Manipulation of Beclomethasone–Hydrofluoroalkane Interactions using Biocompatible Macromolecules

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ABSTRACT: The aim of this work was to physically stabilise beclomethasone dipropionate (BDP) microparticles within a hydrofluoroalkane (HFA) propellant using biocompatible polymers in order to allow the efficient delivery of the steroid to the airways from a pressurised metered dose inhaler (pMDI). BDP microparticles were coated with a number of different 'amphiphilic' macromolecular excipients by spray-drying an aqueous BDP suspension in which the excipients were dissolved. The physical stability of the coated BDP microparticles was assessed both indirectly using a twin-stage impinger (TSI) and directly using '*in-situ*' laser diffraction particle size analysis in a range of nonpolar solvents. The solubility of the formulation excipients within a number of the nonpolar vehicles was determined using an internally manufactured filtration rig and the influence of zeta potential within the microparticle suspensions measured in a series surrogate nonpolar systems. The size of the pure BDP microparticles increased significantly ($p < 0.05$, ANOVA) from 3.13 ± 0.15 μm to 9.86 ± 0.50 μm upon suspension within a nonpolar HFA solvent. However, the addition of poly(vinyl alcohol) (PVA) and poly(vinyl pyrrolidone) (PVP) to the BDP microparticles dramatically reduce this aggregation leading to the production of physically stable suspensions with excellent aerosolisation properties (Stage 2 deposition >40% in the twin-stage impinger). It is postulated that the enhanced physical stability observed when PVA and PVP are coated onto BDP microparticles is partially as a result of steric stabilisation in HFA solvents. However, the large zeta potential associated with the nonpolar microparticle suspensions suggest that charge stabilisation may also influence the physical stability within these systems. J Pharm Sci 95:1060–1074, 2006

Keywords: hydrofluoroalkane; nonpolar; suspension; polymer; beclomethasone dipropionate

INTRODUCTION

Suspending therapeutic agents within volatile nonpolar solvents (dielectric constant $(\varepsilon) < 10$) provides a highly protective environment, which is ideal for the storage of labile pharmaceutical products. Water exhibits low solubility in nonpolar vehicles and the volatile nature of the solvent dictates that such suspensions should be stored in airtight, light protective environments. As a result nonpolar suspension formulations have the capacity to protect a drug from the potentially deleterious effects of oxygen, light and water. In addition, the volatile nature of such solvents provides propulsion to the formulation which is ideal for topical drug delivery.

The physical stability of nonpolar pharmaceutical suspensions (e.g. pressurised metered dose inhalers, pMDIs) is critical to their performance

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because it often not only dictates the dosing reproducibility of the formulation but, as they are 'self-propelled', it can also determine the site of delivery of the suspended particulates.[1] The principles of the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory are often used to explain colloid stability in both aqueous and nonaqueous (nonpolar) suspensions. The DVLO theory states that the stability of the dispersed colloids is governed mainly by electrostatic forces exceeding the attractive van der Waals forces between particles.[2,3] However, there is still debate over the contribution that electrostatic forces make to suspension stabilisation in nonpolar media, which contain very few charged species.[4,5]

In polar solvents a large dielectric polarisability lowers the electrostatic barrier to ionisation thus, charge is ubiquitous. Consequently, charge residing on the surface of particles within polar media can have a direct influence upon the range and strength of repulsive forces within a suspension system. In nonpolar solvents the barrier to charging is up to 40 times larger compared to polar suspension vehicles and therefore, the charge effects within such systems should be insignificant. In the absence of charge, steric mechanisms are thought to physically stabilise nonpolar systems. Hence, excipients such as polymers or surfactants that act to stabilise these systems must display a degree of solubility in the nonpolar vehicle to facilitate chain extension into the continuous phase of the system. This can be problematic in nonpolar solvents that display poor solubility profiles such as hydrofluoroalkanes and short chain hydrocarbons, for example butane and propane. However, several previous studies have implied that the introduction of charge into nonpolar colloid systems can improve physical stability.[6–8] The exact mechanism and/or the relevant pharmaceutical applications of this phenomenon have not yet been fully investigated.

Whilst, several commercially available pharmaceutical products, use nonpolar solvent systems as formulation vehicles in which to suspend therapeutic agents (including several topical and transdermal sprays), pMDIs are by far the most commercially relevant nonpolar suspension systems.[9] Due to the gradual withdrawal of chlorofluorocarbons (CFCs) which have been shown to deplete the ozone, hydrofluoroalkanes (HFAs) are now the suspension vehicle of choice for pMDIs. However, the high volatility of HFAs requires these solvents to be held in sealed canisters under a pressure of up to 4 bar at room temperature hence, the '*in-situ*' analysis of these suspensions can be problematic and as a result the mechanisms of suspension stabilisation within these vehicles, at present, are poorly understood.

As such, the aim of this work was to investigate the influence of charge stabilisation within nonpolar pharmaceutical formulations. pMDIs were used as model systems and the study attempted to determine if, and how, commonly used macromolecular pharmaceutical excipients could modify the physical stability of particulates within HFAs. Where '*in-situ*' analysis was made impossible due to the high volatility of the HFAs, a range of surrogate nonpolar solvents, selected to represent a range of different physicochemical properties and used in previous work to mimic pharmaceutical nonpolar suspensions were employed.[10–12] Micronised beclomethasone dipropionate (BDP) was used as the model drug as it is known to display limited solubility and a poor physical stability in HFA solvents and poly(vinyl alcohol) (PVA), poly(vinyl pyrrolidone) (PVP) and hyaluronan (HA) the amphiphilic stabilisers as they have displayed the unexpected ability to improve the physical stability of BDP in HFA propellants.[13]

MATERIALS AND METHODS

Microparticle Production

The BDP monohydrate and the macromolecular excipients (Tab. 1) were combined by spray-drying an aqueous BDP suspension in which the excipients were dissolved. PVA was used as the suspending agent and was dissolved in deionised water (conductivity 0.5–1.0 μS) by stirring the two components on a heated magnetic stirrer (Stuart Scientific, Surrey, UK) set at 80°C for approximately 20 min[1]. To the PVA solution BDP was added producing a homogeneous suspension after a further 20 min stirring at 25°C. Finally, PVP alone or with HA (which were all readily water-soluble) were added. After a further 20 min of stirring the BDP suspension was spray-dried using a 191 spray-drier (Buchi, Flawil, Switzerland) set with an inlet temperature of 180°C, a material feed rate of 4 mL/min, an atomization flow of 70% and a nozzle air flow of 800 mL/min.

[1]A single batch of this solution was spray-dried alone using identical machine parameters for morphological comparison using scanning electron microscopy.

Table 1. Composition of the Novel Spray-Dried BDP pMDI Formulations

Formulation	BDP (g)	PVA (g)	PVP (g)	HA (g)	Polymer Grades
BDP PVA80	1.0	0.6	—	—	PVA 80%
BDPLW80K15	1.0	0.6	0.1	—	PVA 80% PVP K15
BDP PVA40	1.0	0.6	—	—	PVA 40% in 1% SDS
BDPLW80K15HA	1.0	0.6	0.1	0.1	PVA 80% PVP K15
BDPLW70K15	1.0	0.6	0.1	—	PVA 70% PVP K15
BDPLW88K15	1.0	0.6	0.1	—	PVA 87–89% low M_w PVP K15
BDPMW88K15	1.0	0.6	0.1	—	PVA 87–89% med M_w PVP K15
BDPHW88K15	1.0	0.6	0.1	—	PVA 87–89% high M_w PVP K15
BDPHW88K90H	1.0	0.6	0.6	—	PVA 87–89% high M_w PVP K90
BDPHW88K90L	1.0	0.6	0.1	—	PVA 87–89% high M_w PVP K90
BDPLW98K15	1.0	0.6	0.1	—	PVA 98% PVP K15

Low M_w refers to 13–23 kDa, medium M_w refers to 31–50 kDa, high M_w refers to 124–180 kDa.
The volume of water was increased to 300 mL in this manufacture method in order to decrease the total solid content of the spray-dried suspension.

PVA 70% hydrolysed M_w 13000; PVA 80% hydrolysed, M_w 8000–10000; 87%–89% hydrolysed, M_w 13000–23000; 87%–89% hydrolysed, M_w 31000–50000 and 87%–89% hydrolysed, M_w 124000–180000 were all purchased from Sigma Aldrich, Gillingham, UK. PVP K15, M_w 10000 and PVP K90, M_w 360000 were supplied by Sigma Aldrich, Gillingham, UK. HA (M_w 400000) was a donation from MedPharm Ltd. and the trehalose dihydrate was from Sigma Aldrich, Gillingham, UK. The yield of the spray-drying process was calculated using Eq. 1

$$\%\text{yield} = \frac{\text{Mass}_F}{\text{Mass}_S} \times 100 \quad (1)$$

where Mass_F was the weight of the product from the spray-drying process and Mass_S was the solid content of the initial suspension prior to spray-drying.

Microparticulate Characterisation

The size of the original drug and the spray-dried microparticles was measured using a liquid stired cell placed in a Model 26C4L particle size analyser (Malvern Instruments, Malvern, UK). The optical bench was calibrated using a 3 μm latex standard prior to use. Cyclohexane (Merck, Hoddeson, UK), 1% span 80 (Sigma Aldrich, Gillingham, UK) solution was saturated with BDP and used as the dispersion media. A method validated according to ISO 13320 (data not shown) used a sample sonication time of 40 min and laser diffraction parameters of $\frac{3}{4}$ 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 batch using a standardised sampling procedure.

pMDI Manufacture

A 50.0 mg sample of each microparticle batch was suspended in 20.0 g of HFA 134a (Solkane. Solvay, Southampton, UK) to produce the pMDI formulations. The samples were weighed directly into a clear polyethylene terephthalate (PET) canister (donated by AstraZeneca, Loughborough, UK) and sealed by crimping a 25 μL metered valve (donated by AstraZeneca, Loughborough, UK) onto the vessel. The HFA was filled into the sealed PET canister using a pMDI filler (Pamasol, Pfaffikon, 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. An identical process was followed to produce a second set of HFA suspensions but sealed with continuous valves (donated by 3 M Ltd, Bracknell, UK). The second batch of pMDIs were used to transfer samples into a recirculatory pressure cell (method described below). In addition to the BDP formulations manufactured in-house, a commercial CFC BDP suspension based pMDI, Becotide 50® (AAH pharmaceuticals, Ruislip, UK. Lot E030. Expiry Feb 2006) was used as received.

Determination of Solubility in HFA

The solubility of the excipients in the HFA propellants was either determined visually (PVP K90 and HA) or using a filtration apparatus, designed in-house to allow high-pressure filtration

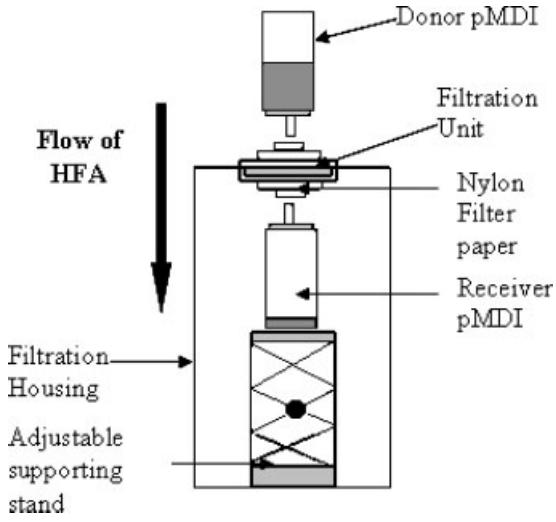

Figure 1. A schematic diagram showing the high pressure filtration unit. During operation the two pMDIs were docked in the filter unit simultaneously. The filter paper was nylon, 0.2 μm (Whatman, UK) cut to fit the diameter of the filtration unit.

of HFA from one sealed system to another (Fig. 1). The excipient to be tested was added to a sealed donor PET pMDI filled with HFA (crimped with a continuous valve) until a suspension was produced. This system was stirred for 12 h to allow equilibration at room temperature. A totally empty receiver pMDI canister was also crimped with a continuous valve and weighed. A nylon filter paper (0.2 μm, Whatman, Brentford, UK) was placed within the filtration unit and sealed using three locking screws to produce an airtight system. The donor and receiver pMDIs were simultaneously docked in the filtration unit (Fig. 1) and the HFA passed from the donor to the receiver pMDI due to the pressure difference. Once the pressure had equilibrated and therefore, the flow of propellant had ceased, the two inhalers were released simultaneously from the filter. The amount of propellant that crossed into the receiver pMDI was calculated by weight. The solute contained within the receiver solution after filtration (if any) was recovered by slowly evaporating the HFA (i.e. carefully removing the crimped valve and exposing the HFA to atmospheric conditions). The residual solid within the pMDI canister after removal of the HFA propellant was redissolved using liquid chromatography mobile phase and this solution was assayed. The solid recovered from the receiver pMDI was used to calculate the solubility of the excipient within the HFA as a weight per weight ratio. The filter in the high-pressure housing was replaced after each filtration operation and its integrity was checked under a light microscope.

The recovery of the high-pressure filtration methodology was determined using BDP, which is known to be slightly soluble in HFA 134a. Initially, the solubility of BDP in HFA 134a was measured using the high-pressure filtration method detailed above. Then a quantity of BDP below its maximum solubility was dissolved in the propellant and filtered as described above. The recovery and BDP solubility were both tested in triplicate. The concentration of BDP after filtration was compared to the initial concentration to determine the recovery. The % recovery (%R) of the BDP was calculated using Eq. 2

$$\%R = \frac{\mathrm{BDP_R}}{\mathrm{BDP_D}} \times 100 \qquad (2)$$

where $\mathrm{BDP_R}$ is the concentration of BDP in the receiver vessel and $\mathrm{BDP_D}$ is the original concentration of BDP in the donor. The % recovery was used to determine whether any binding of the polymers to the filtration apparatus occurred and to correct the calculated solubility using Eq. 3

$$\mathrm{Sol_{corr}} = \frac{\mathrm{Sol}}{\%R} \times 100 \qquad (3)$$

where $\mathrm{Sol_{corr}}$ is the corrected solubility taking into account the recovery from the apparatus, Sol is the solubility of the compound being tested, $\%R$ is defined in Eq. 2.

PVA Quantification

A 30 mg/mL PVA stock solution (100 mL) was made up in HPLC mobile phase. The PVA has dissolved by heated stirring at 90°C. Six standards were produced (using the mobile phase as the diluent) in the range of 1.5–30 mg/mL by serial dilution of the stock solution. PVA was assayed using gel filtration chromatography. The liquid chromatography system consisted of an isocratic Pu 980 Pump (Jasco, Great Dunmour, UK) set at 1 mL/min, an AS 950 autosampler fitted with a 100 μL injection loop (Jasco, Great Dunmour, UK), a CI—10 B integrator (LDC/Milton Roy, UK) and a chart printer (LDC/Milton Roy, UK). A differential refractometer (Waters, Elstree, UK) set at an inlet temperature of 37°C was used for detection. The column (TSKgel G2000 SW column) had a 7.5 mm i.d. and a 30 cm length. Previously filtered (0.45 μm nylon filter paper (Whatman, Brentford, UK)) and degassed 0.1 M phosphate buffer (Sigma Aldrich,

Gillingham, UK), adjusted to pH 7.5 using sodium hydroxide (Sigma Aldrich, Gillingham, UK) was used as the mobile phase. The injection needle was washed in between each injection using HPLC grade methanol (Merck labs, Darmstat, Germany). The method had previously been determined as 'fit for purpose'.[14]

PVP Quantification

The solubility of PVP K90 was determined visually. However, PVP K15 was assayed using HPLC. A 20 mg/mL PVP K15, M_w 10000 (average molecular weight as quoted by Sigma Aldrich, Gillingham, UK) stock solution was prepared in deionised water (conductivity 0.5–1.0 μS) by stirring at room temperature. Five serial dilutions were prepared using deionised water (conductivity 0.5–1.0 μS) to produce calibration standards in the range of 0.08–20 mg/mL. These were analysed by HPLC on a system consisting of an isocratic Pu 980 Pump (Jasco, Great Dunmow, UK) set at 0.6 mL/min, an AS 950 auto-sampler fitted with a 100 μL injection loop (Jasco, Great Dunmow, UK), a CI-10B integrator (LDC/Milton Roy, UK) and a chart printer (LDC/Milton Roy, UK). PVP was detected using a 975 UV/VIS detector (Jasco, Great Dunmow, UK) set at 243 nm. A C_{18} 150 mm × 3 μm column was used in combination with a C_{18} guard column. The mobile phase consisted of 80/20 deionised water (conductivity 0.5–1.0 μS): propan-1-ol (HPLC grade, Merck labs, Darmstat, Germany) and 0.01% TFA (Sigma Aldrich, Gillingham, UK). The method had previously been determined as 'fit for purpose'.[15]

Zeta Potential Measurements

Samples were prepared by suspending approximately 2 mg of the spray-dried microparticles within 10 mL of solvent and sonicating the mixture for 1 min in a decon 5300b ultrasonication bath (Decon laboratories, Hove, UK). The zeta potentials of the samples were measured immediately after suspension within the solvents using a DTS1070 nonaqueous dip cell (Malvern Instruments, Malvern, UK) with a ZetaSizer Nano NS® (Malvern Instruments, Malvern, UK). A total of 20 runs were performed for each sample using a cell voltage of 20 V. Three different suspensions were constructed and measured for each of the four batches of microparticles. A selection of the manufactured microparticles were assessed in the surrogate solvents including, BDPPVA 40, BDPPVA 80, BDPLW80K15, BDPLW80K15HA.

The conversion of the measured electrophoretic mobility (U_e) data into zeta potential (z) was carried out using Henry's Eq. 4

$$U_e = \frac{2\varepsilon z f(K_a)}{3\eta} \quad (4)$$

where ε is the dielectric constant, η is the sample viscosity and $f(K_a)$ is Henry's function. In Henry's function, the parameter K, termed the Debye length is a measure of the thickness of the electrical double layer. In aqueous solutions this $f(K_a)$ is approximated to 1.5, known as the Smoluchowski approximation. However, for small particles in nonaqueous solvents this approximation becomes 1.0 (referred to as the Huckel approximation) and this was used in this set of experiments. The surrrogate solvents used are detailed in Table 2.

Pressure Cell Particle Size Analysis

The size of the microparticles when suspended in HFA 134a was measured using a novel recirculatory pressure cell to determine the physical stability of the microparticles within HFA directly. The development, validation and use of this pressure cell has been described previously.[16]

Table 2. Physicochemical Properties of the Nonpolar Solvents used in the Study

Solvent	Dielectric Constant	Log P	Refractive Index	Viscosity (cP)
Methyltrifluoroacetate	11.64	1.10	1.2907	0.418
Dichloromethane	9.1	1.01	1.4229	0.440
Perfluoropentane	1.5	3.45	1.2383	0.462
HFA 134a	9.5	—	1.17	0.211
HFA 227	4.1	—	1.22	0.267

All physicochemical data used in the table was supplied by the manufacturer or sourced from Beilestein Crossfire chemical database (MDL information systems, GmbH).

Measurements were made in triplicate and three samples were taken from each of the microparticle suspensions.

Impaction Particle Size Analysis

The TSI was employed to determine the deposition characteristics of the microparticles after release from the pMDIs in order to determine the physical stability of microparticles within the HFA indirectly. The TSI apparatus was set up and run using a flow rate 60 L/min. The pMDIs were primed prior to use by discharging approximately 10 shots into a fume cupboard. The pump was allowed to run for 5 s after each discharge and then switched off for 5 s while the inhaler was shaken by hand. A total of 20 actuations were sprayed into the apparatus from each inhaler. After the completion of each run the impinger was taken apart and the drug assayed according to the amounts deposited on the device, stage 1 and 2. The device was washed with 50 mL of liquid and both stage 1 and 2 with 100 mL of fluid. HPLC analysis (described below) was used to quantify the drug within the washing solutions. HPLC mobile phase was used both as the impinger solvent and as the washing solution.

The quantities of the therapeutic agents delivered by the pMDI formulations on each stage of the impinger was determined as a percentage of the total quantity of drug recovered from the device and the impinger. For example, in order to calculate the stage 2% deposition, Eq. 5 was used

$$\%\text{stage2} = \left(\frac{Q_a}{Q_a+Q_b+Q_c}\right)100 \qquad (5)$$

where %stage 2 is the percentage of the drug on stage 2 of the impinger, Q_a is quantity of the drug on stage 2 of the impinger, Q_b is the quantity of the drug on stage 1 of the impinger, Q_c is the quantity of the drug on the device. The fine particle fraction (FPF) in the TSI was defined as the % of the drug on stage 2 of the impinger, that is the % of particles <6.4 μm. A Becotide 50® pMDI was tested in triplicate using an identical TSI method and the quantity of BDP recovered (%*r*) from the apparatus was compared to the label dose using the Eq. 6:

$$\%r = \frac{T_1+T_2+T_3}{LD} \times 100 \qquad (6)$$

where T_1 is the quantity of BDP per shot deposited on the device, T_2 is the quantity of BDP per shot deposited in the TSI stage 1 and T_3 is the quantity of BDP per shot deposited in the TSI stage 2, LD is the label dose.

The chemical analysis of BDP was performed using a Waters Integrated Millennium HPLC system (Waters, Elstree, UK) using a C_{18} 150 mm × 5 μm Hichrome column, an injection volume of 100 μL, a runtime of 7 min, a 70/30 acetonitrile: water mobile phase at room temperature. The study used filtered and degassed (0.2 μm nylon filter, Whatman, Brentford, UK) HPLC grade solvents (Merck labs, Darmstat, Germany). Calibration curves were always run on the day of analysis and the calibration standards were made up in the mobile phase used in the analysis. A total of five standards were made up for each of the three different assays using serial dilution ranging from 100 to 0.2 mg/mL.

RESULTS

Microparticle Characterisation

BDP was successfully combined with numerous grades of PVA and PVP by spray-drying a suspension of the drug with the excipients to form microparticulates. The grade of PVP used during manufacture appeared to have a marked influence on the size of the resultant microparticles. Both batches of spray-dried BDP that employed PVPK90 (batches BDPHW88K90H and BDPHW88K90L) had mean particle sizes that were significantly larger ($p \leq 0.05$, ANOVA, Tab. 3) than the particle sizes of the other batches. In addition, the batch containing the highest concentration of PVPK90 (BDPHW88K90H) was shown to have a significantly larger mean size ($p \leq 0.05$, ANOVA, Tab. 3) compared to those particles containing a lower concentration. The original BDP, measured prior to spray-drying, displayed the smallest median particle size with a Dv, 0.5 of 3.13 μm. An alteration in the molecular weight of PVA had no significant effect ($p > 0.05$, ANOVA) on the Dv, 0.5. The % hydrolysis of the PVA used during spray-drying did have a small effect on the Dv, 0.5 of the microparticles however, there appeared to be no correlation between the size of the microparticles and the % hydrolysis of the PVA (Tab. 3).

The efficiency of the spray-drying process was not directly influenced by the size of the resultant microparticulates but, it was dependent on the type and number of excipients added to the initial

Table 3. Particle Size and Manufacture Yield of the BDP Microparticles (for the % Yield $n = 1$ But for the Rest of the Data $n = 3$, Mean ± Standard Deviation)

Sample	Yield (%)	Microparticle Dv, 0.5 (μm)	BDP Content (%)	pMDI Dv, 0.5 (μm)	TSI FPF (%)
BDP	n/a	3.13 ± 0.15	100.00	9.86 ± 0.50	18.1 ± 2.3
BDP PVA80	10.06	4.22 ± 0.16	92.11 ± 2.20	9.68 ± 0.19	16.3 ± 0.6
BDPLW70K15	19.09	4.02 ± 0.13	64.68 ± 3.79	—	4.1 ± 0.3
BDPLW80K15	14.62	3.37 ± 0.02	74.29 ± 3.80	5.43 ± 0.09	42.1 ± 4.8
BDP PVA40	16.28	4.41 ± 0.03	—	—	6.0 ± 1.4
BDPLW80K15HA	46.15	4.02 ± 0.15	49.46 ± 8.38	5.07 ± 0.30	40.0 ± 7.6
BDPLW88K15	20.60	3.89 ± 0.29	64.39 ± 3.20	—	6.6 ± 1.3
BDPMW88K15	17.44	3.74 ± 0.31	67.03 ± 7.95	—	10.2 ± 1.9
BDPHW88K15	13.76	3.87 ± 0.16	62.51 ± 4.51	—	20.8 ± 1.5
BDPHW88K90H	25.90	8.14 ± 0.27	38.21 ± 3.45	—	4.9 ± 1.6
BDPHW88K90L	28.52	4.58 ± 0.17	62.41 ± 3.56	—	3.7 ± 0.4
BDPLW98K15	20.18	3.22 ± 0.24	66.41 ± 6.28	—	33.4 ± 2.6
Bectotide 50®	—	—	100.00	—	51.2 ± 1.3

pMDI is the particle size of the microparticles measured '*in-situ*', that is, within the HFA using the pressure cell. TSI FPF is the % of the released BDP that has deposited in stage 2 of the twin-stage impinger. Dash indicates not measured.

BDP suspension. For example, whilst the manufacturing yield for the BDP microparticles was typically less than 25%, the inclusion of HA with both PVA, PVP almost doubled the yield (Tab. 3). Furthermore, combining BDP and PVA with high molecular weight PVP (PVP K90) compared to the low molecular weight variety again almost doubled the % yield of the manufacturing process.

The types of excipients added to BDP during the spray-drying process influenced the BDP content and homogeneity of the resulting microparticulates (Tab. 3). Adding higher molecular weight excipients reduced the BDP content of the inhalable powders, whilst the fewer excipients that were added the higher the BDP content remained. For example, adding 0.6 g of PVA to 1 g of BDP (Batch BDPPVA80) only reduced the BDP content in the final microparticles by 8% however, adding only a further 0.1 g of PVP to this mixture (Batch BDPLW70K15) resulted in a 35% loss of BDP content in the final microparticles. The homogeneity (inferred from the standard deviation of the BDP content determinations) of the spray-dried BDP microparticles was excellent across all the manufactured batches apart from BDPLW98K15 and BDPLW80K15HA which both displayed a BDP content standard deviation of >5%.

The morphology of the particles produced from the spray-drying process was analysed using scanning electron microscopy (SEM), as shown in Figure 2. The spray-drying of PVA alone gave rise to numerous surface indentations within the resultant microparticles, presumably caused by the rapid formation of the particles from solution during the process (Fig. 2). Images of the polymers in combination with BDP showed a noticeable reduction in the number of these surface indentations. The BDP prior to spray-drying (Fig. 2d) was much less spherical compared to the BDP microparticles when combined with the polymers.

Physical Suspension Stability

The commercial preparation Becotide 50® is known to exhibit a high degree of physical stability during its self-life of 2 years. The high FPF (51.2 ± 1.3%) delivered by the Becotide 50® confirms that it is well formulated as a physically stable suspension of inhalable particles. The recovery from the impaction apparatus using this methodology was determined to be 105.1 ± 2.5% using the Becotide 50® pMDI.

In contrast to the Becotide 50® formulation, the binary BDP HFA 134a pMDI deposited a relatively small proportion of its dose on stage 2 of the TSI, that is a significant fraction of the microparticles were larger than an MMAD of 6.4 μm after aerosolisation of the dose into the impinger. The low FPF exhibited by the binary BDP suspension infers that the BDP microparticles are aggregating within the HFA solvent. This was confirmed by the pressure cell particle size measurements which showed that the particle size of the BDP

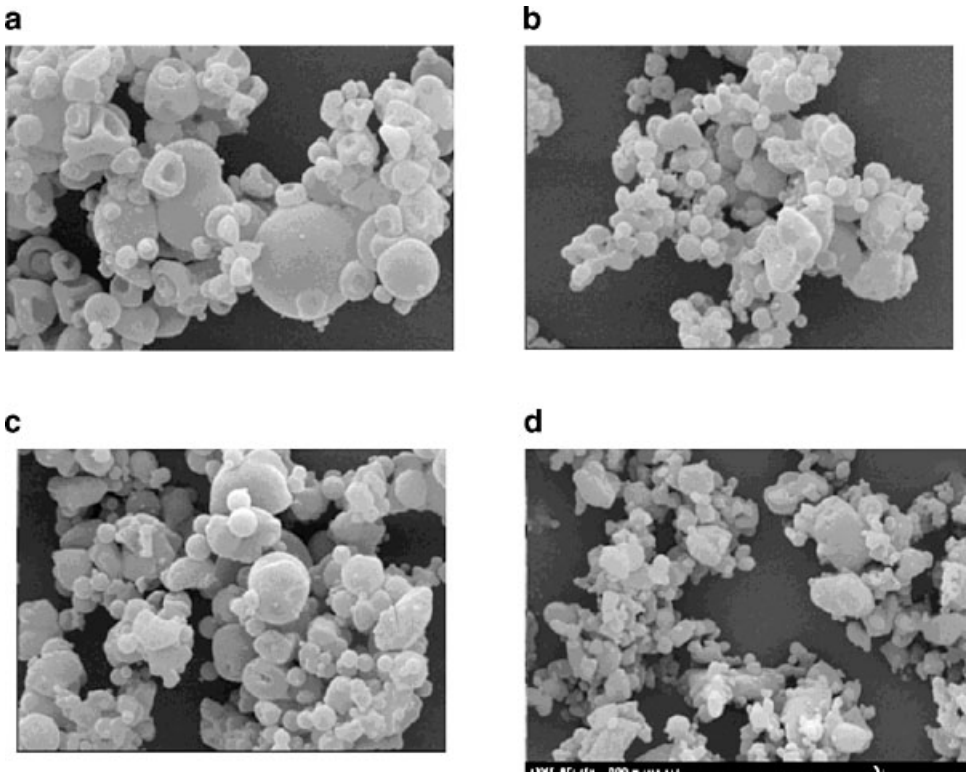

Figure 2. Scanning electron microscopy images of (a) PVA 80% hydrolysed spray-dried alone (b) BDPLW80K15 (c) BDPLW80K15HA (d) BDP.

microparticles was increased significantly ($p < 0.05$, ANOVA) from 3.13 ± 0.15 μm to 9.86 ± 0.50 μm upon suspension within HFA. The addition of PVA to the BDP microparticles did not enhance the stage 2 deposition nor presumably the physical stability of the pMDI formulation compared to the binary BPD/HFA 134a blend (Tab. 3). Again the size measurement using the pressure cell suggested some particle aggregation within the suspension. The median volume based diameter of the BDP PVA80 microparticles increase from 4.22 ± 0.16 to 9.68 ± 0.19 μm upon suspension within HFA. Unfortunately, PVP could not be combined with BDP alone as the polymer did not facilitate the suspension of BDP in water. The hydrophobic nature of BDP means that it cannot be suspended in water alone thus, PVA was a prerequisite component within all the microparticulate mixtures prior to spray-drying.

The combination of PVA and PVP, with or without the addition of HA, gave a significantly larger stage 2 deposition in TSI compared to BDP alone in HFA 134a ($p \leq 0.05$, ANOVA). The stage 2 depositions from the BDPLW80K15 and BDPLW80K15HA HFA suspensions were not significantly different ($p > 0.05$, ANOVA) with approximately 40% of the emitted microparticles depositing in this part of the apparatus. The high FPF implies that both these suspensions displayed a high degree of physical stability. Whilst the particle size of both BDPLW80K15 and BDPLW80K15HA did increase significantly ($p < 0.05$, ANOVA) upon suspension within the HFA solvent, the Dv, 0.5 was still under 6 μm for the microparticle suspensions inferring a high degree of physical suspension stability (Tab. 3).

Adjusting the % hydrolysis of the PVA or changing the molecular weight of either the PVA or PVP within the BDP microparticles suspended in HFA 134a did not improve the stage 2 deposition (Tab. 3). Only BDPLW98K15 produced a significantly larger ($p \leq 0.05$, ANOVA) stage 2 disposition compared to BDP alone in HFA 134a. Thus, manipulating the physicochemical properties of the macromolecular excipients did not appear to further enhance the physical stability of the microparticle suspensions compared to the BDPLW80K15 system.

Determination of Solubility in HFA

The solubility of BDP in HFA 134a was calculated to be 29.85 ± 2.09 μg/g ($n = 3$). However, the recovery from the apparatus was calculated as $78 \pm 4\%$ ($n = 4$). Therefore, the corrected solubility of BDP in HFA 134 was 38 μg/g.

The PVP K90 or HA did not show any detectable solubility either spray-dried or in their original state (i.e. as supplied by the manufacturers) in HFA 134a. In a similar manner, PVA 70% hydrolysed, 80% hydrolysed, PVA 88 % hydrolysed (low, medium or high molecular weight) and PVA 98% hydrolysed, either spray-dried or in their original state showed no detectable solubility in HFA 134a. However, 1.26 mg/g of PVP K15 (corrected for recovery) could be dissolved in HFA 134a.

Zeta Potential Measurements

The magnitude of the zeta potential measurements within the nonpolar suspensions was shown to be dependent upon the physicochemical properties of both the solvent and the particle within the suspension. Regardless of the type of BDP microparticles suspended in the nonpolar systems, the largest electrophoretic mobilities and therefore, the larger zeta potentials were found in solvents with the higher dielectric constants. In addition, the nonpolar suspensions with the largest zeta potentials provided the most reproducible measurements. For example, BDP PVA40 microparticles suspended in methyltrifluoroacetate (MTA) (dielectric constant of ca. 11.64) displayed a well-defined phase plot, showing movement in both directions in the cell (inferred by the positive and negative gradient in Fig. 3) and the zeta potential distribution was very reproducible.

In contrast to the MTA suspensions, the zeta potential of the microparticles within PF (dielectric constant of ca. 1.5) resulted in the very poor quality phase plots and a lack of discrimination interms of zeta potential (Fig. 4). There was not a reproducible positive or negative gradient in the phase plot, which implies that there is very little movement of the particles within the applied electrical field.

The zeta potentials of the PF suspensions were consistently smaller compared the MTA microparticle suspensions. For example, the BDPLW80K15HA microparticles suspended in PF displayed a zeta potential of 18.88 ± 13.25 mV

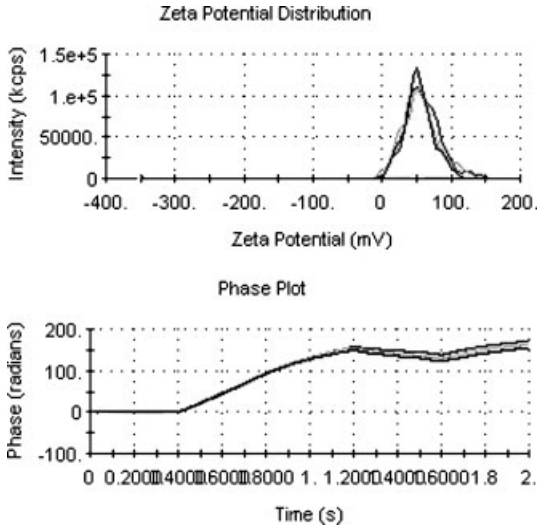

Figure 3. The zeta potential distribution and phase plot for the BDP PVA40 particulates suspended within methyltrifluoroacetate (MTA) ($n = 3$).

which, was significantly smaller ($p \leq 0.05$, ANOVA) compared to the same particles suspended in MTA at 53.60 ± 1.33 mV (Tab. 4). BDP PVA40, BDP PVA80 and BDPLW80K15 all displayed a negative zeta potential when suspended within PF although, in the case of BDP PVA 80 and BDPLW80K15 the zeta potential was smaller than the observed measurement error. When the microparticles were suspended in MTA they all displayed a very similar zeta potential of approximately + 60 mV (Tab. 4). The zeta potential of the microparticles containing HA, when suspended in MTA (BDPLW80K15HA), was significantly lower ($p \leq 0.05$, ANOVA) than the other particle suspensions where the zeta potential was not significantly different ($p > 0.05$, ANOVA) from each other.

The dielectric constant of dichloromethane (DCM) was intermediate between MTA and PF at 9.1. When DCM was used as the suspension solvent the microparticles displayed the greatest differences in terms of the zeta potential. The BDP microparticles containing 80% hydrolysed PVA displayed a zeta potential of approximately + 100 mV (Fig. 4). The addition of PVP to the PVA BDP microparticles, that is BDPLW80K15, reduced the zeta potential of the DCM suspension to approximately + 10 mV (Tab. 4). However, inclusion of HA to the BDP, PVA, PVP microparticles (BDPLW80K15HA) increased the zeta potential of the DCM suspension from + 10 mV (BDPLW80K15) to ca. + 40 mV. The zeta potential of the BDP microparticles containing 40% hydrolysed PVA

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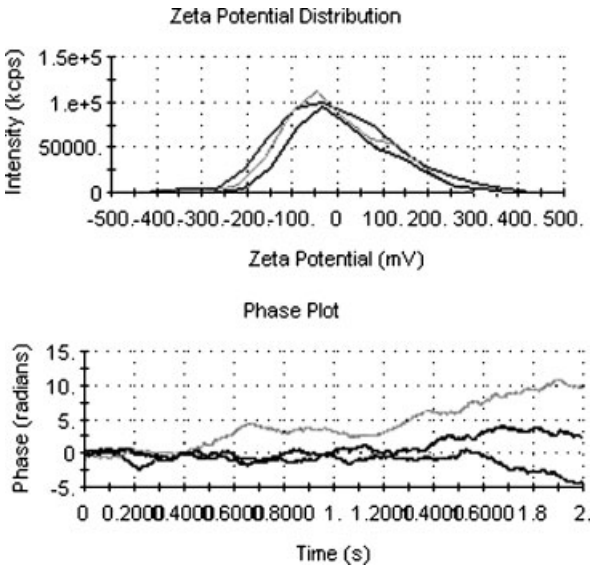

Figure 4. The zeta potential distribution and phase plot for BDPLW80K15 microparticles suspended in perfluoropentane ($n = 3$).

(BDPPVA 40) was not significantly different ($p > 0.05$, ANOVA) to the BDPLW80K15HA microparticles.

Comparing the size of the microparticles prior to and upon suspension within the nonpolar solvents provides a qualitative assessment of the suspension stability (Tab. 4). All of the microparticles when suspended within PF displayed a significantly larger particle size ($p \leq 0.05$, ANOVA) compared to their original size measured in a cyclohexane/span solvent (Tab. 4), indicating that there was significant aggregation of the particles in the system. The particles containing a combination of PVA and PVP with (BDPLW80K15HA) or without HA (BDPLW80K15) displayed the largest particle size when suspended in PF, and the size of these two microparticle suspensions was not significantly different ($p > 0.05$, ANOVA).

Only the microparticles containing 40% hydrolysed PVA (BDP PVA 40) appeared to aggregate in MTA, since these particles displayed a median particle size of >40 μm when suspended in this solvent, compared to a size of 4.42 μm prior to incorporation with the nonpolar vehicle. BDPLW80K15 displayed a particle size that was not significantly different after suspension within the cyclohexane/span mixture and MTA ($p > 0.05$, ANOVA) inferring that the latter provided a physically stable suspension of the inhalable particulates. However, both the BDP PVA 80 and the BDPLW80K15HA microparticles displayed

Table 4. The Particle Size and Zeta Potential Measurements for the Four BDP Microparticles Containing Various Polymers upon Suspension Within Methyltrifluoroacetate (MTA), Perfluoropentane (PF), Dichloromethane (DCM) Compared to the Original Particle Size of the Dry Powdered Formulations Suspended in a Cyclohexane/Span Mixture ($n = 3$, Mean ± standard Deviation)

		Particle Size			Zeta Potential		
Sample	Particle Size Cyclohexane/ Span Dv, 0.5 (μm)*	MTA Dv, 0.5 (μm)	DCM Dv, 0.5 (μm)	PF Dv, 0.5 (μm)	MTA (mV)	DCM (mV)	PF (mV)
BDP PVA 80	4.22 ± 0.16	2.92 ± 0.20	3.43 ± 0.16	20.71 ± 2.61	64.05 ± 6.93	101.11 ± 3.62	−0.35 ± 6.47
BDP PVA 40	4.42 ± 0.03	44.11 ± 1.67	2.84 ± 0.15	24.13 ± 3.07	60.14 ± 4.42	11.25 ± 1.48	−0.83 ± 6.68
BDPLW80K15	3.37 ± 0.02	3.28 ± 0.44	4.79 ± 0.23	32.01 ± 6.44	60.69 ± 3.32	29.50 ± 3.26	−11.26 ± 11.09
BDPLW80K15HA	4.02 ± 0.15	2.09 ± 0.01	5.29 ± 1.98	33.49 ± 3.05	53.60 ± 1.33	34.32 ± 2.39	18.88 ± 13.25

*Size of the original microparticles measured using a laser diffraction stirred cell method first reported in Table 3 are included here for comparison.

a significantly smaller particle size ($p \leq 0.05$, ANOVA) upon suspension within MTA compared to the original size (suspended in cyclohexane/span) thus, implying a degree of dissolution was occurring in the former nonpolar solvent.

The size of the BDPLW80K15HA microparticles was not significantly different ($p > 0.05$, ANOVA) when suspended in DCM compared the original size implying that the system was physically stable. Furthermore, BDP PVA 80 and BDPLW80K15 suspended within DCM showed very similar particle size to the raw material. However, BDP PVA 40 had a smaller particle size when suspended in DCM compared to its original size prior to suspension within the solvent again indicate some dissolution.

DISCUSSION

In this work, the physical stability of inhalable microparticles within HFA propellants was determined both directly through '*in-situ*' laser diffraction and in-directly using a TSI after microparticle aerosolisation. These two methods demonstrated good agreement throughout the study which is an indication that they could be reliably used to estimate the physical stability of the nonpolar suspension systems and is in accordance to our previous work.[16]

Of the numerous macromolecular excipients employed to try and enhance the physical stability of the BDP microparticles within HFA, a combination of PVA and PVP (depending upon the grade of the polymers) with or without HA was shown to be the most effective. These findings concur with those from previous studies.[16,17] The commercially available grades of PVA are known to vary considerably in terms of physicochemical properties. In a comprehensive review of PVA, Pritchard[18] showed that many of the physicochemical properties were determined both by molecular weight and the percentage hydrolysis of the polymer. Importantly the thermal properties, solid-state characteristics and solubility in a range of solvents are all dependent on the % hydrolysis and molecular weight of PVA. In this study whilst varying the grade of PVA was found to have very little effect on the particle size of the manufactured product, it did have significant influence on the physical stability of the pMDI suspension. The fine balance of physicochemical characteristics that are required to stabilise a suspension is illustrated by the fact that only PVA 80% and 98% hydrolysed could be used in combination with PVP K15 to enhance the stability of the BDP pMDIs using HFA 134a.

Increasing the molecular weight of PVP had a dramatic effect on the microparticle manufacturing method and the final HFA pMDI formulation. Breggren and Alderborn[19] showed that varying the PVP grade from K17 to K90, that is increasing the molecular weight of the polymer, altered the physical properties of the microparticles spray-dried from an aqueous solution. In the present study, changing the grade of PVP from K15 to K90 in the initial spray-drying suspension was found to increase the final particle size of the microparticles dramatically. This effect was reduced, by lowering the initial solid content in the suspension delivered to the spray-dryer. However, regardless of the size, microparticles containing PVP K90 aggregated when suspended in HFA 134a. The inclusion of PVP K15 was obviously crucial to the physical stabilisation of the BDP microparticles within the HFA suspensions since without it none of the formulations showed any evidence of physical stability. However, increasing the molecular weigh of PVP reduced the positive influence of the PVA/PVP combination upon suspension stability and made the manufacture of the microparticles problematic.

In order for any of the macromolecular excipients to influence the suspension stability of BDP within an nonpolar system it must modify the interactions of the micronised steroid with other particles, the solvent and the vessel in which they are contained.[20] Typically this occurs when the excipients have an affinity to the solid particulates and adsorption takes place. Adsorption involves the accumulation of molecules at an interface and is favoured by a decrease in free energy of the sorbing system ($-\Delta G$). When adsorption is occurring directly in the suspending media it may be enhanced for 'poor' solvent systems (with respect to the sorbate) as the free energy change is negative ($-\Delta G$) compared to 'good' solvents where the free energy change is positive ($+\Delta G$).[20] If a stabilising compound such as a polymer is rendered insoluble within the dispersion media it can induce flocculation.[21] This was elegantly illustrated by Napper[21] who noted that when ethanol was added to PVA and PMMA microparticles suspended in heptane stabilised with oleophilic chains, flocculation occurs (i.e. particle aggregation) at volume fractions of ethanol that correspond to the upper solubility limit of the polymer. The flocculation of particles caused by the reduction of the stabilising agents solubility was found

to be a reversible process when the stabilising agent was resolubilised.[21] Whilst the classical adsorption theory discussed by both Napper[20] and Bagchi[21] focuses on the suspending media dictating a dynamic adsorption process, the excipients used to stabilise the suspensions in the current work were not simply adsorbed from within the suspension media. Spray-drying a suspended drug with water soluble excipients forces the excipients to accumulate at the surface of the drug prior to suspension within the nonpolar vehicle thus, promoting the process of adsorption irrespective of the properties of the final suspension media. Whilst the polymer should still dominate the interactions between the particles, this could occur through numerous mechanisms. For example, if the excipients that have been spray-dried onto the particles are freely soluble within the suspension media they could potentially be released from the microparticle and any suspension stabilisation could be a dynamic process[21], that is involving continual adsorption and desorption of the excipients at the solid/liquid interface. However, if the excipients are not soluble in the suspension vehicle then desorption from the BDP interface cannot occur and the stability of the suspension will be dependent upon surface effects of the actual microparticles and is heavily dependent upon the properties of that surface.

Applying the traditional theories of nonpolar suspension stabilisation, that is disregarding the contribution of ionic interactions, the enhanced stability of HFA suspensions conferred by the combination of PVA, PVP and HA should be due to steric stabilisation. Therefore, the lack of solubility of all but one of the excipients tested within the HFA propellants was a surprising result. Only PVP K15 which, displayed limited solubility within HFA 134a, could possibly be enhancing the stability of the particles within these propellants via classical steric interactions[22] because this was the only excipient found to be soluble in the HFA propellant and thus possibly extend into the continuous phase of the system.

The characterisation of the electrostatic forces carried out in this study was limited to a series of nonpolar solvents since it was not possible to use HFA propellants within commercially manufactured zeta potential apparatus. Furthermore, BDP showed some limited solubility within the suspending solvents (data not shown) and thus, zeta potential measurements of the raw drug alone in these systems could not be accurately measured and thus, used as a control. Nevertheless, the excipient/BDP particles produced zeta potentials up to 100 mV when suspended within the series of nonpolar solvents. In aqueous solvents, a zeta potential of this magnitude is capable of stabilising suspensions. A direct correlation between zeta potential and physical stability, was difficult to determine. Although a high electrophoretic mobility was associated with two nonpolar suspensions that remained physically stable (including the BDP, PVA, PVP, HA microparticles suspended in DCM) the BDP, PVA + PVP (BDPLW80K15) microparticles demonstrated a low zeta potential in DCM but appeared physically stable. Therefore, it appeared that the zeta potential of the system may have been an important factor in promoting physical stability of some but not all of the nonpolar suspensions.

The dielectric constant of the media used did influence the zeta potential of the nonpolar suspensions. Microparticles suspended in PF, the solvent with the lowest dielectric constant, displayed the lowest zeta potentials, whereas MTA suspensions, regardless of the polymer coating, displayed a constantly large zeta potential. The zeta potential in DCM, which had a midrange dielectric constant, ranged from +100 to +10 mV. As DCM has an identical dielectric constant to HFA 134a this provides some evidence that electrostatic stabilisation may play an important role in the stabilisation of microparticles within HFA propellants. These results may have been slightly affected by the size of the particles because the zetasizer demonstrates poor accuracy when measuring particles with a particle size >10 μm (i.e. the PF suspensions).

Even in inert nonpolar systems the counterion charge in solution must exsist. One trace impurity present in HFA solvents is water. Yu et al.[23] showed the effect of water on zeta potential using a series of homologous solvents. The simple addition of 0.5% water to a nonpolar system provided a one order of magnitude rise in zeta potential. These workers also linked a decrease in the magnitude of zeta potential within suspension systems to a decrease in dielectric constant. Conflicting work by Kosmulski[24] has suggested that the role of water in nonpolar systems is 'overrated' whilst the role of trace impurities such as amines is often overlooked. However, regardless of the source and nature of the counterion, the results obtained in the present study appeared to support the observations that charge can have an important role in pharmaceutically relevant nonpolar systems. The unique aspect of the particle

suspensions used in the current study compared to those previously reported is that all of the water incorporated into the BDP/excipient microspheres is probably located at the particle-solvent interface.[25,26] Water is far less likely to associate with the hydrophobic steroid within the core of the particle than with the excipients at the surface of the microparticle. Therefore, the majority of the ionic species within this system are likely to be situated at the excipient-solvent interface, which will influence the degree of surface charge and hence the zeta potential of the microparticles. Further work is required to determine the exact location of the water in this system and the influence of such trace impurities upon the zeta potential in nonpolar suspension systems.

The perceived distance between the particles within a nonpolar suspension when a macromolecule is adsorbed to the surface is considered to be the primary reason for the negligible electrostatic interactions within such a system.[27] Through calculation of the Haymaker constants (which are related to the mean particle separation distances) it was shown that the adsorption of a polymer with a molecular weight of 10000 would result in a separation distance of 1 order of magnitude greater than would be necessary for van der Waals forces to have a significant influence.[28] However, this theory is related to a dynamic system when a freely or partially soluble stabiliser is added directly to a suspension of the particles. In the present study, the additional excipients are associated with the particles prior to suspension within the hydrophobic propellant. Therefore, little chain extension is likely to occur in the case of PVA and HA as particle separation distances would be dramatically reduced, theoretically allowing electrostatic stabilisation to exert a greater influence.

PVA and PVP exhibit a unique ability to act as stabilisers within several nonpolar systems and this may be because they function synergistically. PVA is made up of two monomers, polyvinyl alcohol (hydrophilic) and polyvinyl acetate (hydrophobic). Due to its amphiphilic nature, PVA is known to form a loop and tail conformation at a hydrophilic/hydrophobic interface. If during the formation of the suspension in the aqueous system the PVA adsorbs to the BDP via the hydrophobic moieties, that is the acetate groups, it may produce a hydrophilic alcohol coating exposed to the exterior of the particle. This external alcohol moiety in addition to trace impurities, for example water may induce the charging of the particle surface. However, once spray-dried and suspended within HFA propellants the PVA alone does not stabilise the BDP particles and the lack of solubility within the propellants suggests that it does not change its conformation or extend into the solvent. PVP K15 is not known to interact with BDP but, it does form miscible composites with PVA in the solid state (i.e. inferring an association can occur between the two polymers).[29,30] If PVP K15 forms a second layer on the BDP particle this may confer an element of steric stabilisation within the HFA propellants thereby reducing the zeta potential of the suspension as PVA is 'coated' by the PVP. The two polymers might be expected act together as an anchor and buoy in a similar manner to multiblock copolymer, the PVP providing physical stability through steric stabilisation.

The addition of HA to the PVA PVP system appeared to increase the physical suspension stability within many of the nonpolar solvents. The microparticles containing HA displayed the smallest increase in size upon suspension within the HFA propellants and the best aerosolisation properties which implies that this system was physically stable. The zeta potential measurements in the series of nonpolar solvents suggested that HA increased the physical suspension stability within these systems by manipulating the zeta potential. Thus, unlike PVP HA may be altering the suspension stability within HFA by altering the charge induced stabilisation within these solvents.

CONCLUSION

Nonpolar vehicles can provide a highly protective environment for the formulation and delivery of labile therapeutic agents but, stabilising suspensions within these solvents can be problematic. Traditional suspension stabilisation theory suggests that within solvents exhibiting a low dielectric constant, the lack of ions dampens the influence of electrostatic stabilisation. Thus, suspension stability in the presence of surface active agents should mainly be as a consequence of steric mechanisms, which require the surface active agent to exhibit a degree of solubility within the suspending solvent.

This work has shown that combination of PVA and PVP can stabilise BDP HFA suspensions possibly through steric hinderance. However, several of the nonpolar suspensions produced during the study, whilst having a relatively low dielectric constant, demonstrated significant eletrophoretic mobility. Using a highly sensitive Zetasizer Nano-

series machine, this study illustrated the ability of PVA and PVP in combination to generate a zeta potential of up to 100 mV in some nonpolar solvents. In suspension vehicles with a very low dielectric constant, for example PF, the zeta potential of the BDP/excipient microparticles could not be reproducibly measured but, when the dielectric constant was increased up to as high as 9.0 (DCM) a large electrophoretic mobility resulting in a zeta potentials of up to 60 mV could be reproducibly detected. As it was previously thought that electrostatic stabilisation was unlikely to influence suspension stability within nonpolar systems the detection of such high zeta potentials was a surprising result.

Although the technical challenges of measuring the zeta potential directly within HFA systems has not yet been solved, this work provides some evidence that both steric hindrance (PVP) and electrostatic stabilisation could be functioning to aid the stabilisation of microparticles within nonpolar systems. The high zeta potential of the particles in DCM which has an identical dielectric constant to HFA 134a indicates that whilst the electrostatic forces didn't seem to be the sole influence on the physical stability of BDP, PVA, PVP microparticles, charge might have an important role to play in pMDI stabilisation. Thus, there is an obvious requirement to develop specialised equipment to allow the direct measurement of zeta potential within high-pressured nonpolar solvents to enable the systematic development of physically stable HFA based suspensions.

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