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High-pressure aerosol suspensions—A novel laser diffraction particle sizing system for hydrofluoroalkane pressurised metered dose inhalers

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Abstract

In this study, a novel laser diffraction particle size analysis dispersion system, capable of sizing particles in situ within suspension hydrofluoroalkane (HFA) pressurised metered dose inhalers (pMDIs), was developed and tested. The technique was compared to four indirect particle sizing methods commonly used to determine the size of particles suspended in HFA pMDIs. The median volume diameter obtained using laser diffraction of both the salbutamol sulphate and fluticasone propionate suspended either in 2H, 3H-decafluoropentane or perfluoropentane (employed as surrogate propellants) was over one-order of magnitude larger than the particle sizes of the drugs suspended in HFA 134a. In contrast, the "in-flight" particle size using the Sympatec inhaler 2000® laser diffraction equipment undersized the particles, predicting higher delivery efficacy compared to the other sizing methods. However, the size of particles suspended in HFAs derived using the novel pressurised dispersion system, showed a linear correlation with the impaction results, $r^2 = 0.8894$ ($n = 10$). The novel pressure cell sizing technique proved to be simple to use, has the ability to be automated and was accurate, suggesting it could be an essential tool in the development of new suspension-based pMDI formulations.

Keywords: Pressurised metered dose inhaler; Laser diffraction; Particle size analysis; Pressure cell; Beclomethasone dipropionate; Salbutamol sulphate

1. Introduction

The replacement of chlorofluorocarbon (CFC) metered dose inhaler propellants with hydrofluoroalkanes (HFAs), a move enforced by Montreal protocol on substances that deplete the ozone layer (1989), has provided an opportunity to reformulate pMDIs, and hence the potential to increase the delivery efficiency from such devices, which is typically poor (Holzner and Muller, 1995; Steckel and Muller, 1998). However, propellant replacement has produced a new problem. The majority of therapeutic compounds are not stable

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when formulated alone as colloidal suspensions within HFA propellants and additional excipients can often be required to prevent aggregation. Excipients traditionally used in CFC-based pMDIs cannot be used in HFA-containing products due to their low solubility. Therefore, to facilitate the reformulation of CFC products and allow the incorporation of novel therapeutic agents within pMDIs, new compounds are required to stabilise suspensions of drug in HFA (Vervaet and Byron, 1999).

Suspension stability is critical to the performance of pharmaceutical pMDIs, as this not only determines the dosing reproducibility of the formulation, but it can also determine the site of delivery (Heyder et al., 1980). It is accepted that numerous factors related to the formulation, drug, patient, device and disease state can affect the optimal particle size required for maximal deep lung deposition. However, for the latter to be obtained, it is generally agreed that a successful inhaleable formulation must store and reproducibly dose a population of sub-10 μm particles to the respiratory tract (Terzano, 2001; Martonen et al., 2002; Musante et al., 2002; Miller et al., 2003). Therefore, particle size is considered a primary endpoint in the preformulation and development of new pMDI products and it is commonly used to predict delivery efficiency and physical stability in vitro (Tzou et al., 1997).

Until recently, CFCs were routinely used as medical propellants. Of the three compounds approved for human use, CFC 11, CFC 12 and CFC 114, only CFC 12 exhibits a boiling point below 0 °C (McDonald and Martin, 2000). Although CFC 12 is highly volatile, when used in pMDIs, it is typically blended with other CFCs in order to reduce the final vapour pressure within the can. A combination of CFC propellants, whilst still volatile can be analysed at room temperature within simple sealed systems (Ashurst et al., 1996). In contrast to CFCs, the HFA propellants, HFA 134a and HFA 227, both have boiling points of less than −15 °C. The similarity of their boiling points means that HFAs cannot be blended in order to reduce their volatility. As HFAs exert a pressure of up to 4 kg cm^{-2}, in situ analysis of propellant suspensions in simple sealed systems is problematic and indirect techniques for measuring particle size, are therefore commonly employed.

The particle size of pMDIs can be measured indirectly using laser diffraction (de Boer et al., 2002a), image analysis (Bower et al., 1997), time of flight (Mitchell et al., 2003; Su et al., 2004), microscopy (Lamprecht et al., 2000; Columbano et al., 2003) and impaction (Weda et al., 2000). Nevertheless currently, inertial impaction is considered as the "gold standard" with which to assess the in vitro performance of inhaled formulations. Impaction techniques are capable of chemical identification and measure the aerodynamic diameter of particles (the importance of which was recently highlighted by Edwards et al., 1997), but they are time-consuming and cumbersome to use (de Boer et al., 2002a). Furthermore, it is difficult to draw conclusive mechanistic information from these indirect techniques, as the properties of the suspension are never physically measured.

Laser diffraction particle size analysis, has been suggested as a suitable alternative to impaction methods to measure the particle size of aerosols (de Boer et al., 2002a,b; Triballier et al., 2003). This technique uses narrow angle diffraction of a high-power laser source to determine the volume of both solid and liquid particulates. The changes in a columnar laser projection resulting from the intersection with a particle field is transposed onto a Fourier transform lens and the resultant laser diffraction pattern is converted into a volume-based size, derived using a mathematical model (Triballier et al., 2003). The non-invasive nature of laser diffraction particle size analysis makes it versatile, quick and simple to perform and cost-effective. In addition, laser diffraction does not require additional chemical analysis and can be easily automated. However, unlike impaction, laser diffraction does not measure the aerodynamic diameter of particulates, and therefore does not take account of particle density. Furthermore, during the mathematical calculation of the volume-based size measured using laser diffraction, it is assumed that particles are all spherical and this can lead to inaccuracies if the particles deviate radically from this presumed shape (Ma et al., 2001).

To try and make direct measurements within pMDI suspensions, researchers have attempted to use solvents with similar chemical properties but higher boiling points as surrogate propellants, in order to carry out studies at room temperature (Dickinson et al., 2000; Young et al., 2003; Ashayer et al., 2004). The most commonly employed solvents include 2H, 3H-perfluoropentane (Rogueda, 2003; Ashayer et al., 2004), perfluorohexane and 2,2,2 trifluoroethanol (Dickinson et al., 2000). These solvents can be used

at room temperature, and therefore analytical techniques such as zeta potential determination, atomic force microscopy and particle size measurement can be performed in situ to investigate the properties of apolar suspensions. However, an ideal surrogate solvent has yet to be identified which matches the key properties of either of the two HFA propellants currently employed commercially in pMDIs. Thus, whilst each of the surrogate systems differ slightly in physicochemical characteristics, it is difficult to extrapolate the findings to real systems and draw definitive conclusions from the scientific work in which the solvents are used.

Several attempts have been made to design specialist equipment that allows the direct in situ measurement of high-pressure suspensions. For example, Malvern Instruments Ltd. designed a specialised cell that prevents the evaporation of volatile solvents. Using this system, it should be theoretically possible to size particles that are suspended within HFA propellants under pressure as liquids. However, the high volatility of HFAs means they must be transferred into sealed systems under pressure. The cell produced by Malvern Instruments Ltd. in its current form does not allow high-pressure filling, and therefore it cannot be filled adequately using highly volatile solvents, such as HFAs.

The protracted indirect methods by which the success or failure of novel stabilising excipients are currently tested has resulted in development programs for new HFA suspension pMDIs becoming inefficient, and therefore expensive. As a potential consequence of this problem since the introduction of HFA propellants no formulation has been marketed that includes a novel HFA suspension stabiliser.

The aim of this work was to develop and test a novel method, to analyse the stability of HFA-based suspensions of therapeutic agents in situ, using laser diffraction particle size analysis. The design of such a system would enable the ability of new excipients to stabilise HFA-based suspensions to be rapidly qualified. Only the better performing excipients would then be selected for further investigation using the more time-consuming impaction models. Such a strategy would enable the formulation development time of suspension pMDIs to be shortened.

The initial aim of the study was to compare several methods of dispersing aersolisable particles within a pressurised HFA system to derive a reproducible sizing system. When the pressure cell particle sizing methodology was developed, this was compared to indirect methods currently used to characterise the physical stability of particles in HFA systems, i.e. "in-flight" laser diffraction measurements, sizing in surrogate propellants and impaction techniques, using four model formulations. Finally, the capability the laser diffraction system to act as a high-throughput screen for HFA pMDIs was assessed. A wide range of HFA-containing pMDIs were manufactured with numerous stabilising excipients and particle sized analysis of the formulations was performed using both impaction and pressure cell measurements in order to assess the predictive ability of the novel in situ particle sizing system to determine pMDI deposition.

2. Materials and methods

2.1. Microparticle production

Fluticasone priopionate and salbutamol sulphate microparticles were recovered by slowly evaporating the propellant from two commercial HFA-bases pMDI preparations (Flixotide 50®, 50 μg per dose AAH Pharmaceuticals, UK, lot D040214, expiry Jun 2005 and Ventolin®, 100 μg per dose, AAH Pharmaceuticals, lot DY577, expiry July 2005) in a fume cupboard. After the propellant had been evaporated, the remaining powder was collected and stored under silica desiccation at room temperature. BDP microparticles were used as received from Airflow Co., UK.

PVA coated BDP microparticles were manufactured by spray-drying 1.0 g of BDP suspended in 100 ml of DI water-containing 600 mg of poly(vinyl alcohol) (PVA) 80% hydrolysed, Mw 8000–10,000 (Sigma–Aldrich, UK). A 191 spray-drier (Bucchi, Switzerland) using an inlet temperature of 180 °C, material feed rate of 4 ml min^{-1}, atomisation flow of 70% and nozzle air flow of 800 ml min^{-1} was employed to process the aqueous suspension.

2.2. Microparticulate characterisation

The particle size of the microparticles produced in Section 2.1 was measured using a liquid stirring cell placed in a Model 26C4L particle size analyser (Malvern Instruments, UK) so as to determine the orig-

inal size of the microparticles prior to suspension in the HFA propellants. The optical bench was calibrated using a latex standard prior to use. A saturated cyclohexane (Merck, UK), 1% span 80 (Sigma–Aldrich) solution was used as the dispersion media. A method validated according to (ISO 13320, 1999) (data not shown) used a sample sonication time of 40 min and laser diffraction parameters of three-fourth power stirring rate, 2000 sweeps, a measurement path length of 14.5 mm and a 63 mm lens for each measurement. Three measurements were made of each sample and three samples were taken from each microparticle batch using a standardised sampling procedure.

A helium pycnometer (Micromeretics, UK) was used to measure the density of the all the microparticles. The machine was calibrated as per the manufacturers instructions using a calibration sphere and in addition, an aluminium standard (Micromeretics) was used to check the operation of the equipment. Approximately, 100–150 mg of each sample was measured five times and three samples taken from each batch, again using a standardised sampling protocol.

2.3. pMDI manufacture

A 50.0 mg sample of each BDP microparticle batch (Section 2.1) was suspended in either 20.0 g of HFA 134a (Solkane, Solvay, UK) or 17.5 g[1] of HFA 227 (Solkane) to produce the pMDI formulations. The microparticles were weighed directly into a clear polyethylene terephthalate (PET) canister (donated by AstraZeneca, UK) and sealed by crimping a 25 μl metered valve (donated by AstraZeneca) onto the vessel. The HFA was filled into the sealed PET canister using the Pamasol MDI filler (Pamasol, Switzerland) until the desired weight was attained. Ultrasonication was applied to the microparticle suspension for 1 min to ensure dispersion of the powder in the HFA. The Flixotide 50® and Ventolin® 100 pMDIs were used as received.

An identical process was followed to produce other HFA suspensions of drug (including those containing the drug particles obtained from commercial preparations as described in Section 2.1). However, these containers were sealed with a continuous valve (donated by 3M Ltd., UK). These second HFA suspension pMDIs were used to transfer samples into the re-circulatory pressure cell.

[1] 17.5 g of HFA 227 produces an equivalent volume to 20.0 of HFA 134a due to density differences.

2.4. Comparison of direct and indirect methods to determine HFA suspension stability

The feasibility of using surrogate propellant systems to predict the size of particles suspended in HFA solvents was determined by directly comparing the volume-derived particle size obtained using two surrogate solvents with that derived directly within HFA 134a and HFA 227 using the pressure cell system (methods described below). However, in order to compare, the volume derived diameters obtained by laser diffraction in the liquid stirred cell, the Sympatec Inhaler 2000® and the pressure cell (methods described below), the data from these measurements were converted to a MMAD using the measured density and compared to the twin-stage impinger results using Eq. (1). The percentage of particles < 6.4 μm MMAD, i.e. the fine particle fraction (FPF), was compared across the four techniques. Fluticasone propionate, salbutamol sulphate, BDP and BDP with PVA (all suspended within HFA 134a when appropriate) were used as the test formulations in all comparisons.

$$\mathrm{MMAD} = \mathrm{MMD}\sqrt{\frac{\rho}{\rho_0}} \quad (1)$$

where the MMD is mass median diameter (or volume median diameter × density), ρ the particle density and ρ_0 is the unit density (i.e. 1 g cm^{-3}).

2.4.1. Surrogate propellant particle size analysis

2H, 3H-decafluoropentane (Apollo scientific, UK) and perfluoropentane (Apollo scientific) were used as surrogate propellants. Samples were dispersed in the solvents prior to laser diffraction using sonication for 1 min (duplicating the manufacture of the pMDI formulations). An identical laser diffraction method was used to determine the particle size each of the four test formulations in the two surrogate propellants as that employed to determine the size of the raw material (Section 2.2).

2.4.2. Pressure cell particle size analysis

Two pressure cells were designed in-house. The first was a simple sealed unit consisting of a plastic cell with

two optical borosilicate glass surfaces. The particle size of a sample was measured in this cell by placing a predetermined weight of solid inside the unit, which was sealed and filled with HFA 134a through a specially adapted valve. The measurements were performed on a Mastersizer X particle size instrument (Malvern Instruments) using identical operational parameters as per the validated method described previously (Section 2.2). A second pressurised cell, manufactured by Malvern Instruments Ltd., was also employed, but the re-circulatory system and filling mechanism for this cell was designed in-house. The re-circulatory system could be filled with HFA using a specially designed valve and samples were injected into the system via a high-pressure sample input port. Samples were allowed to equilibrate in the HFA and were measured using two methods of re-circulation, either stop-flow re-circulation (which involved re-circulating the system on full power for 10 s then switching the pump off) or continuous re-circulation (which involved continuous re-circulation during the measurement sequence at three-fourth full power of the high-pressure pump). The particle sizing instrument was set up to match the previously used parameters (Section 2.2).

2.4.3. "In-flight" laser diffraction particle size analysis

The Sympatec Inhaler 2000® (Sympatec, UK) was used to characterise the particle size of the HFA-based pMDI formulations post-actuation, i.e. "in-flight". The equipment used the R2 lens (0.45–87.5 μm) and was set up specifically to measure the size of particles emitted by pMDIs. The optical bench was validated prior to use with the manufacturer's calibration standards. Method development experiments were performed using the two commercial inhalers and determined the effects of flow rate through the machine with a view to establishing experimental trigger conditions, data collection speed and the effect of the refractive index disturbance of HFA (as detailed previously (Smyth and Hickey, 2003)). Using these data, a set of experimental conditions to test the pMDIs was determined.

2.4.4. Impaction particle size analysis

The twin-stage impinger apparatus was set up and operated according to the USP, drawing air through the apparatus at 60 l min^{-1}. A total of 20 actuations of each inhaler were sprayed into the apparatus "mouth piece" with a 5 s gap between sprays. Chemical analysis was performed using a validated assay on a Waters Integrated Millennium HPLC system (Waters, UK) with the parameters detailed in Table 1. The study used filtered and degassed (0.2 μm nylon filter, Watman, UK) HPLC grade solvents (Merck labs, Germany). The washing solutions for the impinger equipment were the same as the mobile phase for all of the therapeutics except for salbutamol, which used 0.05 M phosphate buffer (Sigma–Aldrich, UK).

2.5. Pre-formulation screening

To test the capability of the pressure cell laser diffraction system to be used as a pre-formulation screen, six novel sets of excipients were combined with BDP, spray dried (Section 2.1) and suspended in either HFA 134a or HFA 227 (Section 2.3). The particle size of these six novel BDP formulations were compared using the twin-stage impinger (method as per Section 2.4.4) and the pressure cell system (method as per Section 2.4.2). To allow direct comparison of the two techniques, the derived volume diameter distribution obtained by laser diffraction was converted

Table 1
Summary of the HPLC methodology used in the study

Parameter	Beclomethasone	Salbutamol	Fluticasone
Column (C_{18})	150 mm × 3 μm	150 mm × 3 μm	250 mm × 5 μm
Mobile phase	70/30, ACN:H_2O	8/92, ACN:P	72/25, MeOH:AmAC
Flow rate (ml min^{-1})	1	0.75	1
Injection volume (μl)	100	10	20
Temperature (°C)	Room temperature	40	40
Runtime (min)	12	6	9

Columns used for the assay of beclomethasone and salbutamol were sourced from Hichrome, UK, and that for the analysis of fluticasone was base-deactivated, purchased from Capital HPLC, UK. ACN corresponds to acetonitrile, P to 0.05 M phosphate buffer, AmAC to 0.6% ammonium acetate solution, MeOH to methanol.

into mass median aerodynamic diameter using Eq. (1). The derived FPF for these six formulations and the four pMDIs used in Section 2.4 for both twin-stage impinger and pressure cell measurements were compared.

3. Results

3.1. Microparticle characterisation

The size of the microparticles prior to incorporation into HFA propellant was similar for each of the four test formulations (see Table 2). The BDP alone had the lowest median size (Dv, 0.5) but this was only approximately 1 μm smaller than the largest formulation, BDP with PVA. The salbutamol sulphate contained the greatest size range of particles, 90% of the cumulative size distribution (Dv, 0.9) for this material was over 2 μm larger than the other microparticle batches.

The density of the fluticasone was significantly larger ($p < 0.05$, tested using ANOVA) compared to the other microparticle batches (see Table 2), which were not significantly different from each other ($p > 0.05$, tested using ANOVA).

3.2. Comparison direct and indirect methods to determine HFA suspension stability

3.2.1. Method development

The two different pressure cells and the two methods of pressure cell re-circulation were directly compared using the BOP microparticles in HFA 134a. The Dv, 0.5 obtained for the BDP particles using the stop-flow re-circulatory method in the Malvern adapted cell, the continuous re-circulation method in the Malvern adapted cell, and sealed pressure cell manufactured in-house were 11.16 ± 1.64, 9.86 ± 0.50 and 10.77 ± 2.24 μm, respectively. There was no significant difference in the derived median volume diameter either between the re-circulation methods or the two cell types ($p > 0.05$, tested using ANOVA). Therefore, the simplest methodology to perform practically, i.e. the continuous re-circulatory method was used in all future experiments of this type.

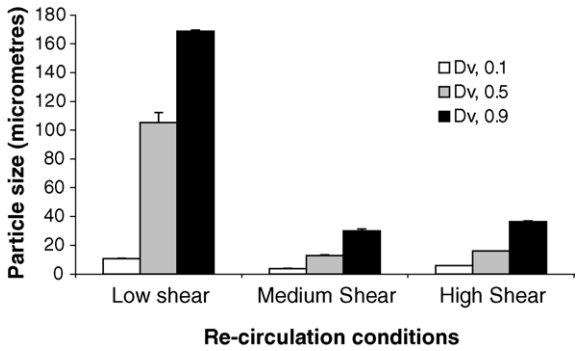

Fig. 1. Effects of re-circulation rate on particle size of BDP in HFA 134a ($n = 9$, mean ± S.D.). Dv, 0.1 represents the particle diameter at 10% of the cumulative undersize curve for the volume-based particle distribution (Dv, 0.5), 50% the cumulative undersize (i.e. the median) and (Dv, 0.9), 90%.

The Dv, 0.5 for the BDP particles suspended in HFA 134a, in order to achieve a low-, medium- and high-laser obscuration levels (which corresponded to approximately one-order of magnitude increase in the suspension concentration) were 10.29 ± 0.10, 10.79 ± 0.54 and 11.15 ± 0.13 μm, respectively. Therefore, variation in the laser obscuration, and thus suspension concentration, was shown to have no significant effect on the median particles size of BDP within the pressure cell ($p > 0.05$, tested using ANOVA). However, altering the shear applied to the BDP particles within HFA 134a did influence the median volume diameter (see Fig. 1). Increasing the re-circulation rate from a low HFA flow rate (i.e. applying low shear), to medium flow rate (i.e. medium

Table 2
The density, median particle size (Dv, 0.5) and the 10 and 90% cumulative dimensions (Dv, 0.1; Dv, 0.9) of the four test formulations prior to incorporation into the pMDI formulations ($n = 3$, mean ± S.D.) measured using laser diffraction in a liquid stirred cell

Sample	Dv, 0.1 (μm)	Dv, 0.5 (μm)	Dv, 0.9 (μm)	Density (g cm^{-3})
BDP	1.83 ± 0.21	3.13 ± 0.15	4.90 ± 0.36	1.336 ± 0.007
Salbutamol sulphate	2.10 ± 0.01	3.78 ± 0.30	11.60 ± 1.68	1.363 ± 0.046
Fluticasone propionate	2.25 ± 0.05	4.07 ± 0.29	8.11 ± 0.97	1.426 ± 0.015
BDP + PVA	2.54 ± 0.02	4.22 ± 0.16	7.99 ± 0.24	1.359 ± 0.021

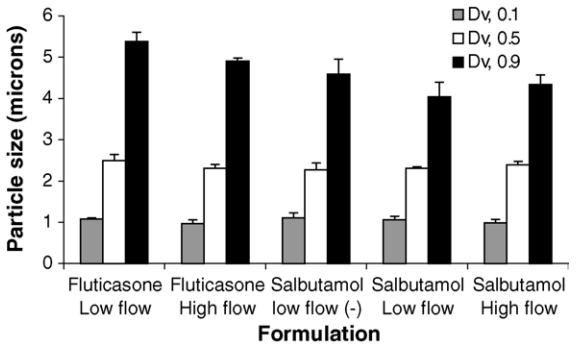

Fig. 2. Effects of flow rate and blank HFA subtraction (−) on the particle size distributions of two commercial inhaler preparations in HFA 134a ($n = 3$, mean ± S.D.).

shear) reduced the particle size of the BDP particles suspended in HFA by almost one order of magnitude. In contrast, varying the re-circulation rate from medium to high changed the median particle size by less than 8%, indicating at either medium or high re-circulation rates the particles were at their smallest stable size (see Fig. 1).

Inverting a plain canister of HFA 134a and spraying the vapour directly across the optical bench of the Sympatec inhaler 2000® produced an identical "ghost effect" to that previously reported by Smyth and Hickey (2003) (data not shown). The refractive index disturbance was present in the top four channels of the three lenses tested on this optical bench, and hence during the subsequent measurements, the data obtained from these channels was suppressed to eliminate this effect from the true diffraction result. Varying the flow rate of air through the extraction hood of the laser diffraction apparatus from 20.9 to 29.7 l min^{-1} (termed low and high in Fig. 2) generated a small, but statistically significant ($p < 0.05$, ANOVA) change in the median particle size of the aerosol emitted form the two commercial inhalers (Fig. 2). Manipulation of the data capture speed or experimental trigger sensitivity had only limited effects on the particle size of the therapeutics contained within the commercial formulations (data not shown). In addition, each of the three particle size indices detailed in Fig. 2 showed no significant difference ($p > 0.05$, tested using ANOVA) with or without (detailed on in Fig. 2 as salbutamol (−)) the subtraction of the particle size data obtained from measuring the HFA 134a propellant alone. As a consequence of these results, the following machine parameters were used to compare the particle sizes of the drugs present in the different formulations using the sympatec machine, flow rate 29.7 l min^{-1}, 0.3% optical concentration trigger and 5 ms data collection with no blank HFA subtraction.

3.2.2. Surrogate propellant particle size comparison

The four microparticle batches dispersed within perfluoropentane produced aggregated suspensions with a large Dv, 0.5 (see Table 3). In this propellant, the two BDP suspensions were found to contain particles with a similar median particle size around 30 μm. In addition, When fluticasone and salbutamol particles were suspended in perfluoropentane they both appeared to form aggregates, as they displayed median particle diameters of 82.66 and 48.53 μm, respectively, when analysed by laser diffraction. Conversely, in 2H, 3H-decafluoropentane the particles from the 2 BDP formulations displayed median diameters of 3.60 and 3.85 μm, respectively. The particle size of the two BDP formulations in 2H, 3H-decafluoropentane was not significantly different ($p > 0.05$ tested using ANOVA) from the original particle size of the formulations (see Table 2) implying that these particles are forming a physically stable single particle suspension in this solvent. Both the fluticasone and salbutamol particles showed some aggregation when suspended

Table 3
The density, median particle size (Dv, 0.5) and the 10 and 90% cumulative dimensions (Dv, 0.1; Dv, 0.9) of the four test formulations suspended in four apolar solvents

Formulation	PF (μm)	DecaF (μm)	HFA 134a (μm)	HFA 227 (μm)
BDP	33.42 ± 10.88	3.60 ± 0.48	9.86 ± 0.50	6.25 ± 1.33
BDP + PVA	28.61 ± 4.96	3.85 ± 0.19	9.68 ± 0.19	Soluble
Fluticasone	82.66 ± 11.82	11.09 ± 0.68	4.22 ± 0.41	–
Salbutamol	48.53 ± 11.82	15.41 ± 0.80	4.32 ± 0.27	–

BDP, BDP monohydrate; PF, perfluoropentane; DecaF, 2H, 3H-decafluoropentane ($n = 3$, mean ± S.D.).

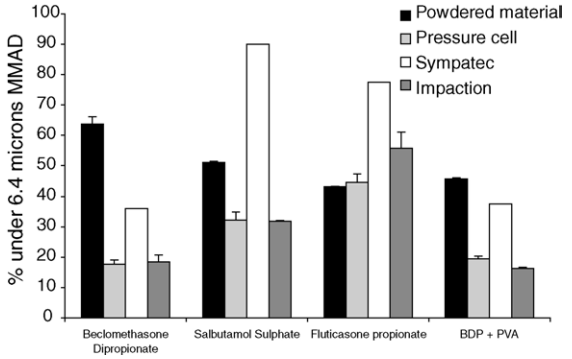

Fig. 3. The percentage of particles with an aerodynamic diameter of <6.4 μm in the four-test formulation compared using four particle sizing techniques ($n = 3$, mean ± S.D.).

in 2H, 3H-decafluoropentane, displaying median particle diameters of between 10 and 20 μm (see Table 3). All of the formulations suspended within the surrogate propellants produced significantly different ($p < 0.05$, ANOVA) median particle sizes compared to the true particle size, determined either in HFA 134a or HFA 227 (see Table 3).

3.2.3. Pressure cell, "in-flight", stirred cell and twin-stage impinger measurement comparison

Comparing the particle measurements obtained for the two BDP formulations and the two commercial preparations using the four sizing methods both before and after formulation within the propellant showed that the suspension of the respirable particles within HFA 134a resulted in some degree of aggregation for three of the four formulations (Fig. 3). Only the fluticasone propionate was found to have a similar median particle size both prior to incorporation within the propellant suspension, within the HFA vehicle and after expulsion from the formulation. The excellent stability of the fluticasone particles in the propellant resulted in this formulation displaying the greatest deposition upon the second stage of the impaction apparatus.

The size of the particles measured within the spray plume of the formulations, i.e. "in-flight" was consistently smaller in comparison to the sizes obtained for the same particles using either the pressure cell or the impaction equipment. In three of the four formulations analysed the percentage of particles < 6.4 μm using the Sympatec inhaler 2000® was twice the figure obtained using the twin-stage iminger (Fig. 3). In contrast, there

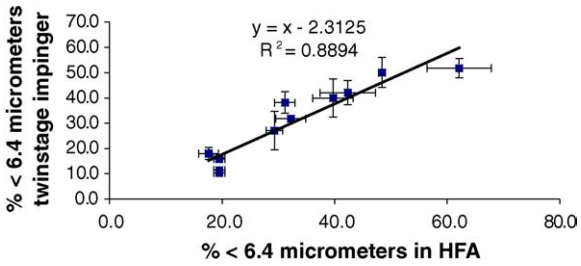

Fig. 4. Comparison of the percentage of particles with an aerodynamic diameter of <6.4 μm in eight novel HFA-based pMDI formulations and two commercial HFA pMDI preparations, measured using the twin-stage impinger and the pressurised laser diffraction system, r^2 is the linear correlation coefficient ($n = 3$, mean ± S.D.).

was good agreement between the particle size derived in the pressure cell, i.e. the particles suspended in the HFA propellant, and the impaction data. The percentage of BDP and salbutamol particles < 6.4 μm was not significantly different ($p > 0.05$, ANOVA) when measured using the pressure cell or impaction equipment. BDP formulated with PVA did show a small but significant difference ($p < 0.05$, ANOVA) in percentage of particles with an aerodynamic diameter of <6.4 μm when measurements were made using the pressure cell and the impaction equipment, whilst the fluticasone pMDI was oversized by the pressure cell on the basis of the data obtained by impaction.

3.3. Pre-formulation screening

A strong linear correlation ($r^2 = 0.8894$) was obtained when the percentage of particles < 6.4 μm for the eight novel HFA formulations and the two commercial preparations was compared using data obtained from both the impinger and the pressure cell, see Fig. 4. The greatest impinger stage 2 deposition of the eight novel BDP formulations was just over 50%, which was a five-fold increase upon the binary mixture of BDP and HFA 134a. This indicates the linear trend between these two measurements was applicable over a wide range of particle size results.

4. Discussion

Previous work comparing surrogate solvent systems has shown that at present there is not an ideal HFA sur-

rogate commercially available (Dickinson et al., 2000; Rogueda, 2003). Data in this study agreed with these conclusions and showed that particles from the four test formulations, which have a wide range of physicochemical characteristics, interacted with the surrogate propellants in a radically different manner compared to the HFA solvents. There was no correlation between the particle size in either of the surrogate solvents and the HFA propellants, which infers that predicting the particle size of HFA suspensions using the surrogate propellants 2H, 3H-decafluoropentane or perfluorpentane is not a valid sizing technique.

The data obtained using the Sympatec inhaler 2000® particle size analyser was found to consistently undersized the particles within the pMDI formulations compared to those obtained using the impinger and the pressure cell. Such discrepancies could be due to the application of the Fraunhoffer theory to the data (used by this machine to covert the diffraction pattern into a volume distribution), which has previously been reported with multi-modal particle size distributions (Annapragada and Adjei, 1996). However, this explanation is unlikely, as the similarity of particle size distributions should have consistently propagated the error, i.e. still ranked the formulations correctly, which it did not. A second explanation for the apparent undersizing of the formulations using the Sympatec inhaler 2000® may lie in the loss of particle size information in the four suppressed data channels, which was necessitated by the refractive index disturbance discussed previously. Little work has been performed to investigate the effects on the particle size measurement caused by the refractive index distortion seen in this study. The loss of data is presumably caused by vapours crossing the laser beam, but very little can be done to practically to prevent this when measuring pMDIs (Smyth and Hickey, 2003). However, the most plausible explanation of the lack of correlation between the data obtained using the Sympatec inhaler 2000® and other particle sizing techniques is the disparity between the type of dispersion in which the particle size measurement is taking place. The Sympatec machine measures the size of particles directly as they exit the inhaler device. During this time, the particles are travelling at a high velocity, the propellant is evaporating, particles may be forming, aggregating and de-aggregating and resulting in a complex multi-phase dispersion. To try and measure a more stable dispersion, it is possible to increase the distance between the mouthpiece of the inhaler and the beam to allow the plume to equilibrate, but the further the particles have to travel prior to intersection with the laser beam, the lower the particulate concentration, and hence the worse the signal to noise ratio. Recent work by Haynes et al. (2004) also showed that the pMDI plume was a very dynamic environment in which to measure particles size close to the point of actuation. This group found that by separating the point at which the pMDI is actuated from the laser, by using a heated impinger throat, the laser diffraction/impinger measurement of particle size could be better correlated (Haynes et al., 2004).

The particle size distribution determined in this study using the novel pressure cell methodology correlated better with the data from the impaction equipment, in comparison to that derived from the Sympatec inhaler 2000®. The pressure cell particle sizing methodology described in this work was based on the premise that after the application of a moderate shear force if the particles do not retain their original particle size, the suspension would be considered in some way unstable. For example, if the particles appeared to be smaller than the original particle size, dissolution was possibly occurring and if they were larger, the particles may have been irreversibly aggregating. Thus, the greatest potential source of error with this technique is not as a result of the actual measurement as with the Sympatec, as such measurements are known to be accurate due to the wealth of validation data concerning particle sizing in suspensions (Witt and Rothele, 1996; Muhlenweg and Hirleman, 1998; (ISO 13320, 1999), but is centred upon the degree of shear applied to the formulation. If the shear is applied to the particles is too great then this will not represent the typical shaking and actuation of the particles prior to the actuation of a dose and may result in the undersizing of the drug particles. Conversely, if the shear applied is insufficient there will be a tendency to oversize the particles in the suspension. Such a problem will be more pronounced when a flocculated rather than a deflocculated suspension is sized. However, the strong correlation between the particle size results obtained using this technique and those derived from the impaction studies using an extensive range of formulations, including flocculated suspensions (Fig. 4) suggest that the degree of shear applied to the suspensions only confered a small source of error in the particle size measurements of pMDI suspen-

sions when this parameter was optimised to produce a smooth re-circulatory flow (i.e. medium or high shear). In addition, whilst it is accepted that the mathematical conversion of volume particle diameter measurements provides only a relatively crude estimate of aerodynamic diameter, absolute precision is not required in this instance. It is envisaged that pressure cell studies would be employed to screen for the most promising pMDI suspensions of a drug, and therefore providing a linear correlation exists between this technique and impaction data, absolute accuracy in predicting the precise percentage deposition is not required.

The pressure cell sizing technique was not only shown to be superior to several indirect methods of determining pMDI suspension stability, but it also displayed the potential to act as a diagnostic tool to identify why certain HFA suspensions might produce only low pulmonary deposition. Fluticasone propionate was the only drug that did not change in particle size both prior too and after suspension in HFA 134a, suggesting a stable suspension existed. This excellent suspension stability led to an excellent FPF in the twin-stage impinger for fluticasone. In contrast, the suspension of salbutamol sulphate increased in particle size within the propellant, thus implying a degree of irreversible aggregation, not broken down by the shear imparted during the re-circulation process. Hence, although the salbutamol suspensions displayed an element of stability a small degree of aggregation led to a lower FPF compared to fluticasone. The BDP microparticles increased dramatically in size upon suspension in HFA 134a, leading to the conclusion that the vast majority of the particles aggregated irreversibly. The pressure cell predicted a low fine particle fraction would be obtained from the BDP/HFA 134a suspension and this was found to be correct when the same formulation was assessed using the impaction technique. The physical stability of the BDP suspension was not improved by the addition of PVA, since this formulation had similar particle size characteristics to the binary BDP/HFA formulation, which implies that PVA did not act as a suspension stabiliser for BDP in HFA solvents.

5. Conclusion

This study highlights the requirement for the development of an efficient, accurate and precise methodology for the particle sizing of pharmaceutical aerosols. Although laser diffraction methods have been suggested as a suitable replacement for impaction particle sizing techniques (which can be labour intensive) accuracy in determining absolute particle size is difficult to attain using current equipment, which is obviously a crucial problem when attempting to develop respirable dosage forms. The major difference between aerosolised drug particles emitted from complex dosage forms as pressurised metered dose inhaler or dry powder inhalers and simple suspensions or solid dispersions, is that during the measurement sequence dynamic processes are occurring within the aerosol cloud. Typical examples of this include, a solvent rapidly evaporating during the actuation of a pMDI or the de-aggregation of a carrier and drug during the actuation of a DPI. There has been little evidence to show that these dynamic processes do not affect the particle size results derived from laser diffraction equipment. Perhaps as a consequence, several recent studies have failed to correlate absolute particle size measurements determined within impaction apparatus to the same measurements made within aerosol clouds using novel laser diffraction dispersion systems (Smyth and Hickey, 2003; Berry et al., 2003a,b; Haynes et al., 2004).

Currently, the United States Food and Drug Administration (FDA) does not accept data obtained by laser diffraction in lieu of results obtained from impaction studies for the regulatory submissions of new medicinal products. A great wealth of data comparing laser diffraction to other particle sizing methods will be required for this stance to be altered. However, the future development of a pressurised particle sizing system as employed in this work would allow laser diffraction particle size analysis to be employed in combination with other more time-consuming methods. Such a technique can be used at a pre-formulation stage of suspension pMDI development to perform compatibility screening of drug-excipient-propellant interactions. Screening of HFA suspension formulations using laser diffraction and subsequent assessment with impaction equipment will not only assist the production of required regulatory data, but also improve the efficiency of pMDI formulation development. The novel method developed and tested in this work, which uses basic laboratory equipment, and can be very simply and cost effectively automated, could

play a vital role in the future of suspension pMDI development.

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