the investigation of the dissolution behaviour of pharmaceutical dosage forms in vitro to provide some predictive estimates of their behaviour in vivo.

The flow through cell method has been officially approved in the British Pharmacopoeia (2000) for the assessment of the dissolution of drugs from different dosage forms. Dissolution testing is a sensitive and reliable predictor of bioavailability for some drugs and is one of the most important quality control tests performed on pharmaceutical dosage forms.

The aerosol particles of the inhaled drug which enters the pulmonary region in the non-ciliated area undergo dissolution in the lung fluids, and the dissolved fraction of the dose will be available for absorption across the alveolar membrane (Dolovich et al., 1987). The dissolution rate for drug particles is determined by the rate at which solvent–solid forces of attraction overcome the cohesive forces present in the solid. The dissolution process is most frequently described in the form of a dissolution profile of cumulative percent of drug dissolved over time. Although this is the most exact representation, it is useful in practice to summarise and quantify dissolution kinetics in terms of one or more parameters, including dissolution rate constant or dissolution rate coefficient.

Following administration of a drug substance by an inhalation aerosol, it is generally accepted that less than 20% of the emitted dose reaches the respiratory tract (Barnes, 1995). A narrow particle size range (typically 1–5 μm) reaches the respiratory tract and may undergo dissolution. Drug particles greater than >5 μm may impact in the mouth and throat. The fate of the inhaled drug particles depends on the physicochemical and physical properties of the drug substance and the site of deposition in the respiratory tract.

Particles that deposit in the ciliated airways are cleared primarily by mucociliary clearance (Dolovich et al., 1987). Drug particles that penetrate deep into the lungs and deposit in the peripheral non-ciliated areas of the lungs can be cleared by many mechanisms, including dissolution (Dolovich et al., 1987).

Medications for managing respiratory diseases are generally delivered using metered-dose inhalers or dry powder inhalers. These devices deliver solid particles to the respiratory tract, and those particles that deposit in the non-ciliated regions of the respiratory tract need to be in solution in order to facilitate their local and systemic effects. Aerosol particles from the emitted dose that reach the non-ciliated regions of the lung have very small particle size and the dissolution rate of a drug is expected to be very high because of the large surface area to weight ratio of the particles. However, glucocorticoids are poorly water-soluble compounds, and hence the dissolution process is likely to be a rate-limiting step in glucocorticoid's action and systemic absorption.

Glucocorticoids are very hydrophobic compounds with lipophilicity in rank order: FP > BD > TA (Rohdewald et al., 1990). The fate of particles deposited in the pulmonary region can be described as in Fig. 1. The insoluble particles will probably be engulfed by alveolar macrophages and transported to the mucociliary clearance system. Subsequently, drug particles will be carried up to the pharyngeal region where they may be expectorated or swallowed. If a particle is swallowed, it may be solubilised upon passage through the gastrointestinal tract and enter the blood stream. Finally, systemic effects may be elicited with subsequent metabolism and excretion of the drugs (or their metabolites) via the urine or bile. Particles that do not undergo disintegration and dissolution would be excreted in the faeces. Insoluble particles in the alveolar region which are not phagocytised may become sequestered in pulmonary tissue (Clark, 1974). If sequestered for a long period, some solublisation may occur and, depending on the chemical nature of the material adverse tissue reactions, may lead to fibrotic response. Alternatively, benign pneumoconiosis may result with no immediate adverse response seen.

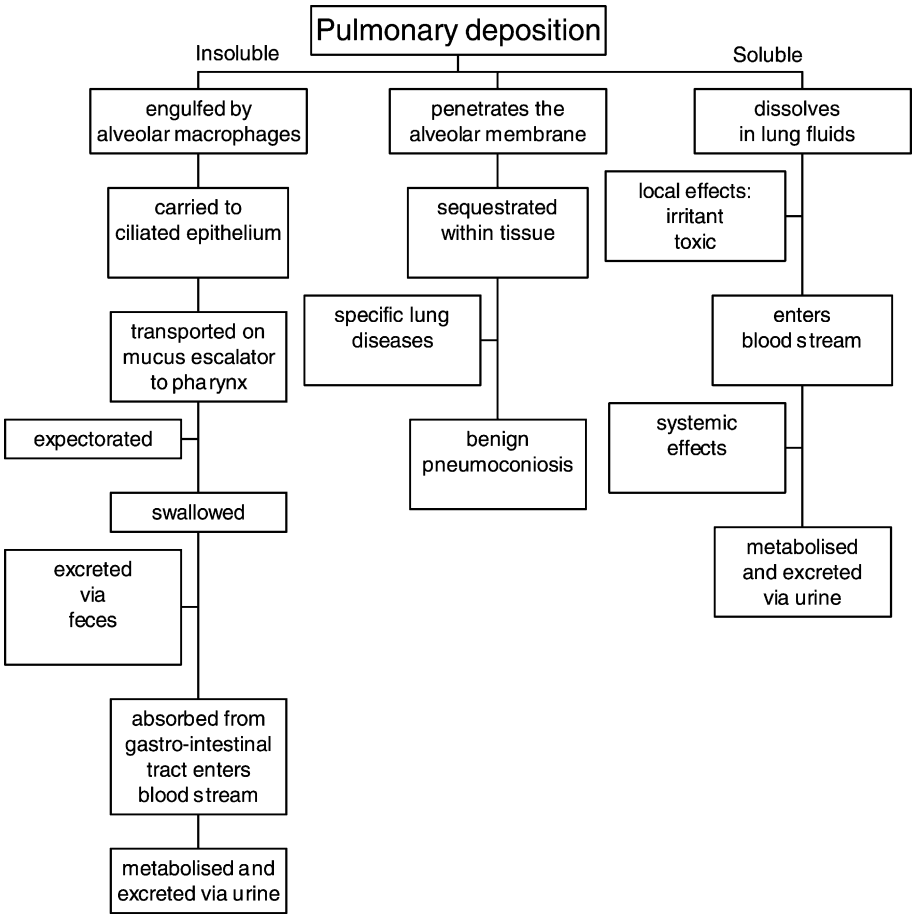

Fig. 1. Fate of inhaled particles deposited in the pulmonary region. Adapted from Clark (1974).

Soluble particles which reach the pulmonary region will dissolve in the lung fluid and may exert local toxic, irritant or pharmacological effects. Xenobiotics can also enter the blood stream and exert systemic effects and ultimately be subject to excretory mechanisms.

To date no single in vitro test system has yet emerged as the ideal choice for performing dissolution measurements as a tool to estimate in vivo solubility in the lung fluids. The objective of these studies is to study the dissolution of three inhaled glucocorticoids. Specifically, this study will examine the dissolution profile of BD in water, and different types of simulated lung fluids (SLFs). In addition, the dissolution profiles of TA and FP are investigated in different SLFs using a new flow through dissolution system.

The four experimental aims of this study were to investigate: (1) baseline solubility and partition coefficient (K) of the different glucocorticoids. The solubility of TA, BD and FP was studied in various dissolution media which include water, SLF (Table 1) and SLF modified with various concentrations of L-α-phosphatidylcholine (DPPC) (0.01, 0.02 and 0.05%). (2) The influence of the dissolution media, including water, SLF, SLF modified with DPPC (0.1, 0.02 and 0.05%), acidic and alkaline SLFs on the dissolution profile of BD in water. (3) The influence of the dissolution media flow rate on the dissolution profile of BD. (4) The influence of 0.02% DPPC on the dissolution profile of TA and FP compared to SLF alone.

Table 1
Compositions of actual lung fluid, simulated lung fluids and the modified simulated lung fluid by DPPC (mEq./l)

Ion	Actual[a]	Simulated lung fluid[b]	Modified simulated lung fluid with 0.02% DPPC
Calcium, Ca^{2+}	5.0	5.0	5.0
Magnesium, Mg^{2+}	2.0	2.0	2.0
Potassium, K^{+}	4.0	4.0	4.0
Sodium, Na^{+}	145.0	145.0	145.0
Total cations	156.0	156.0	156.0
Bicarbonate, HCO_3^{-}	31.0	31.0	31.0
Chloride, Cl^{-}	114.0	114.0	114.0
Citrate, $H_5C_6O_7^{3-}$	–	1.0	1.0
Acetate, $H_3C_2O_2^{-}$	7.0	7.0	7.0
Phosphate, HPO_4^{2-}	2.0	2.0	2.0
Sulphate, SO_4^{2-}	1.0	1.0	1.0
Protein	1.0	–	–
L-α-Phosphatidylcholine	–	–	200 mg
Total anions	156.0	156.0	156.0
pH	7.3–7.4	7.3–7.4	7.3–7.4

DPPC: L-α-phosphatidylcholine.
[a] Diem and Lenter (1970).
[b] Moss (1979).

2. Materials and methods

FP (analytical standard) was donated by Glaxo Wellcome Group Research (UK). BD was obtained by emptying a commercial Pulmicort Turbuhaler® Astra Pharmaceuticals (NSW, Australia). TA, DPPC (from frozen egg yolk, 99% TLC) and ethyl alcohol (HPLC grade) were purchased from Sigma Chemicals (MO, USA). Methanol, acetonitrile and dichloromethane (HPLC grade) were purchased from Selby Biolab Scientific (Victoria, Australia). Analytical grade acetic acid was purchased from Rhone-Poulenc Chemicals (Victoria, Australia). Cellulose acetate membrane filters (25 mm diameter) of 0.45 μm pore size were purchased from Sartorius Company (Victoria, Australia), and a pre-filter (25 mm diameter) from Gelman Sciences (Michigan, USA).

Glucocorticoid dry powder products used in this study were Flixotide Accuhaler® 250 μg FP (Allen and Hanburys, Division of Glaxo Wellcome, NSW, Australia), Pulmicort Turbuhaler® 200 μg BD (Astra Pharmaceuticals, NSW, Australia), and the metered-dose inhaler Azmacort® 200 μg, TA (Rhone-Poulenc Rorer Pharmaceuticals Inc., Pennsylvania, USA). Magnesium chloride hexahydrate, sodium chloride, potassium chloride, sodium phosphate dibasic anhydrous, sodium sulphate anhydrous, calcium chloride dihydrate, sodium acetate trihydrate, sodium bicarbonate and sodium citrate dihydrate were all analytical grade purchased from Ajax Chemicals (NSW, Australia).

2.1. Particle morphology

Samples of the aerosol particle from FP, BD and TA were collected using the Andersen Mark II Cascade Impactor (Graseby Andersen, Atlanta, CA) as described (Section 2.3.3). The samples were then mounted in the microscope chamber under vacuum prior to image analysis. The scanning electron microscope was Joe JSM, 6000F (Tokyo, Japan). The magnification was set to 10,000× for FP and TA and 15,000× for BD, the spot size was 5 mm and the electrical power was 5 kV.

2.2. Solubility studies

2.2.1. Solubility of BD and TA

The solubility of BD and TA in water, SLF and SLF modified with 0.01, 0.02 and 0.05% DPPC (Table 1) was determined by placing excess BD or TA in dialysis bags (molecular weight cut-off 12,000–14,000 Da; Spectrum Laboratories, Inc., USA). The dialysis bags were tied and placed into Pyrex screw-capped test tubes containing either water, SLF and SLF modified by 0.01, 0.02 and 0.05% DPPC. The tubes were then oscillated at 90 cycle/min in a 37 °C water bath for 30 h, and samples were withdrawn from the outer solution (1 ml) and analysed for drug content at the following time intervals: 1, 2, 3, 4, 5, 6, 12, 18, 24 and 30 h. The BD and TA concentrations in water and SLF were determined by measuring the absorbance of the sample using UV spectrophotometry (HITACHI, U2000, Japan) at 244 nm against an appropriate standard curve. The amount of BD and TA dissolved in the SLF modified by the addition of 0.01, 0.02 and 0.05% DPPC was determined by measuring the absorbance of the sample at 244 nm after dilution with ethanol (1:4). The amount of glucocorticoid dissolved in SLF modified with DPPC corresponds to the sum of the amount

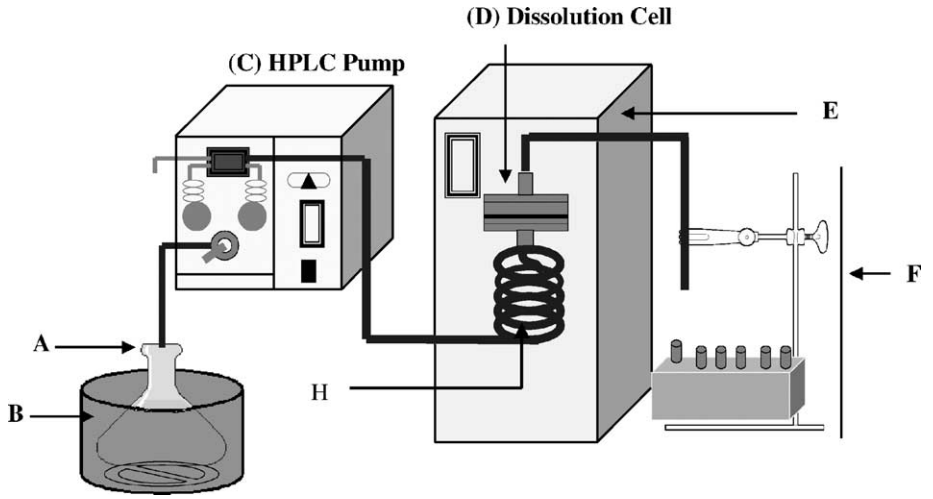

Fig. 2. Schematic diagram of the dissolution apparatus. Dissolution medium reservoir (A), water bath (B), HPLC pump (C), dissolution cell (D), HPLC column oven to control the temperature (E), sample holder (F) and heating coil (H).

dissolved in the aqueous phase and the amount bound to or associated with DPPC. The amount dissolved in the aqueous phase of the dispersion which corresponds to solubility in water was determined after ultracentrifugation of the sample dispersion (Beckman xl-9 ultracentrifuge, USA) at 56,000 × *g* for 30 min at 37 °C followed by measuring the absorbance of the supernatant layer at 244 nm.

2.2.2. Solubility of FP

The saturated solubility of FP was measured at 37 °C by placing an excess of the pure FP (analytical standard) in different dissolution media (water, SLF, SLF modified by 0.01, 0.02 and 0.05% DPPC) in 5 ml glass vial with a teflon-lined screw cap with a magnetic stirrer. After 24 h the samples were withdrawn and centrifuged at 15,000 rpm (37 °C) for 5 min (Jouan Model CT4-22 Centrifuges, Saint Herblain, France). The concentration of FP in the samples was measured as described in detail in Section 2.4.3.

2.3. Dissolution apparatus

A flow through dissolution apparatus was employed to conduct the dissolution studies of inhaled glucocorticoid particles. A schematic diagram of the dissolution apparatus is shown in Fig. 2. The main parts of the apparatus include the dissolution media reservoir (A) equilibrated at 37 °C in a water bath (B), HPLC pump (C) (Waters model 510 California, USA) delivering the dissolution medium from the dissolution reservoir to the dissolution cell (D) (Fig. 3) through a silicon tube (0.5 mm i.d). The dissolution medium was equilibrated to 37 °C before passing through the dissolution cell by means of a pre-heating coil (H) placed in an HPLC column oven (E). The dissolution

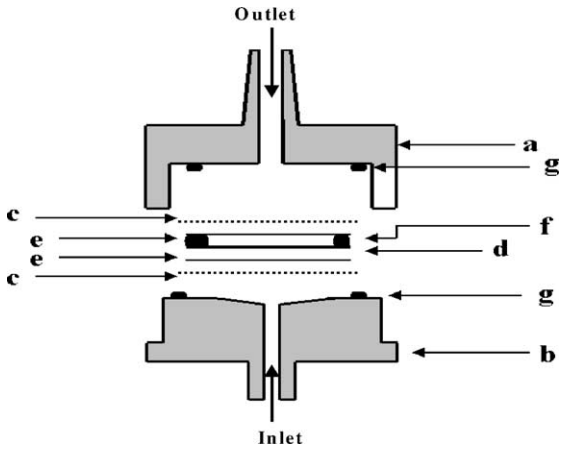

Fig. 3. Cross-section of the dissolution cell: upper (a) and lower (b) parts of a stainless steel filter holder, stainless steel screen support filters (c), glass fibre filter containing the drug particles (d), a 0.45 μm pore size cellulose acetate membrane filter (e), teflon ring 1 mm thickness (f) and casket ring (g).

media pass the aerosol particle bed which is immobilised onto the filter by the constant pressure applied through the HPLC pump. The dissolution medium is finally collected in a fraction collector (F).

2.3.1. Dissolution cell

A detailed diagram of the dissolution cell is shown in Fig. 3. Glucocorticoid particles obtained using impaction were collected onto a glass pre-filter for dissolution studies. The dissolution cell consists of a modified 25 mm Millipore filter holder (Millipore, USA).

2.3.2. Dose collection

The base of the impactor and stage number zero of Andersen Mark II Cascade Impactor (Graseby Andersen, Serial No. 3025, Atlanta, GA, USA) and the USP induction port was used to collect the fraction of the dose intended for the dissolution. A custom-designed stainless steel ring was placed at the connection point of the USP induction port with the inlet part of the Andersen Cascade Impactor to hold the fibre glass filter and the stainless steel screen support filter as shown in Fig. 4. A single dose of BD (200 μg) delivered from the Pulmicort Turbuhaler®, FP (250 μg) delivered from the Flixotide Accuhaler® and TA (200 μg) delivered from Azmacort® inhaler was dispersed into the fibre glass filter through the USP induction port with the aid of a vacuum pump (Erweka, Heusenstamm, Germany) calibrated at 60 l/min to simulate the inspiratory flow rate. The flow was allowed to continue for a further 20 s before the pump was switch off to ensure complete evacuation of the dose from the device. The flow rate was measured by a mass flow meter (Model 822S-H-4-OV1-V1; Sierra Instruments, Monterey, CA, USA).

The induction port was dismantled and the fibre glass filter containing the drug was removed and inserted between two 0.45 μm cellulose acetate membrane filters. A teflon ring of 1 mm thickness was placed between the two membrane filters, the sandwich-like filters containing the drug particles were placed into the dissolution cell supported by two stainless steel support filters at both ends.

2.3.3. Dissolution media

The composition of human interstitial lung fluid (Diem and Lenter, 1970), the SLF and SLF modified with DPPC used in this study are shown in Table 1. A comparison between the electrolyte composition of actual and SLF shows that these fluids are virtually identical. The protein components of actual lung fluid were represented by an ionically equivalent amount of citrate in the SLF as suggested by Moss (1979).

2.3.4. Dissolution testing

The dissolution medium which is equilibrated to 37 °C was pumped upward through the dissolution cell by means of an HPLC pump calibrated to give a constant flow of 0.7 ml/min (Fig. 2). The dissolution medium was pumped to flow through the aerosol particles previously collected and immobilised on the glass fibre filter between 0.45 μm membrane filters. The dissolved fraction of the dose which passed the upper filter was collected separately for individual

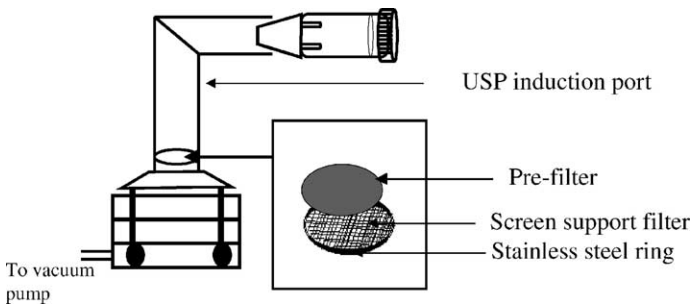

Fig. 4. Schematic diagram shows the sample collection procedure of the drug particles intended for dissolution studies of glucocorticoids delivered from drug powder inhaler.

analysis at 3-min intervals for BD and TA and at 20-min intervals for FP. Drug concentration was determined by an appropriate method of analysis for drug content as described in Section 2.4. The cumulative percent after a time period was determined by dividing the total mass dissolved after the time interval by the total mass collected on the filter multiplied by 100%. The total amount of glucocorticoid deposited on the filter was determined from the sum of the total cumulative amount dissolved plus the amount retained on the filter after the dissolution period time. The amount of glucocorticoid retained on the filter was determined by washing the sandwich-like filters with methanol. An aliquot of dexamethasone-21-acetate of the internal standard in methanol was added to the methanolic washings, which was then evaporated to dryness at 35 °C under a stream of nitrogen. The dry residue was reconstituted in mobile phase and injected directly into the HPLC system.

2.4. Sample preparation and chemical analysis

2.4.1. Analysis of BD and triamcinolone in water and SLF

Analysis of dissolution samples of BD and TA in water and in SLF was performed on a Beckman System Gold HPLC apparatus as described previously (Feddah et al., 2000, 2001).

2.4.2. Analysis of BD and triamcinolone in modified SLF

Sample preparations of BD and TA in modified SLF were performed using method of Li et al. (1997). Samples of BD and TA in modified SLF with DPPC were injected into the HPLC after extraction of the drug using a solid-phase extraction Sep-Pak C_{18} cartridges (pre-conditioned by rinsing twice with 3 ml ethanol and twice with 3 ml water). A 24-port manifold (Supelco, Bellefonte, USA) was used to accommodate the cartridges and operated at approximately 5.1×10^3 Pa. Each sample was aspirated through the cartridge dropwise. The cartridges were then dried under vacuum before each glucocorticoid was eluted with 5 ml of methanol, which was then dried at 35 °C under a stream of nitrogen. The dried residue was reconstituted in 1 ml mobile phase and was injected into the HPLC.

2.4.3. Analysis of FP

Analysis of FP was performed using minor modifications of an LC-MS/MS assay (Li et al., 1997). A dissolution sample (1 ml) was spiked with flumethasone (50 μl corresponding to 100 ng/ml) as an internal standard and extracted with dichloromethane (4 ml) for 30 min using a roller mixer. The organic layer was collected and evaporated to dryness under a stream of nitrogen at 35 °C. The dried residue was reconstituted in 200 μl of mobile phase and injected into the LC-MS/MS system which consisted of a Finnigan/Mat TSQ 700 LCMS/MS (Sanjose, CA, USA) and a Hewlett Packard HP 1090 Liquid Chromatograph (Palo Alto, CA, USA) coupled to the TSQ 7000 and controlled by its software. Chromatography was performed with an Alltima C8 (5 μm particle size, 150 mm × 2.1 mm; Alltech, NSW, Australia) column at ambient temperature and a mobile phase of methanol:water (75:25), flow rate was maintained at 0.3 ml/min and an injection volume of 30 μl.

2.5. Statistical analysis

Data are expressed as the mean ± standard deviation (S.D.) of replicate determinations ($n \geq 3$). Statistical analyses were performed using SPSS (SPSS Inc., Illinois, USA). Significance of difference in means of paired samples was assessed using paired-sample *t*-test. Analysis of variance of two or more groups of data was performed using analysis of variance (ANOVA). All values of P were based on two-tailed tests and P values of less than 0.05 were considered as statistically significant.

3. Results

The size distributions of the primary particles indicated by D (v, 0.5) (particle diameter at below which 50% of the particles reside) for FP delivered from the Flixotide Accuhaler®, BD from the Pulmicort Turbuhaler® and TA from Azmacort® measured by the Laser diffraction technique were 1.9 ± 0.1, 2.0 ± 0.1 and 2.1 ± 0.1 μm, respectively. The morphology of the aerosolised particles is shown in the typical scanning electron microscope images (Fig. 5).

Fig. 5. Scanning electron microscope images of FP (a), BD (b) and TA (c).

The images demonstrate that the particles consist of irregularly shaped crystals ranging in size from <1 to 10 μm, 1 to 6 μm and 1 to 8 μm for FP, BD and TA, respectively.

3.1. Solubility and partition coefficient (K)

3.1.1. Solubility

The solubility and the extent of solubilisation of BD, TA and FP at 37 °C in different dissolution media are indicated in Fig. 6. There was no change in solubility after 18 h. The solubilities of BD, TA and FP in the different concentrations of modified SLFs represent the total solubility in both the aqueous phase and lipid phase (DPPC). Hence, the solubility of drug in the lipid phase could be calculated from the difference between the total solubility and the solubility in water.

3.1.2. Partition coefficient

The partition coefficient of the different glucocorticoids between the aqueous and the lipid phase (DPPC) can be calculated from the solubility data as follows: $K = (\%\,\text{in lipid}/\%\,\text{in water}) \times F$. Percent lipid: w/w percentage of glucocorticoids in lipid phase. Percent water: w/w percentage of glucocorticoids in aqueous phase. F: weight ratio of the aqueous phase over lipid phase. Thus, K is the ratio of the glucocorticoids in a unit mass of lipid over that of aqueous solution. There were no significant differences in the partition coefficient of the glucocorticoids at different concentrations of DPPC.

3.2. Dissolution

The effect of dissolution medium on the dissolution profile of BD was studied using various dissolution

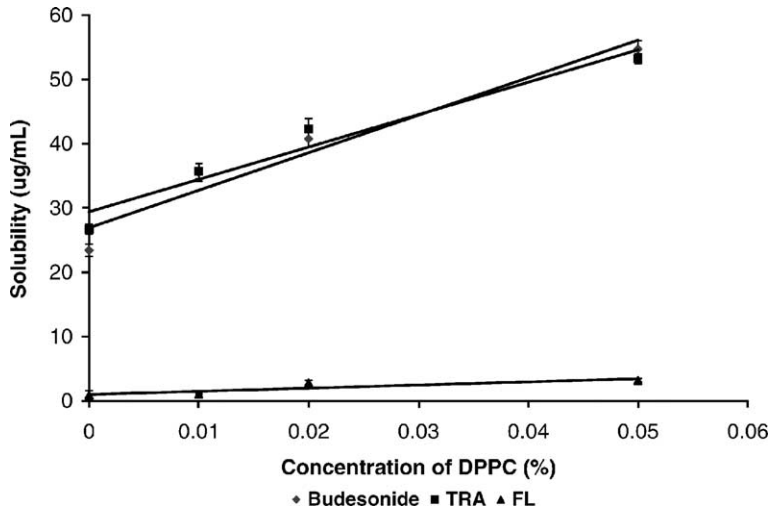

Fig. 6. Influence of concentration of DPPC on the solubility of BD, TA (TRA) and FP (FL). The vertical bars indicate the S.D. of three determinations.

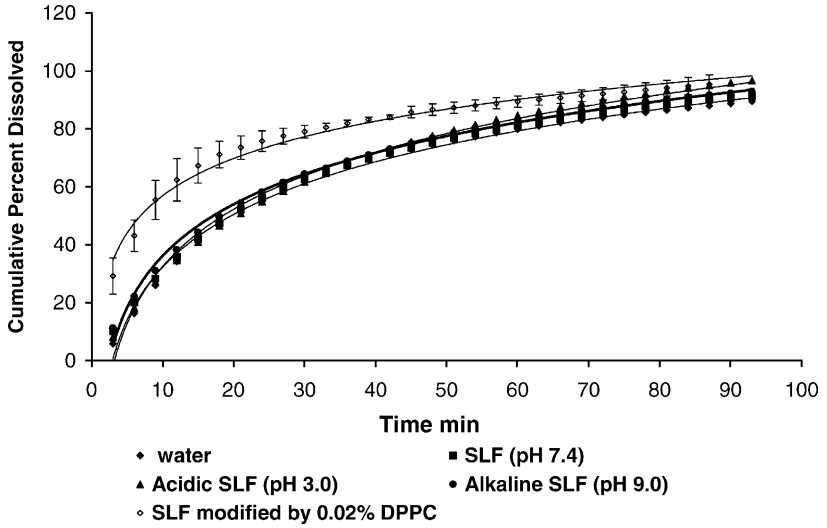

Fig. 7. Dissolution profile of BD in water, simulated lung fluid (SLF), acidic SLF (pH 3.0), alkaline SLF (pH 9.0) and modified SLF by the addition of 0.02% DPPC. A fitted line using a logarithmic function.

media, including water, acidic SLF (pH 3.0), alkaline SLF (pH 9.0) and SLF modified with 0.02% DPPC. The results demonstrate that except for SLFs modified with DPPC, the dissolution of BD in the various dissolution media exhibits a similar dissolution profile as shown in Fig. 7. The pH of the dissolution media has no significant effect on the dissolution of BD. However, the presence of DPPC in the dissolution medium significantly ($P > 0.05$) increased the dissolution rate of BD. The dissolution profile of BD and TA indicates

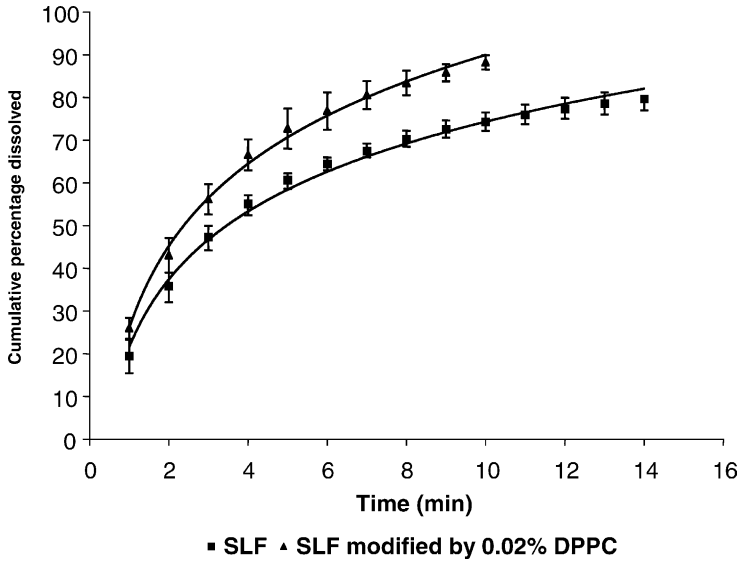

Fig. 8. Dissolution profile of TA in simulated lung fluid (SLF) and in modified SLF by 0.02% DPPC. The vertical bars indicate the S.D. of five determinations. A fitted line using a logarithmic function.

Table 2
Dissolution rate coefficients[a] for BD and TA and dissolution rate[b] of FP in simulated lung fluid and in modified simulated lung fluid with 0.02% DPPC at 37 °C

Glucocorticoid	SLF (mean ± S.D.)	Modified SLF with 0.02% DPPC (mean ± S.D.)
Bd[a]	0.033 ± 0.002 min^{-1}	0.074 ± 0.015
Ta[a]	0.054 ± 0.002 min^{-1}	0.087 ± 0.002
FP[b]	0.058 ± 0.006 ng/min	0.129 ± 0.011

Mean ± S.D. of five determinations.
[a] Derived from the Weibull dissolution model.
[b] Derived from the slope of the linear dissolution profile.

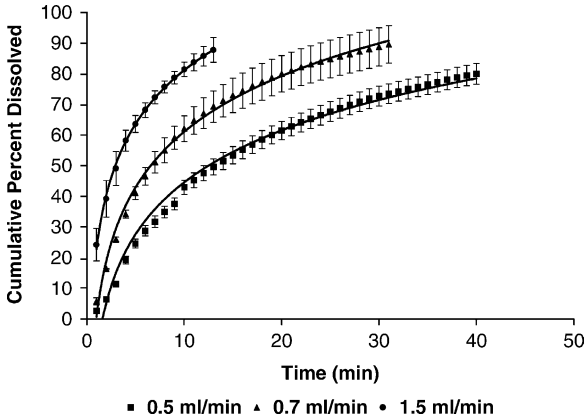

Fig. 10. Influence of flow rate of the dissolution medium of 0.5, 0.7 and 1.5 ml/min on the dissolution profile of BD. The vertical bars indicate the S.D. of five determinations. A fitted line using a logarithmic function.

that the dissolution rates are not constant which may reflect the polydispersity in the particle size distribution in which the small particles dissolve quickly at the beginning of the dissolution process leaving the large particles to dissolve more slowly, which determines the shape of the dissolution profile. Although it is possible that this non-linearity is also due to the change (decrease) in total drug substance surface area that occurs during the dissolution process.

The dissolution rate in this study was characterised by means of a Weibull modelling approach in the logarithmic form in which $1/t = K_w$ represents the dissolution rate coefficient (Loth and Schäfer, 1985).

The dissolution rate coefficient of BD and TA and the dissolution rate of FP increased significantly ($P < 0.05$) with the addition of 0.02% DPPC to the SLF (Fig. 6), while no significant differences ($P < 0.05$) were detected in the dissolution rate coefficient

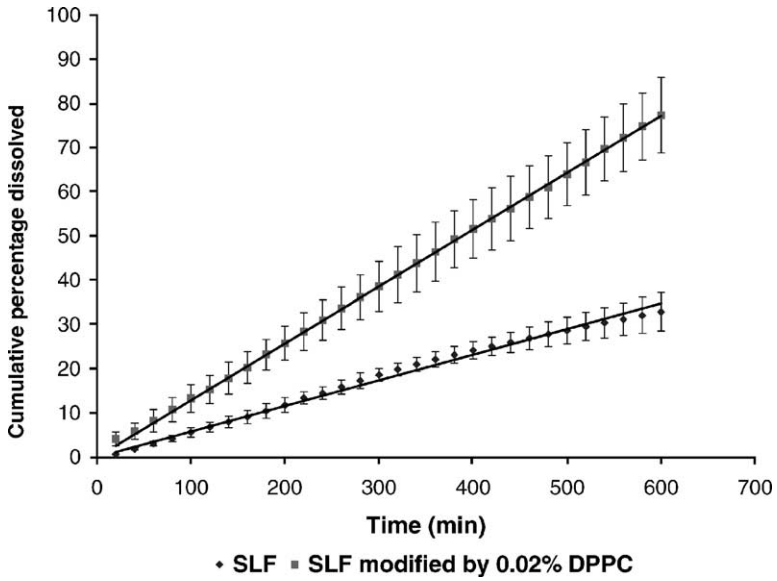

Fig. 9. Dissolution profile for FP in simulated lung fluid (SLF) and in SLF modified by DPPC. The vertical bars indicate the S.D. of five determinations. A fitted line using a linear function.

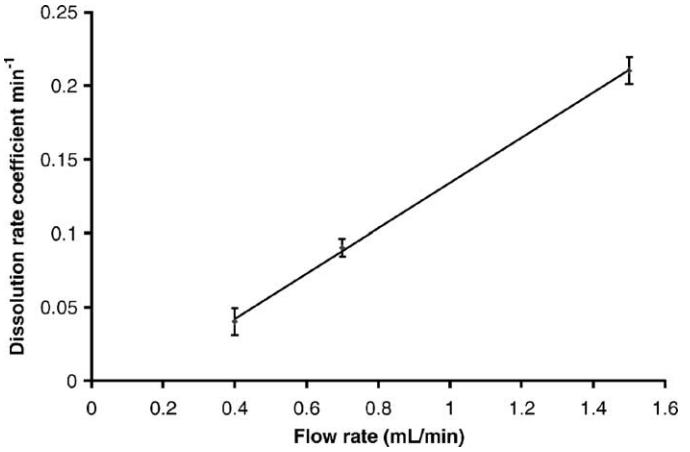

Fig. 11. Influence of flow rate of dissolution medium on the dissolution rate coefficient of BD in water. The vertical bars indicate the S.D. of five determinations.

of BD in water ($0.031 \pm 0.004\,min^{-1}$), acidic ($0.034 \pm 0.002\,min^{-1}$) and alkaline SLF ($0.035 \pm 0.002\,min^{-1}$).

The addition of 0.02% DPPC to SLF also increased the dissolution rate of TA and FP compared with SLFs alone. The dissolution profile of TA in SLF and in SLF modified with 0.02% DPPC is shown in Fig. 8. The dissolution rate coefficient increased from 0.054 to $0.087\,min^{-1}$ with the addition of 0.02% DPPC as indicated in Table 2.

The dissolution profile of FP in SLF and in SLF modified with DPPC is shown in Fig. 9. The dissolution profile of FP in both media over the testing period was modelled to linear equation ($R^2 = 0.993$ and 0.998). Thus, the dissolution rate was calculated from the slope of the linear relationship between cumulative percent dissolved and time. The dissolution rate increased from 0.058 to 0.129 ng/min with the addition of 0.02% DPPC.

The influence of flow rate on the dissolution profile of BD in water was investigated at three different flow rates, 0.4, 0.7 and 1.5 ml/min. The dissolution profiles of BD at the different flow rates are shown in Fig. 10. The dissolution rate coefficients of BD in water at a flow rate of 0.4, 0.7 and 1.5 ml/min were 0.045, 0.09 and $0.21\,min^{-1}$, respectively. The results demonstrate that the dissolution rate of BD in water increased in a linear manner with increasing flow rate of the dissolution media as shown in Fig. 11. A low dissolution flow rate may be desirable in the study of glucocorticoid dissolution because the volume of the samples to be analysed can be kept to a minimum. The 0.7 ml/min flow rate was chosen in this study to maintain sink conditions.

4. Discussion

4.1. Solubility and partition coefficient

The use of the equilibrium dialysis method to determine the solubility of poorly water-soluble glucocorticoids was selected because it enabled drug equilibration to be achieved. Furthermore, it overcomes the inherent wetting problem of these highly hydrophobic powders and minimised errors associated with sample processing. The aqueous solubility of TA estimated in this study is in agreement with previous studies which report values of 20.7 ± 0.13 and 25.5 μg/ml (Charanjit et al., 1976; Block and Patel, 1973). The aqueous solubility of BD estimated in this study is in agreement with a previous study which reported a value of 23 μg/ml (van Amerogen et al., 1992). The solubility of FP has not been previously reported.

The addition of DPPC to SLF resulted in a concentration-dependent increase in the solubility of the three glucocorticoids as indicated in Fig. 6. This suggests that there is no minimum critical DPPC

concentration required to improve solubility of glucocorticoids. In agreement with the results in this study, a concentration-dependent increase in the solubility of BD was reported with the addition of Survanta® (a commercial lung surfactant) (Li and Zimmerman, 1996) and with the addition of DPPC (Wiedmann et al., 2000).

The distribution of glucocorticoids between DPPC and the aqueous solution was found to be different among the different glucocorticoids used in this study. The glucocorticoids with higher aqueous solubility had a lower partition coefficient. There were no significant differences in the partition coefficient of the glucocorticoids at different concentrations of DPPC. Since the addition of DPPC enhances the solubility characteristics of glucocorticoids, it is of value to compare it with native lung surfactant. Native lung surfactant is secreted by type 2 cells that are located in the alveoli and the lower respiratory bronchioles of the lung. It consists of an aqueous dispersion mixture of lipids and proteins. The composition of native lung surfactant is greater than 90% lipids, with the remaining 10% composed of mixtures of proteins. For the lipids, 95% are phospholipids of which 90% are DPPC and another 8–10% are phosphatidyl glycerol. The phosphatidylcholines are also largely saturated with DPPC, representing 40% of the phospholipids present (Morton, 1989).

4.2. Dissolution studies

The flow through dissolution apparatus offers advantage of sink conditions with the continuous flow of fresh dissolution medium and output of filtered solvent containing the dissolved drug. This flow through non-recycled system is a suitable technique to study the dissolution kinetics of poorly water-soluble drugs, such as glucocorticoids. There are many advantages of using this system, including the flexibility that permits changes to be readily made even within a test run, of important factors, such as temperature, flow rate, viscosity, surfactant concentration and pH. In addition, all the drug particles collected on the filter experience essentially the same intensity of solvent flow, thus agitation conditions are related to the solvent flow rate in a meaningful way, and the flow rate of the dissolution media is easy to define and to control.

The dissolution profiles of BD and TA in the different dissolution media appear to follow the Nernst–Brunner equation (Eq. (1))

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \frac{DS}{h}(C_\mathrm{s} - C_\mathrm{b}) \quad (1)$$

where $\mathrm{d}m/\mathrm{d}t$ is the change in mass per time (dissolution rate); C_b, concentration of dissolved solid in the bulk phase at time t; C_s, saturation solubility of the solid in the diffusion layer; h, thickness of the diffusion layer around the particle; S, surface area of the solid exposed to the dissolution medium, D, diffusion coefficient of the drug in the unstirred layer surrounding the particles.

From Eq. (1) it is possible to identify the variables that will affect the dissolution of drugs from powders and solid formulations, such as size of drug particle and viscosity and temperature of the dissolution media. There did not appear to be any significant changes in dissolution rate or extent at the various pH's used to simulate respiratory acidosis and alkalosis.

Interestingly, FP exhibited a pseudo zero-order dissolution profile over a period of 8 h in the SLF and in the modified SLF with 0.02% DPPC. After 8 h the dissolution appeared to start to deviate from linearity. The reasons for this difference is not apparent, however, the solubility and hence dissolution for FP is extremely slow compared to the other glucocortiocids and was not complete during the 10-h time interval of this study. The results could be attributed to the dispersion of the original powder and to the very poor water solubility of FP in the dissolution media consistent with its very low solubility (Fig. 6). The solubility of FP is so low that it necessitates HPLC-MS analysis of the samples. However, drug particles of FP were dispersed through the induction port of the Andersen Cascade Impactor using a flow rate of 60 l/min. The aerosolised particles were collected on a filter and Flixotide® Inhaler contains the drug particles (FP) mixed with lactose as dispersity, which prevents the aggregation of the drug particles, and enhances the disparity of the drug particles during inhalation. Furthermore, using the flow through non-recycled systems permits the drug particles to experience essentially the same intensity of solvent flow rate. The dissolution media also contain surfactant (DPPC), which increases the wettability of the drug particles and prevents aggregation. These in vitro

observed effects with FP also appear to correlate with in vivo clinical observations where the slow rate of flucticasone dissolution results in delayed distribution of this drug.(Esmailpour et al., 1997). High retention of inhaled glucocorticoids in the airways may prolong and promote topical anti-inflammatory activity with low delivery into the serum.

In summary, this flow through dissolution system technique is able to evaluate the dissolution of poorly soluble glucocorticoids administered as aerosols. This technique may be used during aerosol product development to examine dissolution which may have fiscal and time constraint implications during formulation development. Due to its practicality it could also be used for comparative studies between different dosage forms especially at the beginning of developing a new dosage forms. Importantly, the investigation of the dissolution behaviour of the pharmaceutical dosage forms in vitro may provide estimate of their release behaviour in vivo. This technique could be used to examine aerosol formulations which are intended to extend the residence time of drugs delivered to the lungs which may alter onset and duration of activity by sustaining therapeutic drug levels. As more aersol products come off patent, this technique could also be used to evaluate interchangeability of generic versus innovator aerosol products.

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