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RESEARCH PAPER

Influence of Suspension Stabilisers on the Delivery of Protein-Loaded Porous Poly (DL-Lactide-co-Glycolide) (PLGA) Microparticles via Pressurised Metered Dose Inhaler (pMDI)

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ABSTRACT

Purpose This work investigates the feasibility of delivering large (≈ 25 μm) porous poly (lactide-co-glycolide) (PLGA) microparticles containing a model protein via pressurised metered dose inhaler (pMDI).

Methods Porous PLGA microparticles were prepared by modified double emulsion method as pMDI suspension based systems containing suspension stabilisers in 1,1,1,2,3,3,3-heptafluoropropane (HFA 227). Physical suspension stability was assessed by visual and optical suspension techniques. Aerosolisation characteristics were investigated using aerosol particle sizing, dose delivery through the valve (DTV) and shot weight.

Results An optimum concentration of suspensions stabiliser was required to achieve physical pMDI suspension stability; values of; 0.0075%w/w PVP K30 or 0.075%w/w PEG 300 were required. Formulations that exhibited good physical stability also showed optimum aerosolisation characteristics. When employing 0.0075% PVP K30 DTV at the start and end of can life was 98.11(±10.01) % and 75.06 (±7.01) % respectively *verses* values of 37.39 (±11.12) % and 5.57 (±1.72) % without the inclusion of PVP K30.

Conclusion Porous PLGA microparticles show potential as macromolecule/protein carrier and also to target lower regions of the lungs when prepared as pMDI suspension formulations in HFA 227 using suspension stabilisers to achieve consistent dose delivery through the life of the pMDI, however, inter-relationship between the device and the formulation need to be considered to achieve suitable respiratory delivery.

KEYWORDS Heptaflouropropane (HFA 227) and suspension stabilisers · Metered dose inhalers · Porous microparticles · Suspension

ABBREVIATIONS

APS	Aerodynamic particle sizer
BSA	Bovine Serum Albumin
DCM	Dichloromethane
DPIs	Dry Powder Inhalers
DTV	Dose Delivered Through the Valve
EE	Encapsulation Efficiency
FDA	Food and Drug Administration
FEP	Fluorinated ethylene propylene
HFA	hydrofluoroalkane
HFA 227	1,1,1,2,3,3,3-heptafluoropropane
MMAD	mass median aerodynamic diameter
OSCAR	optical suspension characterisation
PBS	Phosphate buffered saline (PBS)
PEG 300	polyethylene glycol 300
PET	Polyethylene terephthalate
PLGA	poly (lactide-co-glycolide)
pMDI	Pressurised Metered Dose Inhaler
PVA	polyvinyl alcohol
PVP K30	polyvinylpyridone K30
SDS	sodium dodecyl sulphate
SEM	Scanning Electron Microscopy
USP	United States Pharmacopeia
VMD	volume mean diameter

INTRODUCTION

The pressurised metered dose inhaler (pMDI) has been available for 60 years and is the most popular dosage form for the delivery of locally acting drugs to the lungs. They comprise of either dissolved or micronized drug suspended in a liquefied

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hydrofluoroalkane (HFA) propellant packaged in an aluminium can fitted with a metering valve. The propellant provides the driving force to expel the drug as aerosolised droplets to the lower regions of the lungs. In order to exert a therapeutic effect the mass median aerodynamic diameter (MMAD) should be in the region of0.5-5 μm (1). The general lack of solubility of compounds in HFAs has enabled pMDIs to be considered for the delivery of polymeric microspheres for the controlled delivery of both locally and systemically acting drugs (2). Over recent decades a variety of formulation approaches have been adopted in particular porous polymeric microspheres and the use of nanoparticle technology (3–6). Nanoparticles are particularly suited for pulmonary drug delivery as their size allows access to the peripheral airways (7) depending on the mode of administration selected, but also ensures that they escape phagocytic and mucocilairy clearance mechanisms (8). In contrast porous polymeric microparticles has been shown to be suited to deep lung delivery as the relatively large porous particles can deposit in lower regions of the lungs due their relatively small MMAD, and therefore can potentially target lower regions of the lung (9–11). In addition, large porous particles deposited in the pulmonary region may escape clearance mechanisms therefore permit drug release for longer periods of time and more efficiently (12,13). The delivery of porous microparticles has been researched extensively with respect to Dry Powder Inhalers (DPIs) however to date there is limited research with respect to pMDIs this in part due to conformational stability of the macromolecules and sedimentation of particles in the HFA propellants. Conformational stability has been observed in HFAs for selected macromolecules (14,15). Formulation strategies that can stabilise macromolecules and suspension stabilisers may provide a use of pMDI for delivery proteins and peptides.

Polymeric technologies has the potential for stabilisation of macromolecules and the key issue being uniform dose delivery from the pMDI is required throughout the life of the device. The challenges include the formulation of a suspension that doesn't aggregate and remains suspended for the time to deliver the dose and the delivery of an accurate dose of aerosolised material throughout the life of the product. To address suspension stability and aid valve functionality suspensions stabilisers are often included in pMDIs to lubricate the valve mechanism (16,17) and provide suspension stabilisation (18–21). Traditionally oleic acid and sorbitan mono-oleate (Span 85) were used, however they are insoluble in HFAs (1), therefore alternatives which exhibit solubility in HFAs and have Food and Drug Administration (FDA) approval including polyvinylpyridone (PVP K30) and polyethylene glycol (PEG 600)have been identified and the suspensions stabilisers are thus to be employed in this study (22).

The objective of this study was to prepare and characterise pMDI suspension formulations containing porous PLGA microparticles. Consistent Dose delivery through the valve (DTV) and thus delivery of active to the lungs is a considerable challenge, therefore the study aims to explore PVK K30 and PEG 300 over a concentration range to achieve suspension stability, valve functionality and aerosolisation characteristics (23). The study describes the use of two FDA approved propellant and suspension stabiliser blends to potentially deliver porous microparticles for pulmonary administration via pMDIs.

MATERIALS AND METHODS

Materials

PLGA 50:50 (molecular weight, M_w 45–75,000 kDa), Bovine Serum Albumin (BSA), Oleic acid, Tween 80, PEG 300 and polyvinyl alcohol (PVA) (M_w 13–23,000 kDa, 98% hydrolysed), were purchased from Sigma Aldrich, UK. Dichloromethane (DCM) was obtained from Fisher Scientific, UK. For pMDI studies Polyethylene terephthalate (PET) vials (21 ml) (precise plastics, London, UK), 300 ml aluminium cans, continuous valves (1 in.), non-metering valves, 12 ml Fluorinated ethylene propylene (FEP) lacquered aluminium cans and 50 μl spraymiser metered valves were supplied by 3 M Healthcare Ltd, UK. HFA 227 and HFA 134a was supplied by Solvay Fluor and DuPont respectively.

Methods

Preparation of Porous PLGA Microparticles

Microparticles were prepared by double emulsion solvent evaporation technique modified from Bodmeier and McGinity, 1987 (24). The oil phase (250 mg PLGA in 2 ml DCM) was homogenised (Silverson, UK) at 10,000 RPM for 4 min with 2.5%w/w PVA internal aqueous phase containing 6 mg BSA to produce the primary emulsion. This was added drop-wise under constant agitation to 1.5%w/w PVA (75 ml), homogenised for 6 min at 10,000 RPM and left for 3 h to allow DCM evaporation. The resultant porous microspheres were recovered by centrifugation (Beckman-Coulter Instruments Inc., UK) at 16,000 RPM (20, 413 g) for 40 min and were then freeze-dried using a VirTis AdVantage 2.0 BenchTop freeze dryer (SP Industries, Ipswich, UK). for 48 h prior to analysis.

Determination of Protein Loading in Porous Microparticles

Five mg samples of microparticles were dissolved in 500 μl DCM at 37°C. Once dissolved 500 μl of 5mMol sodium dodecyl sulphate (SDS) in phosphate buffered saline (PBS) was added and centrifuged at 15,000 RPM for 15 min. The

upper aqueous layer was recovered and the process repeated twice. The harvested solution was assayed using a BCA assay™ (Pierce, Rockford, USA) for the presence of protein. Encapsulation efficiency (EE) is expressed as the ratio of the actual and theoretical loading.

Size and Porosity of Porous Microparticles

Particle size analysis was determined using a Malvern Mastersizer X (Malvern Instruments) fitted with a 100 mm receiver lens, and 15 ml magnetically stirred cell. A representative sample of microspheres was dispersed in 0.01%w/v Tween 20. The data generated are presented as 10^{th} (D0.1), 50^{th} (D0.5, volume mean diameter; VMD) and 90^{th} (D0.9) percentile of the cumulative particle under size frequency distribution.

Density determination was completed using helium pycnometry (AccuPyc 1330, Micrometrics, UK) to determine particle density. This density determination was adopted as it takes into account the volume due to interparticle pores. Briefly the apparatus was calibrated using polished steel balls following which the sample cell was filled approximately 2/3 full with porous microparticles. Automated analysis was started following on-screen instructions. Five measurement sequences were performed for each porous microparticle sample to ensure the sample volume agrees within 0.5%. The density was automatically calculated for each sample subject to analysis by determining the volume occupied by a known mass of solid that is equivalent to the volume of gas displaced by the powder. A total of $n=3$ porous microparticle samples were subject to analysis.

Scanning Electron Microscopy (SEM)

Surface morphology of microparticles was assessed by SEM using a Cambridge Instrument Stereoscan 90B. Samples were prepared by placing a representative sample onto an aluminium specimen stub, and were sputter coated with gold prior to imaging.

Preparation of pMDIs

Prior to the preparation of pMDIs series of cans were prepared containing the HFA227/PEG 300 or PVP K30 combination. PVP K30 was investigated over a concentration range of 0%w/w to 0.1%w/w with 0%w/w to 0.5%w/w PEG 300 adopted. Briefly the appropriate quantity of PEG 300/PVP K30 was added to a 300 mL aluminium can and a one-inch continuous valve was crimped in place (Pamasol 555G, laboratory scale crimper, Switzerland). The can was cooled to −40°C using a Cryogen bath (Neslab-Cryotrol, CB80, Neslab instruments, UK) following which approximately 300 g of propellant (HFA 227) was pressure-filled into the can via the valve. The cans were left to equilibrate for 24 h prior to use.

Uniform dispersions of porous microsphere suspensions in HFA227/PEG 300/PVP K30 were prepared in transparent PET vials to assess physical suspension stability and 12 ml FEP cans fitted with a 50 µl spraymiser metered valve for aerosolisation studies. PET vials fitted with continuous valves were prepared adopting a transfer fill technique. FEP cans were initially prepared with a continuous valve prior to cold transfer and subsequent fitting of metering valve.

A 28 mg aliquot of porous microparticles was added to a PET vial (or FEP can) and a continuous valve was crimped in place using a manual pMDI filler. The vial (or can) was cooled for 10 min using a Cryogen bath following which 19.726 g of propellant blend manually filled into the vial (or can) via the valve. The filled PET vial was left to equilibrate to room temperature, valve up prior to analysis for 24 h. The cans were subject to cold transfer to fit with a 50 µl metering valve. The cans fitted with continuous valves were cooled to −40°C for 30 min following which the cans were opened, and the contents transferred to a FEP can. A 50 µl metering valve was crimped in place.

Analysis of Porous Microsphere Suspension Stability

Visual assessment was employed to assess suitability of suspensions with respect to sedimentation height and packing, aggregation, flocculation and particle redispersibility following shaking. PET vials containing porous microsphere suspensions were hand shaken for 30 s after which photographic images were taken at 0, 2, 5 and 10 min time points. Further analysis was conducted using an optical analyser: optical suspension characterisation (OSCAR). OSCAR was performed over an analysis period of 2 min to assess physical suspension stability. The photo-detectors positioned 2 mm above and below the suspension surface and adopting the dilute concentration setting (19). Each sample was vortex mixed for 5 s and left to settle for 7 s prior to analysis. Voltage signals from the photo-detectors were converted to digital values and processed to produce transmission voltage (mV) *verses* time (seconds) graphs.

Dose Delivered Through the Valve and Weight Per Actuation

The cans were primed and weighed before measurement of weight per actuation. Twenty five individual shots were fired and the weight recorded after each shot to represent the weight per actuation (shot weight) at the start of the can. Shots were fired to waste following an additional individual shot weights were recorded (middle of the can) and the procedure was repeated to give 25 shots at the end of the can life.

The average shot weight of was determined at the start, middle and end of the can in groups of $n=5$ shots of which a single group was selected; shot weights 10–15 refer to the start of the can. Shots 73–78 refer to the middle of the can shot weight and 136–141 represent the shot weight at the end of the can. The samples were analysed to determine the dose delivered through the valve (DTV). Micro BCA assay was used to deterimine the amount of BSA delivered.. The DTV was calculated based on the concentration of microparticles fired from the can, adjusted based the encapsulation efficiency determined on a representative sample. Statistical analysis using ANOVA of variance was adopted to compare formulations.

Determination of Aerosol Parameters using Aerodynamic Particle Sizer (APS)

Aerodynamic particle sizer (APS) (Model 3320, TSI instruments, St Paul, MN) fitted with a United States Pharmacopeia (USP) throat and 20 cm throat extension was used to determine MMAD and aerosol deposition. Analysis was performed with a laser power at minimum 75% and a flow rate at 28.3 (±0.1) litres/minute adopting a 10-second analysis period in correlated mode. Selected pMDI formulations that exhibited physical stability were analysed using this method. The can was shaken for 5 s, primed, and the first 5 actuations fired to waste, following which the pMDI was actuated in to APS for a total of 5 shots with a 30 s interval between actuations. The results were processed to determine MMAD. The same process was employed for the determination of aerosol deposition with an additional 20 actuations through the APS. The filter, plates, throat were dismantled, rinsed with 20 ml DCM. The BSA was extracted via centrifugation at 12,000 RPM using 5 ml aliquot of d.d water following which the proportion of BSA was determined using the Micro BSA assay as previously described and the equation applied (Fig. 1):

$$\%\mathrm{FPF} = \frac{\mu\mathrm{g\ microparticles} < 4.71\mu\mathrm{m\ diameter}}{\mathrm{Total}\ \mu\mathrm{g\ microparticles\ sprayed}}$$

RESULTS AND DISCUSSION

Characterisation of Porous PLGA Microparticles

Porous microparticles were successfully prepared (Fig. 1) with a VMD of 20.84 (±9.00) µm and MMAD of 4.80 (±1.12) µm. The density of the porous microparticles was determined as 1.384 (±0.105) g/cm^3 at 20°C. An encapsulation efficiency of 42.07 (±8.89) % and BSA loading of 1.00 (±0.19) % was calculated. Particle size analysis show the MMAD is smaller than that of the physical particle size of the respective porous microparticles.

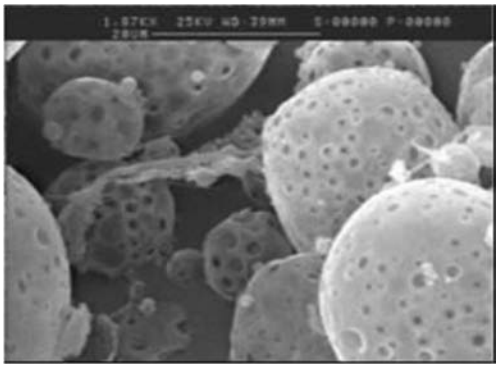

Fig. 1 SEM illustrating porous morphology of microsphere (scale bar dimension, 20 μm).

Visual Analysis of Porous Microsphere Suspension Stability

The physical stability of PLGA microsphere suspensions in HFA 227 had greater physical stability than HFA 134a (data not shown), which is in agreement with previous studies employing chitosan (3). This may be attributed to the greater density of HFA 227 being closer to that of the microparticles compared to HFA 134a as a result formulations prepared in HFA 134a were not continued. All formulations prepared with HFA 227 exhibited creaming on standing due to the density of HFA 227 being greater than that of the porous microspheres, 1.409 g/cm^3 verses 1.384 (±0.105) g/cm^3 at 20°C respectively.

Both visual and OSCAR assessment illustrated the stabilising effect of PVP K30 and PEG 300 on porous PLGA microparticle suspension formulations in HFA 227, with an optimum concentration required. Suspensions without stabilisers showed a thin creamed layer, 2.0 mm in thickness and a translucent supernatant following shaking. The creamed layer started to form 30 s following the cessation of shaking, with a complete layer formed after 60 s (Fig. 2a). This is in agreement with other studies which found poor physical suspension characteristics (4).

Following the inclusion of 0.0001 to 0.01%w/w PVP K30 improved suspension stability was observed; a loosely packed creamed layer which was slower to form in conjunction with a more opaque supernatant was observed on standing (Fig. 2b). However, above 0.025%w/w PVP K30 a reduction in supernatant opacity, depth of sedimentation layer and increase in formation of creamed layer was observed, indicative of physical instability. The aforementioned effects were more pronounced as PVP K30 concentration increased. From visual assessment the optimum PVP K30 concentration was 0.001-0.0075%w/w (Fig. 2).

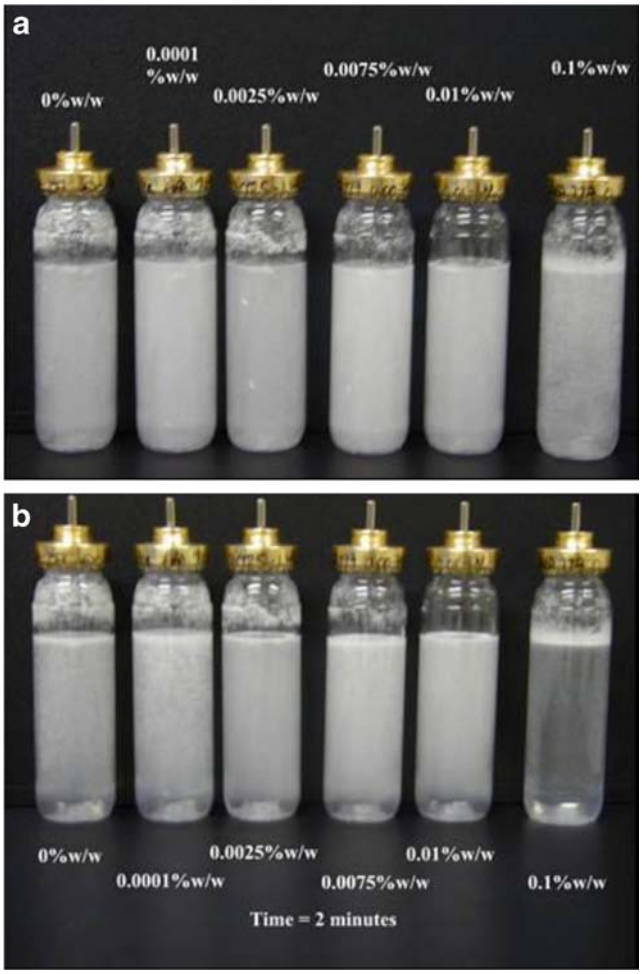

Fig. 2 Visual Analysis of Porous Microsphere Suspensions in HFA 227 containing PVP K30 (**a**) Immediately following the cessation of shaking and (**b**) Following 2-minutes standing.

A slow rate of creaming in conjunction with an opaque supernatant and ease of re-suspension is a perquisite to for acceptable dose reproducibility from a pMDI. A good stabilisation excipient for pMDI should be well solvated and block particle-particle interaction (22). The stabilisation effect following the inclusion of PVP K30 or PEG 300 can be attributed steric stabilisation of suspensions in accordance with the DLVO theory of suspensions in low dielectric solvents (25–27). Below the optimum PVP K30 concentration, 0.001 to 0.005%w/w the barrier may not be sufficient to keep the particles at a large enough distance apart resulting in the creaming rate and the opacity of the supernatant is lower than that of formulations adopting the optimum PVP K30 concentration (0075%w/w). Above this concentration the steric barrier produced could be too large. The increase in size of the steric barrier results in the particles kept too far apart; hence a more flocculated system was exhibited.

The same effect was exhibited following the inclusion of PEG 300 in HFA 227 however the concentration of PEG 300 required was greater than PVP K30 (data not shown). When employing PEG 300 over a concentration range of 0–0.5%w/w suspensions were also produced, with an optimum concentration required to achieve suspension stability. Following the inclusion of PEG 300 up to 0.075%w/w the rate of creaming was reduced with a loosely packed layer that was easy to redisperse on shaking. Above 0.075%w/w PEG 300 increased flocculation was observed, characterised by the presence of larger more loosely packed particles. In addition an increased rate of creamed layer formation and supernatant clarity was observed. The differences in suspension stability may be attributed to the molecular weight and the long chain structure of PVP K30. In addition PVP K30 is able to hydrogen bond at the surface of the suspension solid thus achieving a large degree of anchoring at the surface (28). The results are in agreement with Wright, 1994 (29) that suggest there is some hydrogen bonding between PVP K30 and HFA 227 that may help PVP K30 chains favour extended confirmations in solution.

Optical Analysis of Porous Microsphere Suspension Stability

The experimental data obtained using the OSCAR system are semi-quantitative indication of suspension behaviour with respect to time. A formulation is deemed stable if a low transmission value is observed over a 2 min analysis period. Visual and OSCAR assessment correlates and suggest an optimum value of 0.0075%w/w PVP K30 to achieve suspension stability. This is based on a comparison of the transmission values obtained when employing 0.0075%m/m PVP K30 compared to other values across the range 0.001-0.0075%w/w PVP K30. The detrimental effect of 0.1%w/w PVP K30 is also observed compared to the excipient free formulation (Fig. 3). The optimum concentration range for suspensions containing PEG 300 was further characterised from 0.001 to 0.075%w/w following visual analysis to 0.075%w/w PEG 300 (Fig. 3b). Visual and OSCAR results correlate with each other and highlight the stabilising effect following the inclusion of surfactants. The inclusion of 0.5%w/w PEG 300 in the formulation did not result in the degree of flocculation observed by the formulations employing PVP K30, and is supported by the low transmission values obtained when 0.5%w/w PEG 300 was employed (Fig. 3b). The data also suggest the optimum concentration range when adopting PEG 300 is greater than that of PVP K30.

Effect of Suspension Stabiliser Concentration and Type on DTV

Based on Visual and OSCAR analysis limited formulations were subject to aerosolisation characteristics; low, optimum and high surfactant concentrations were selected (Table I).

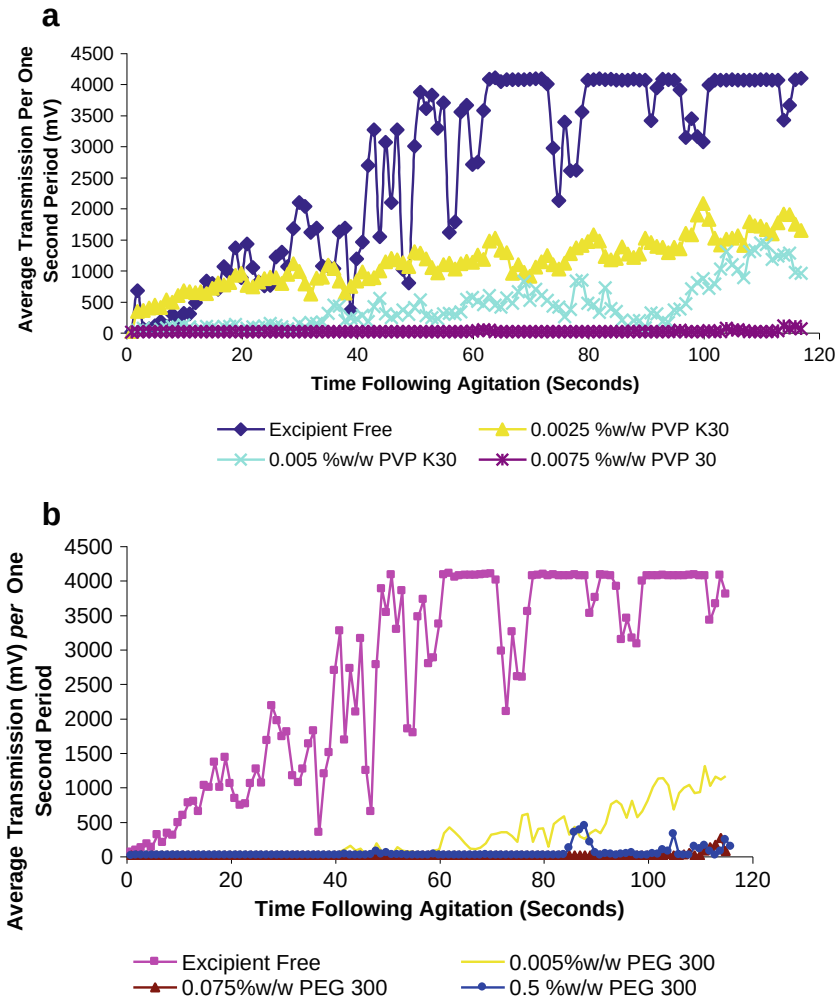

Fig. 3 Average transmission *per* 1 s interval *verses* analysis time for porous microsphere suspensions employing (**a**) PVP K30 in HFA 227 and (**b**) PEG 300 in HFA 227 ($n=3$).

The inclusion of stabiliser resulted in more consistent shot weight throughout the life of the can for HFA 227 formulations exhibiting the greatest suspension stability (Table I). Weight *per* actuation was used to assess valve performance with respect to the valve lubrication function of excipients throughout the life of the can, with a consistent shot weight throughout the life of the can indicative of consistent dose delivery through the valve (DTV). A more consistent shot weight was obtained when incorporating suspensions stabilisers at the optimum physical stability concentration; 0.075%w/w PEG 300 and 0.075%w/w PVP K30 respectively. However, more variable shot weight values were obtained when employing 0.0075%w/w PVP K30 suggesting PEG 300 may be a more superior valve lubricant than PVP K30.

In line with shot weight data, generally higher and more consistent DTV was observed following the inclusion of surfactants in the suspension (Table II). Without the inclusion of surfactants, suspensions exhibited low DTV values that decreased throughout the life of the can, from 37.39 (±11.12)% at start to 5.57 (±1.72)% at the end suggestive of fast creaming rate reducing the dose in the can due to the inverted orientation of the valve (30). A proportion of the observed decline may also be attributed to valve functionality, illustrated by the decline over time (Table II).

The inclusion of a low concentration of suspension stabilisers (0.005%w/w PEG 300 and 0.001%w/w) resulted in increased DTV however, the type of stabiliser influenced the DTV throughout the life of the can. When employing 0.005%w/w PEG 300, 42.60 (±6.68) % was delivered through the valve at the start compared to 86.89 (±7.39) % when employing 0.001%w/w PVP K30. The type of stabiliser is significant with respect to DTV following ANOVA of variance ($p<0.05$). However, when employing low surfactant concentrations DTV was not maintained throughout the life of the can (Table II). A decline to 5.49 (±2.77) % was observed at the end of can life for 0.005%w/w PEG 300 suspension. The reduction in DTV attributed to poor physical stability is supported by previous studies (19,30).

Table 1 Effect of Stabiliser Type and Concentration on Shot Weight

Suspension stabiliser type & concentration (%w/w)	Weight of shot fired from the can (mg) (±sd $n=5$)		
	Start	Middle	End
0	76.76 (±10.19)	76.08(±3.82)	77.30(±4.43)
0.005 PEG 300	77.56 (±7.06)	76.46 (±5.92)	74.76 (±3.70)
0.075 PEG 300	78.12 (±1.81)	76.16 (±1.84)	74.46 (±1.69)
0.5 PEG 300	78.28 (±2.20)	76.78 (±2.41)	74.98 (±1.63)
0.001 PVP K30	76.68 (±9.52)	74.66 (±6.09)	74.56 (±7.89)
0.0075 PVP K30	77.68 (±2.85)	76.78 (±2.41)	75.72 (±2.99)
0.05 PVP K30	82.78 (±5.10)	74.84 (±2.64)	68.30 (±14.28)

Porous microparticle suspensions that exhibited optimum physical stability also exhibited high and consistent DTV. When employing 0.075% PEG 63.86 (±1.72) % of porous microparticles were delivered at the start of can life compared to 56.84 (±19.93) % at the end of can life. Similar results were obtained for formulation containing 0.0075% PVP K30, a DTV of 98.11(±10.01) % was obtained at the start compared to 85.92 (±3.62) % at the middle of can life and a value of 75.06 (±7.01) % at the end. The change in DTV are statistically significant ANOVA of variance ($p<0.005$) between the start and end of the can when employing PVP K30 but no statistical difference was observed (when employing PEG 300 at the optimum concentration.

Higher DTV values were achieved when employing PVP K30 compared to PEG 300. This may be due to the long term stabilising power of PVP K30 compared to the PEG 300. The variation in the stabilising power of PEG compared with PVP is supported by the findings of Ashayer *et. al.*, 2004 when comparing the stabilising power between particles and the aluminium surfaces of the can (31). The results confirm that physical stability is a prerequisite to ensuring consistent DTV is achieved throughout the life of the can and thus regulatory compliance. However, optical assessment techniques alone may not be sufficiently selective and highlights the complexity of the development of successful pMDIs.

Effect of Suspension Stabiliser Concentration and Type on Aerosolisation Characteristics of pMDI

There appears to be no relationship between stabiliser concentration and the FPF of porous microparticles delivered via pMDI (Fig. 4). A reduction in throat deposition with increased actuation deposition was exhibited following the inclusion of stabilisers with the exception of following the inclusion of 0.000 1%w/w in which there was no overall reduction in throat deposition (Fig. 4). Following the inclusion of 0.005%w/w PEG 300, throatdeposition was reduced, with 21.14 (±2.63) % of porous microparticles detected but an increased actuator deposition, 17.74 (±3.64) %. A similar effect was shown following the inclusion of 0.001%w/w PVP K30, throat and actuator deposition values of 43.87 (±5.96) % and 13.59 (±3.06) % were obtained (Fig. 4b). The reduction in throat deposition may be attributed to a reduction in the percentage of porous microsphere particles successfully entering the APS instrument due to an increase in actuator deposition. High actuator deposition may be due to the inclusion of non-volatile excipients reducing the rate of propellant evaporation. The slow evaporation rate will produce larger droplets that may fail to pass through the actuator, resulting in the droplets depositing in the actuator, thus the increased actuator deposition. Alternatively the addition of a high stabiliser concentration may lead to high cohesiveness of porous

Table 2 Effect of Stabiliser Type and Concentration on Dose Delivered Through the Valve

Suspension stabiliser (%w/w)	DTV (%) (±sd $n=3$)			
	Start	Middle	End	MMAD (μm) (±sd $n=3$)
0	37.39 (±11.12)	32.12 (±8.81)	5.57 (±1.72)	4.81 (±1.12)
0.005 PEG 300	42.60 (±6.68)	56.76 (±11.88)	5.49 (±2.77)	4.93 (±2.05)
0.075 PEG 300	63.86 (±1.72)	71.40 (±4.94)	56.84 (±19.93)	5.31 (±1.06)
0.5 PEG 300	71.97 (±7.79)	54.82 (±8.77)	47.75 (±4.92)	6.22 (±2.01)
0.001 PVP K30	86.89 (±7.39)	32.46 (±9.11)	27.49 (±4.05)	4.73 (±1.35)
0.0075 PVP K30	98.11 (±10.01)	85.92 (±3.62)	75.06 (±7.01)	5.70 (±2.36)
0.05 PVP K30	58.55 (±15.93)	42.03 (±13.96)	41.14 (±18.52)	6.01 (±3.12)

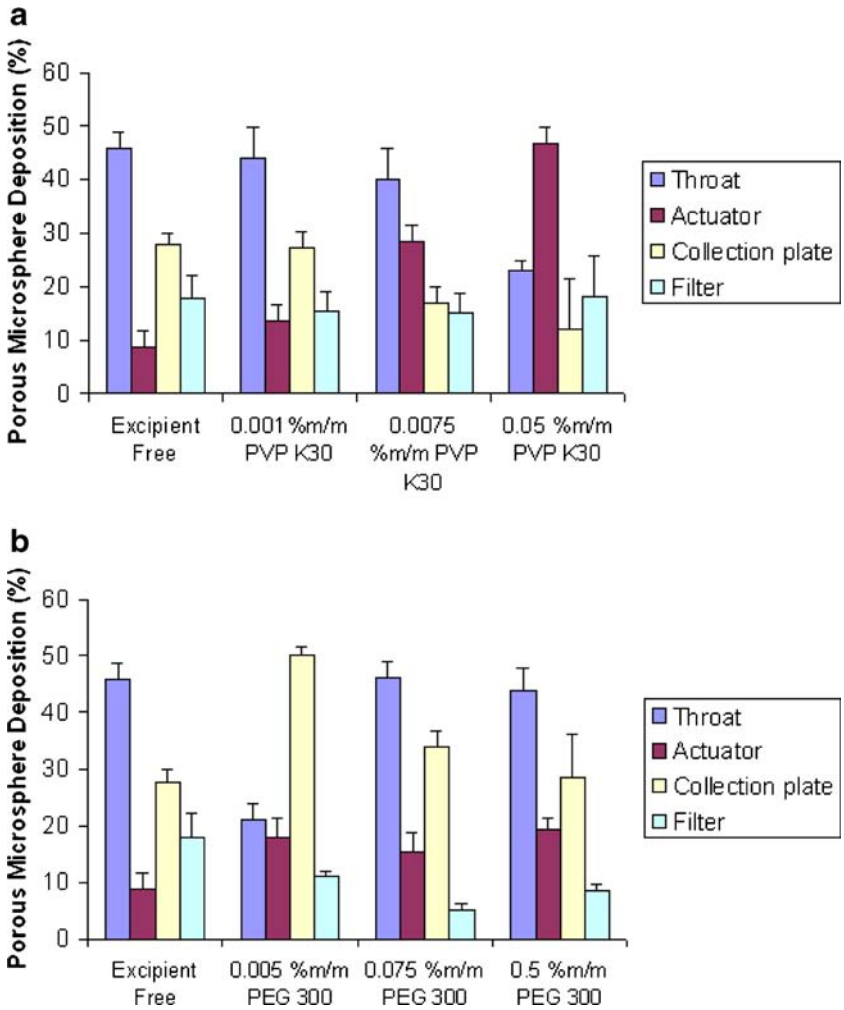

Fig. 4 Influence of suspension stabiliser type and concentration of the Deposition pattern of porous microparticle pMDI suspensions (**a**) PVP K30 concentration and (**b**) PEG 300 concentration. Data are mean (±s.d. $n=3$).

microspheres, which are not separated when the propellant evaporates therefore, resulting in the production of larger particle sizes (32).

The slow rate of propellant evaporation is supported by an increase in detected collection plate deposition following the inclusion of PEG 300, although no direct relationship was exhibited. Without the inclusion of PEG 300, 27.69 (±2.19) % of porous microparticles were deposited on the collection plate, compared to 33.77 (±4.78) % when employing 0.075%w/w PEG 300.

As expected with an increase in collection plate and actuator deposition an increase in MMAD observed. Without the inclusion of suspension stabilisers a MMAD of 4.81 (±1.12) μm was obtained with an increase to 5.31 (±1.06) μm and to 6.22 (±2.01) μm as PEG 300 concentration increased from 0.075%w/w to 0.05%w/w PEG 300. However, the increase in MMAD resulted in a reduction in FPF with the inclusion of suspension stabilisers. The reduction in FPF can be attributed to the production of larger slower evaporating droplets due to the inclusion of non-volatile components. It has been well documented that a slow evaporation rate will result in an increase in particle size of the emitted aerosol (17). The above results are in agreement with the findings of Brambilla, *et. al.*, 1999 that illustrated the addition of non-volatile excipients including PEG depressed the fine particle dose emitted from pMDIs and is a concentration dependant effect (33). Without the inclusion of suspension stabilisers a FPF of 17.89 (±4.16) % was obtained, reducing to 4.91 (±1.10) % following the inclusion of 0.075% PEG 300 and 14.92 (±0.36) % following the inclusion of 0.0075%w/w PVP. However, the change in FPF did not appear to be influenced by the concentration of suspension stabiliser (Fig. 4) and is supportive of previous studies conducted by Gupta, *et. al.*, 1990 and Kulkarni, *et. al.*, 1991 who showed suspension stabiliser type did not discernibly affect the FPF of microparticles (2,34). Although the pMDIs were successful in delivering porous microparticles within the respirable range, the values are low as commercially available pMDI products deliver approx 30% of total emitted dose to the lungs (35,36). This may be attributed to the relatively large MMAD of the particles used in the study when formulated as suspensions that is above the particle size range defined for both local and systemic drug delivery (0.5- 5 μm). As a result the porous microparticles employed throughout this study have limited

applications for pulmonary drug delivery when employing the current microsphere formulation parameters. They do however demonstrate the potential for large porous microparticles composing of PLGA to target the lower regions of the lungs.

CONCLUSION

The study has successfully demonstrated a formulation approach to deliver porous PLGA microparticles to the lungs via pMDIs when employing suspensions stabilisers. Without the inclusion of suspension stabilisers suspensions were produced that were physically unstable and showed inconsistent shot weight and DTV throughout the life of the can. Improved physical stability was observed following the inclusion of an optimum concentration of suspension stabiliser; 0.075%w/w PEG 300 and 0.0075%w/w PVP K30 characterised by slow rate of creaming, and ease of dispersion on shaking. Suspensions that exhibited greatest physical stability also achieved the most consistent dose DTV throughout the life of the can thus emphasising the physical stability is a prerequisite to achieving consistent dose delivery and thus regulatory compliance. Although improved physical stability was observed with the inclusion of suspension stabilisers a reduction in FPF was observed due steric stabilisation mechanism of action coating the particles, thus increasing the MMAD. This study shows that although particle engineering provides an opportunity to produce porous particles for antigen delivery within the respirable range it highlights the importance of valve lubrication and the inter-relationship between device and formulation.

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