

# *Spray dried progesterone formulations for carrier free dry powder inhalation*

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## Research paper

# Spray dried progesterone formulations for carrier free dry powder inhalation

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## ABSTRACT

Low oral absorption and extensive first pass metabolism of progesterone is reported for many oral formulations which warrants investigation into other routes of administration. It is the aim of this study to investigate the generation of inhaled formulations of progesterone through a spray drying approach with a focus on how spray drying impacts the physicochemical properties of progesterone. Formulations of progesterone with L-leucine and hydroxypropyl methylcellulose acetate succinate (HPMCAS) are reported to this aim. X-ray diffraction, spectroscopy and thermal analysis were used to characterise these formulations and confirmed that progesterone crystallises as the Form II polymorph during spray drying regardless of the solvent used. The resultant formulations showed higher aqueous solubility than progesterone Form I starting material and the addition of HPMCAS was shown to temporarily enable a supersaturated state. Thermal analysis was used to show that the Form II polymorph was sensitive to transformation to Form I during heating. The addition of L-leucine to the formulations reduced the temperature for the polymorphic transformation by  $\sim 10^\circ\text{C}$ . However, when HPMCAS was added to the formulation, the Form II polymorph was prevented from transforming to the Form I polymorph.

Cascade impaction was used to determine the aerosol performance of the spray dried powders and showed promising lung deposition profiles (mass median aerodynamic diameter  $5\ \mu\text{m}$ ) with significant variation depending on the organic solvent used and the ratio of organic to aqueous phase in the feedstock. However, further optimisation of formulations was required to direct more progesterone into the alveolar regions. The addition of HPMCAS was seen to increase the alveolar deposition and therefore formed a formulation with a lower fine particle fraction and mass median aerodynamic diameter. The most suitable formulation for inhalation was formed from a 50:50 acetone:water mixture and showed an ED, PPF and FPD of 81.7%, 44.5% and 7.3 mg respectively.

Therefore, HPMCAS is suggested as a suitable excipient to increase solubility, prevent polymorphic transformation and improve inhalation properties of spray dried progesterone formulations.

This study highlights the use of spray drying to form inhalable progesterone powders with higher solubility which may broaden the application of this medicine.

## 1. Introduction

Hormone replacement therapy (HRT) is required for many women suffering from obstetric and gynaecological conditions. Progestogens, such as progesterone (PROG) are a major component of these therapies due to their high efficacy and reduced side-effect profile compared to other hormones. Other therapies use oestrogens which are associated with many side effects including an increase in breast cancer rate [1,2].

Currently, formulations of PROG are available for oral, vaginal, or transdermal routes of drug administration, with the oral route preferred. These oral formulations contain micronised PROG in the form of a soft gel capsule to enhance the poor absorption from the GI tract by improving water solubility [3]. Following oral administration, PROG is extensively metabolised in the digestive tract, lumen wall and liver [4]. This limits the overall bioavailability and makes multiple dosing throughout the day necessary [5]. In addition, by-products of this metabolism are pharmacologically active and associated with side

effects [4].

As such, alternative routes of administration are being explored, for example delivery of PROG through the pulmonary route [6]. The lungs have great potential for systemic drug delivery due to high levels of vascularisation, a very thin diffusion pathway and minimal pH or enzymatic activity [7,8]. Dry powder inhalers (DPI) are a key delivery system for the inhaled route, but they largely rely on the use of a coarse carrier, such as lactose, to ensure good aerosolisation and lung deposition [9,10]. Limitations associated with the use of coarse carrier use have initiated a shift in technology towards carrier-free formulations generated through technologies such as spray drying [11,12]. At the time of writing, no previous research with respect to developing PROG for inhalation using a carrier free spray drying method exists.

The addition of small molecule and polymer excipients can increase stability and modify solution properties as well as the aerosolisation performance of inhaled powders [13–15]. Leucine (LEU) is one such small molecule which significantly increases aerosol performance,

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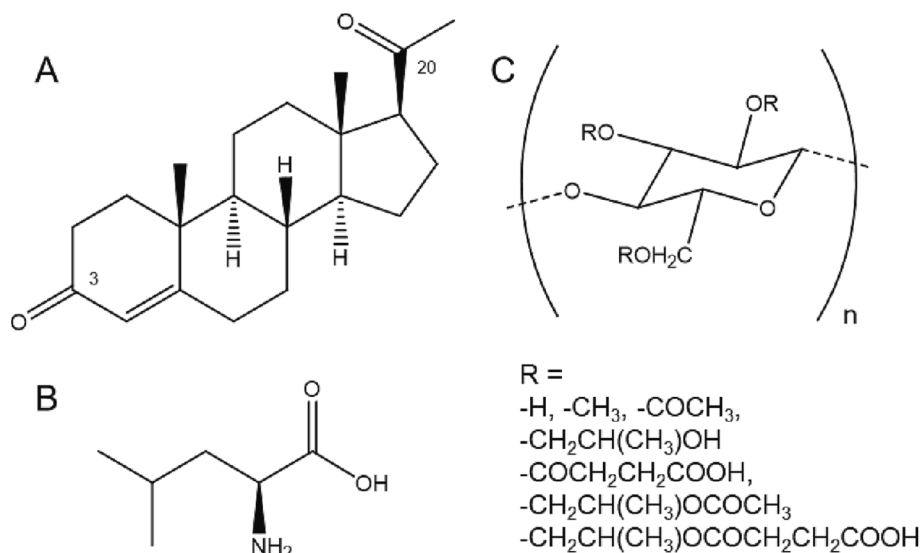


Fig. 1. Structures of progesterone (A), L-leucine (B) and HPMCAS (C). Key progesterone carbon atoms are numbered.

therefore, formulation with PROG would be beneficial [16]. In addition, solid dispersions formulations containing PROG and polymers (including hydroxypropyl methylcellulose acetate succinate (HPMCAS)) have been reported to increase aqueous solubility [17]. Although these powders were not tested for inhalation properties, the increased solubility is a significant property when developing inhaled formulations for systemic delivery [19].

One challenge of formulating PROG, LEU and HPMCAS through spray drying comes from their different solubility profiles, especially since PROG and LEU favour organic and aqueous solvents respectively [20]. Therefore, the spray drying feedstock needs to be a mixture of miscible organic and aqueous phases allowing PROG and LEU to be in solution together. In this work, the solvent type and aqueous to organic ratio in the feedstock was varied to investigate the influence on inhalation properties for the spray dried product with or without HPMCAS. PROG LEU formulations with and without HPMCAS are contrasted to investigate the influence of excipients on formulation stability, solubility and aerosolisation performance. Physicochemical properties, thermal behaviour, aqueous solubility measurements and *in-vitro* aerodynamic performance are reported. It is the aim of this research to explore the utility of a carrier free spray drying method to enable the development of a PROG DPI formulation. In this work we systematically varied spray drying process parameters to prepare inhalable formulations of PROG using a series of 2-fluid nozzle spray drying methods.

## 2. Material and methods

### 2.1. Materials

Progesterone (PROG) and L-leucine (LEU) (Fig. 1) were obtained from Sigma-Aldrich (Dorset, UK). Ethanol (EtOH), HPLC water, acetone (ACE), acetonitrile and propan-2-ol (IPA) were obtained from Fisher-Scientific Limited (Leicestershire, UK). Hydroxypropyl methylcellulose acetate succinate HG grade (HPMCAS) was obtained from Shin-Etsu Chemical Co (Tokyo, Japan) (Fig. 1). Gamble's solution was obtained from Pickering Laboratories (Mountain View, California). All chemicals were used as received.

### 2.2. Preparations of spray dried formulations

Spray drying experiments were performed using a B-290 spray dryer (Büchi, Labortechnik AG Switzerland) operated in closed loop mode with a nitrogen atomising gas and nitrogen drying atmosphere. The

Table 1

Spray-drying methods for PROG-LEU formulations.

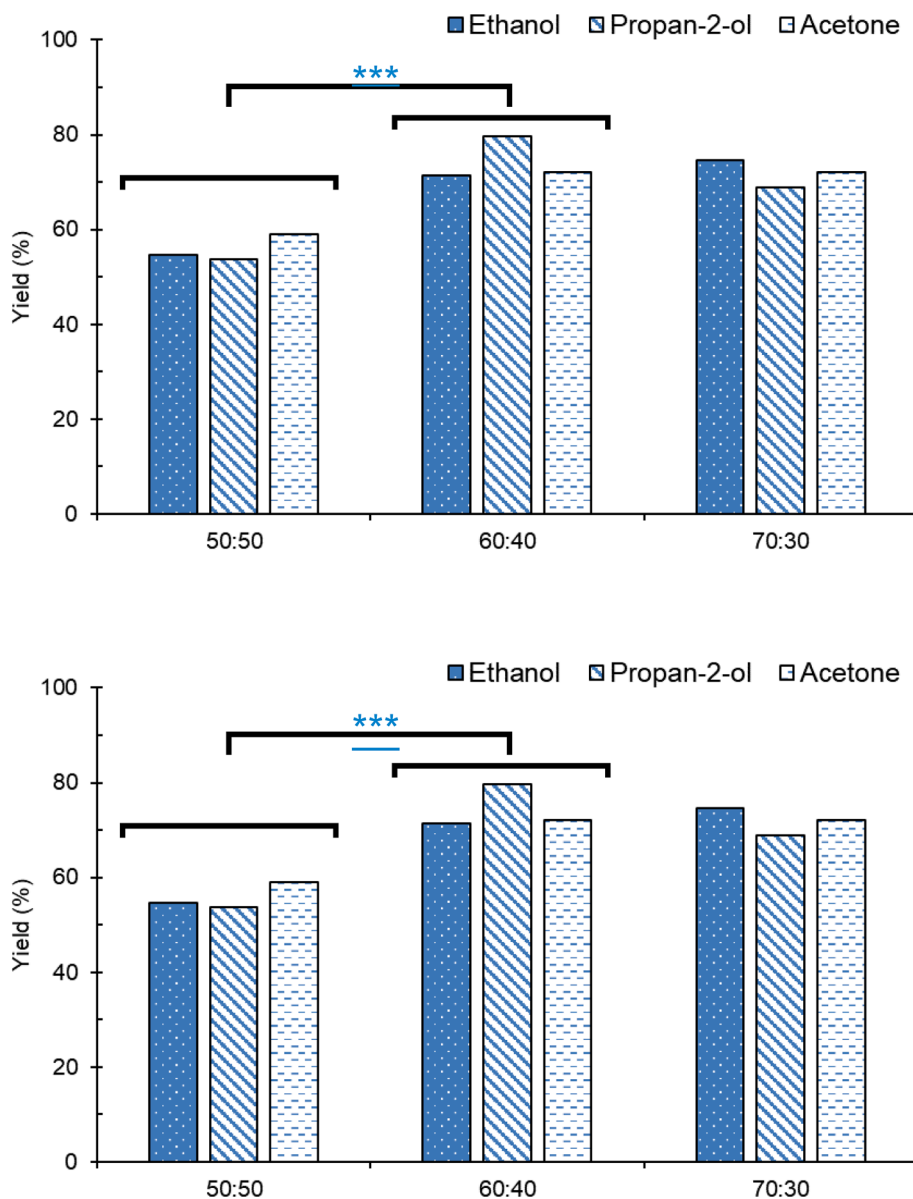
Experiment	Organic Solvent	Solvent Ratio (v/v, Aqueous:Organic)
1	EtOH	50:50
2	EtOH	70:30
3	EtOH	60:40
4	IPA	60:40
5	IPA	70:30
6	IPA	50:50
7	ACE	70:30
8	ACE	50:50
9	ACE	60:40
PROG-SD	ACE	0:100

aspirator was set to 100% generating a vacuum of −100 mbar with the atomising gas flow valve set to 40 mm which corresponds to a 660 L/h flow rate. For each experiment the inlet temperature was set to 110 °C and experiments were started when the outlet temperature stabilised to 60 °C. A two fluid nozzle with nozzle diameter 0.5 mm was used at a 5 mL/min pump rate.

Feedstocks were prepared according to Table 1 to batch size 1 g and contained PROG and LEU in a 9:1 mass ratio with varying types and proportions of organic phase. Initially, PROG and LEU were dissolved separately in organic and aqueous solvents respectively. Organic and aqueous phases were then combined to create one feedstock with total final concentration of 1% w/v. Combining the two phases resulted in a clear solution following stirring. Process yield was calculated as a percentage of feedstock solids retrieved from the spray dryer sample collection point. A PROG sample without LEU was also generated, to be used as a reference, from a 1% w/v acetone solution using the same spray drying conditions (PROG-SD).

Following spray drying, collected powder was transferred to vials, weighed, and stored in a desiccator.

Following initial analysis of processing yields and formulation composition, run 7 was chosen for re-run with the addition of 20% w/v HPMCAS since it showed high yield and required improved inhalation properties. The method was similar except for the HPMCAS was added to the aqueous phase with LEU. Organic and aqueous phases were combined in the same way to produce a feedstock with 1% w/v solid content containing 10% w/v LEU, 20% w/v HPMCAS and 70% w/v PROG.



**Fig. 2.** % yield for spray dried PROG-LEU formulations calculated as collected mass from the total solid content in the feedstock. Mean values of 50:50 and 60:40 formulations taken and statistical difference calculated (One-way ANOVA, Significance levels: \*\*\* =  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ , NS =  $p > 0.05$ ).

## 2.3. Physicochemical characterisation

### 2.3.1. Powder X-ray diffraction (PXRD)

Transmission capillary PXRD data were collected for all spray dried formulations using a Bruker D8 Advance diffractometer equipped with a monochromatic  $\text{CuK}\alpha_1$  source. Samples were packed into a 0.7 mm borosilicate glass capillary then scanned in the range  $4^\circ$  to  $45^\circ$   $2\theta$  using step size  $0.0171^\circ$  with a count time of 1.4 s per step. Data for the starting materials and PROG-SD were also collected using the same settings to be used as a reference.

### 2.3.2. Variable temperature PXRD (VT-PXRD)

PXRD data were also collected over a range of  $100$ – $130^\circ\text{C}$  to monitor the polymorphic transformation of PROG. Temperature control was achieved using an Oxford Cryosystems Cryostream Compact device. The same data collection parameters as previous PXRD experiments were used.

### 2.3.3. Fourier -Transform infrared spectroscopy (FTIR)

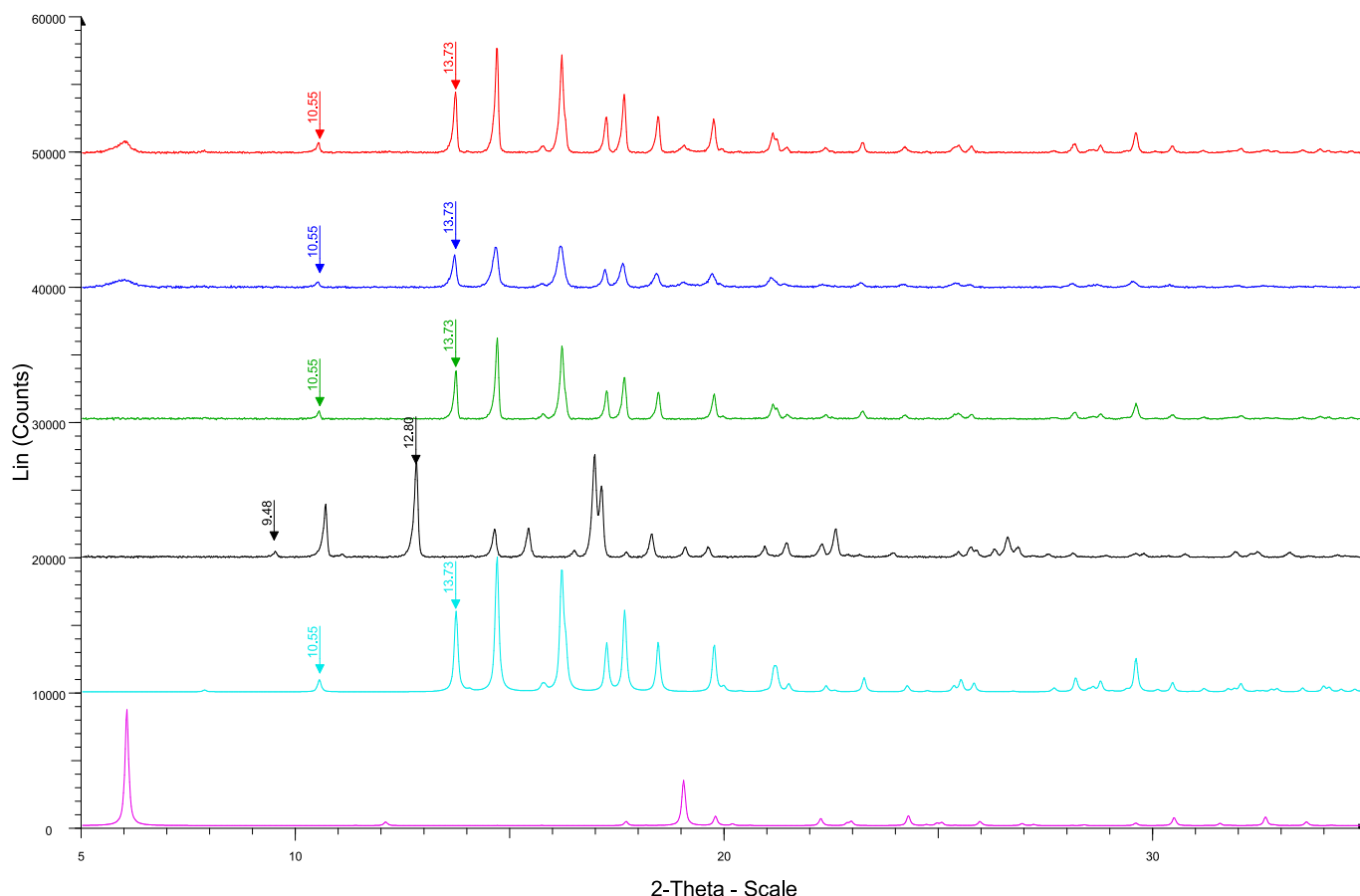
FTIR spectra were collected using a Perkin-Elmer 100 FTIR Spectrometer equipped with a diamond attenuated total reflectance (ATR) accessory (Shelton, CT, USA). Sample was analysed in the solid state and transmission was recorded from an average of 16 scans over the range  $650$ – $4000\text{ cm}^{-1}$  with a resolution of  $4\text{ cm}^{-1}$ .

### 2.3.4. Thermogravimetric analysis (TGA)

Thermal degradation and any moisture loss from formulations was analysed using TGA. Samples were heated to  $400^\circ\text{C}$  at  $20^\circ\text{C}/\text{min}$  in a  $\text{N}_2$  atmosphere (TGA, TA Q50) (New Castle, DE, USA). Results were analysed using TA Universal Analysis software to identify degradation temperature.

### 2.3.5. Differential scanning calorimetry (DSC)

Thermal properties of samples were analysed using DSC following identification of degradation point using TGA. For DSC experiments, samples were hermetically sealed into aluminium pans with pierced lids to allow for any pressure release. All samples were heated to  $150^\circ\text{C}$  at



**Fig. 3.** PXRD data comparing PROG-LEU (red), PROG-LEU-HPMCAS (blue), PROG-SD (green), PROG starting material (Form I, black), PROG Form II (light blue) and L-leucine (pink). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

10 °C/min in a N<sub>2</sub> atmosphere using a TA Q2000 DSC instrument (New Castle, DE USA). Results were analysed using TA Universal Analysis software to identify thermal events.

#### 2.3.6. Scanning electron microscopy (SEM)

Images of samples were obtained using a Quanta 600F scanning electron microscope (Hillsboro, ORE, USA) under high vacuum. Samples were attached to carbon tabs, mounted on aluminium pins, then sputter-coated with gold for 3 min at 30 mA (Emitech K550).

### 2.4. Physical property analysis

#### 2.4.1. Progesterone concentration analysis via UV-HPLC

PROG separation was achieved with an Agilent 1100 system fitted with ACE 3 C18 column (75 × 4.6 mm, 3 µm) using a gradient elution method with water:acetonitrile mobile phase over 20 mins. The flow rate was set to 0.8 mL min<sup>-1</sup>, sample volume to 20 µL, column temperature 25 °C and UV detector at 245 nm. A linear calibration curve was generated ( $r^2 > 0.99$ ) from a stock solution of PROG in 50:50 acetonitrile:water over a range of 0.1 mg mL<sup>-1</sup> to 200 mg mL<sup>-1</sup>.

#### 2.4.2. In-vitro aerodynamic performance – Andersen Cascade impaction (ACI)

Aerodynamic properties of the samples were determined using an Andersen Cascade Impactor (Copley Scientific, Nottingham, UK). The device was fitted with a pre-separator, to collect non-inhalable powder boluses, followed by 8 stages with cut off size: 8.6, 6.5, 4.4, 3.3, 2, 1.1, 0.54 and 0.25 µm. Formulations equivalent to 20 mg of PROG were loaded into a size 3 gelatine capsule and dispersed through the apparatus from a RS01 DPI device (Berry Global, IN USA). 20 mg equivalent

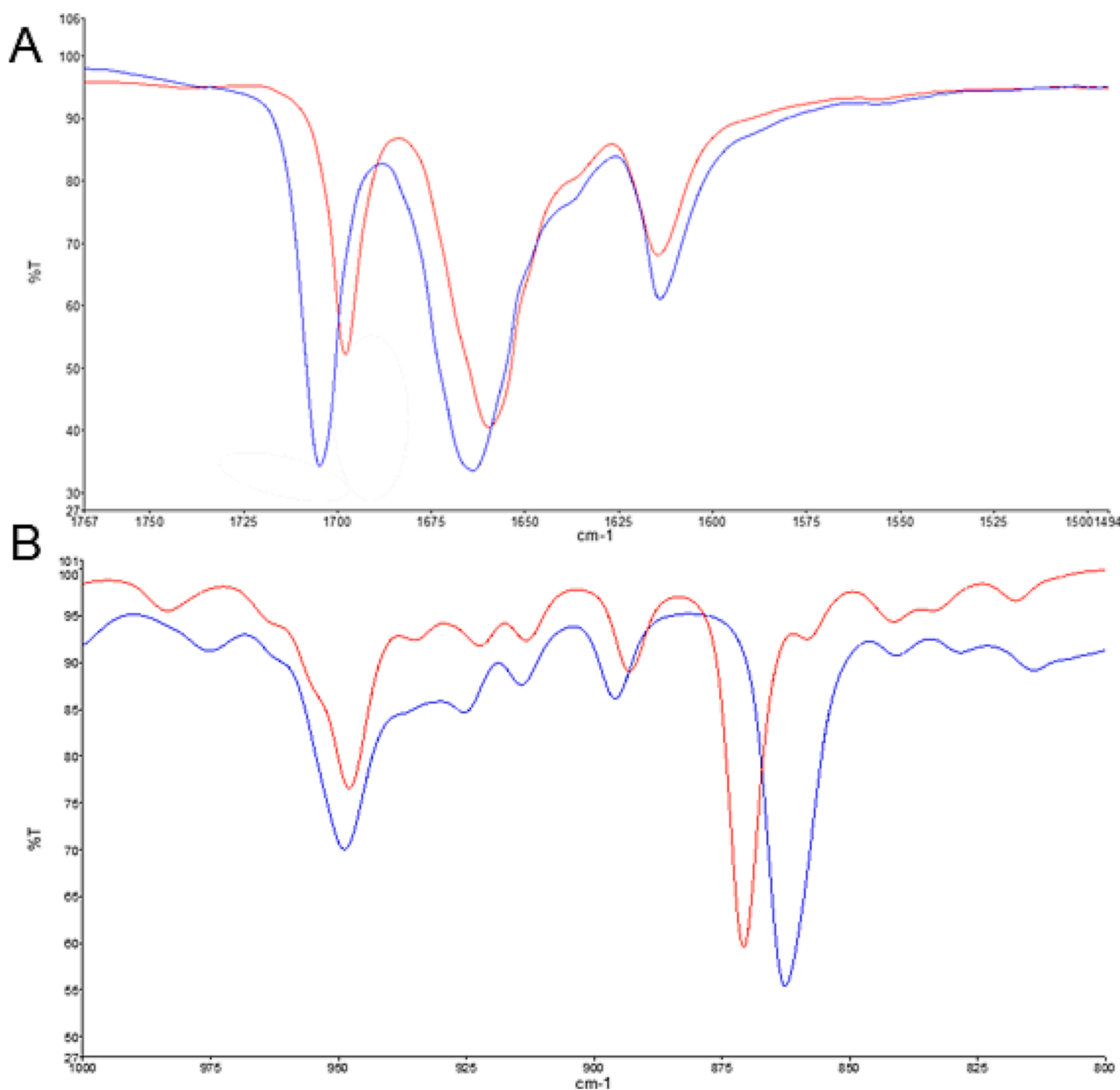
of PROG was used as a theoretical inhaled dose based on an 80% first pass metabolism rate of the standard oral dosing unit [21] and this dose is within current licenced carrier free powder inhalation limits [22]. The instrument operated at 60 L/min over 4 sec and the flow rate was verified with a flow meter prior to testing according to (USP (601) ) [23]. Following each run, particles were collected from each stage by washing with 20 mL of absolute ethanol. These were then filtered with 0.22 µm syringe filter (Ministart®) and PROG concentration was determined through UV-HPLC analysis.

Emitted dose (ED) was calculated as % of original PROG dose collected from all stages including the pre-separator. Mass median aerodynamic diameter (MMAD) was calculated as the aerodynamic particle size at which 50% of the ED is larger and 50% of the ED is smaller following a log-normal distribution of aerodynamic particle size against cumulative percentage mass. Geometric standard deviation (GSD) was calculated to describe the spread of data around the MMAD using equation 1 as per USP (601).

$$GSD = \sqrt{\frac{\text{Quantity with Size} < 84.1\mu\text{m}}{\text{Quantity with Size} < 15.8\mu\text{m}}}$$

Equation 1: Formula used to calculate geometric standard deviation for the formulations.

Fine particle fraction (FPF) was calculated as the % of ED which has an aerodynamic diameter < 5 µm. Fine particle dose (FPD) was calculated as the mg of PROG found in the FPF which practically corresponds to the sum of PROG collected from stages 3–7 of the ACI for flow rate of 60 L/min.



**Fig. 4.** FTIR data comparing PROG-SD (Blue) and PROG starting material (Red, Form I) at two different regions of infrared spectrum (A) 1750  $\text{cm}^{-1}$  to 1500  $\text{cm}^{-1}$ , B) 1000  $\text{cm}^{-1}$  to 800  $\text{cm}^{-1}$ ). PROG-SD does not match PROG Form I for either of these characteristic regions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

#### 2.4.3. In-vitro solubility analysis

The solubility of the samples was assessed in deionised water over a period of 24 h. 5 mg of each sample was added to 5 mL of deionised water in triplicate, which was a sufficient quantity to prevent complete dissolving. Three sets of experiments were mixed on a mechanical mixer for 1 h, 3 h or 24 h at 25 °C. At the required time point, samples were centrifuged at 13,000 rpm for 5 mins then the supernatant was diluted, and PROG concentration determined using UV–Vis spectroscopy at 245 nm, following generation of a calibration curve ( $r^2 > 0.99$ ). A repeat experiment was conducted comparing the saturated solubility of PROG-LEU and PROG-HPMCAS with PROG Form I reference in simulated lung fluid (SLF, Gamble's solution) at 24 h.

#### 2.5. Statistical analysis

Statistical analysis was carried out using SPSS software (IBM SPSS version 27.0, SPSS Inc.). Data were compared with appropriate test

following test for normality and variance. In each case, statistical significance was defined as  $p < 0.05$  with significance levels: \*\*\* =  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$  and NS =  $p > 0.05$ .

### 3. Results and discussion

#### 3.1. Preparation of spray dried (SD) formulations

Spray dried formulations were prepared and the % yield was calculated following the methods outlined in section 2.2 (Fig. 2). All formulations showed over 50% yield with the maximum yield being ~ 80%. Spray drying is established as a highly reproducible process and as such single repeats of the formulations were used for formulation analysis [24]. Process yield optimisation is a highly important area of spray dried formulation and, although beyond the scope of this current work, it is highlighted as a point for future development.

Process yield results show that the ratio of organic to aqueous



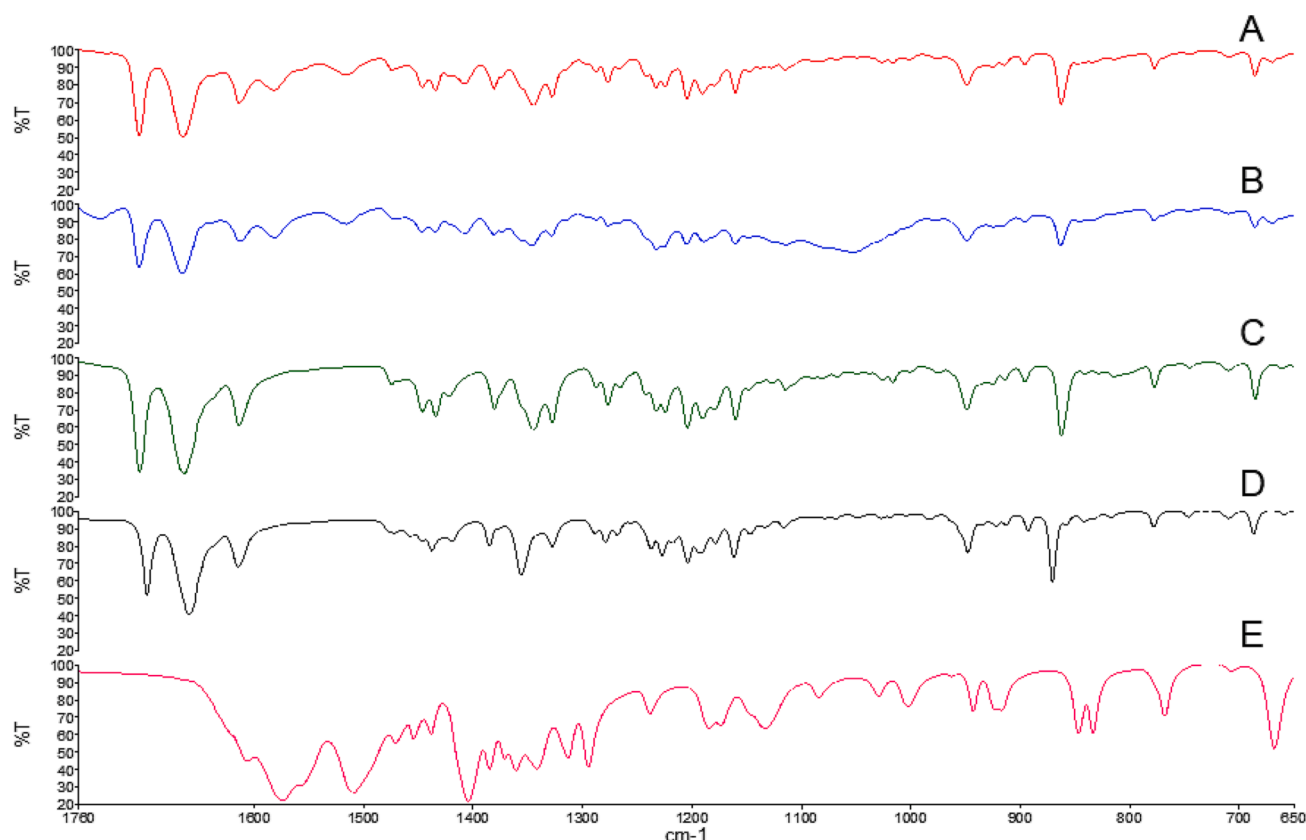


Fig. 5. FTIR spectra for A) PROG-LEU, B) PROG-LEU-HPMCAS, C) PROG-SD, D) PROG Form I reference and E) L-leucine reference.

solvents rather than the organic solvent used has the greater influence. Taking the mean of each solvent ratio, the 50:50 solvent ratio showed a significantly lower yield than both 60:40 and 70:30 (One-way ANOVA,  $p < 0.05$ ). There was no significant difference between the yield of the 60:40 and 70:30 solvent ratios; the 60:40 ratio is considered to be the most suitable due to high yield and reduced use of organic solvent.

Thermogravimetric analysis (TGA) was carried out to investigate the levels of residual organic solvent in the samples following the spray drying process. There was no clear weight loss seen for any of the samples in the 40 °C to 110 °C region which would be expected if there was evaporation of any residual organic solvent or water from the samples. This temperature range corresponds with the boiling points of ACE, EtOH, IPA and water at 56 °C, 78 °C, 83 °C and 100 °C respectively. An example TGA trace showing weight response to heat for the 70:30 samples are shown in Fig. S1.

For the formulations to comply with the ICH residual solvent requirements a minimum solvent content of 5000 ppm is required since ACE, EtOH and IPA are class 3 organic solvents. To ensure there is compliance to this standard, further residual organic solvent analysis using higher sensitivity headspace gas chromatography is highlighted as a point of future work. This would ensure that spray dried samples comply with the ICH requirements for class 3 organic solvents in pharmaceutical formulations prior to any clinical testing [25].

### 3.2. Effect of the spray drying process on progesterone

#### 3.2.1. Progesterone polymorphism

PXRD analysis was used to determine the nature of PROG following spray drying. Comparison of powder patterns with reference materials showed a change of polymorph from PROG Form I starting material (Sigma Aldrich) to PROG Form II. Fig. 3 shows the comparison of starting material with PROG-SD, PROG-LEU and PROG-LEU-HPMCAS. Form II is present exclusively in all experiments regardless of

feedstock solvent composition. This is most clearly seen by the loss of peaks at 9.48 °2 $\theta$  and 12.80 °2 $\theta$  and the appearance of peaks at 10.55 °2 $\theta$  and 13.73 °2 $\theta$ . Spray drying PROG from solution is the cause of the Form II polymorph being exclusively present in all formulations since the diffraction differences are seen regardless of excipients or solvents used (Fig. S1).

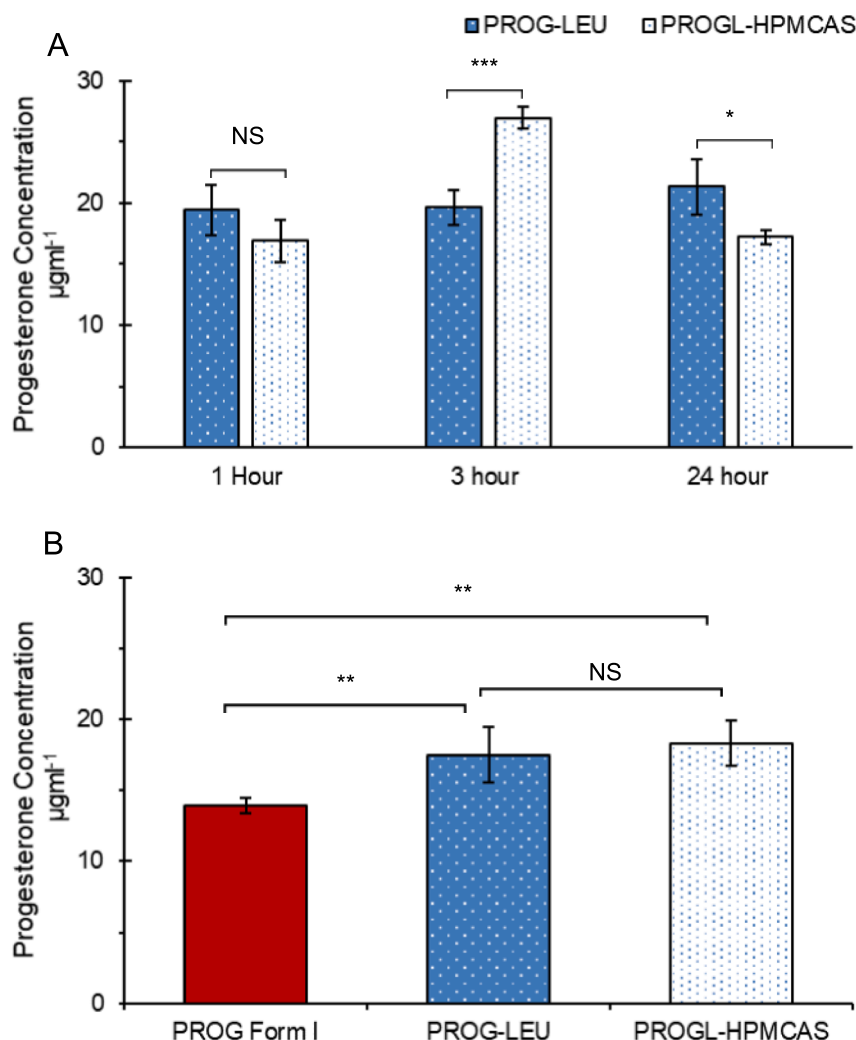
Similar observations are also seen when analysing FTIR data for the spray dried formulations. Comparing the PROG-SD, PROG-LEU and PROG-LEU-HPMCAS to PROG Form I reference shows a slight difference in the positioning of both ketone carbonyl stretching frequencies at  $\sim 1700$  cm $^{-1}$  (C<sub>20</sub>) and  $\sim 1660$  cm $^{-1}$  (C<sub>3</sub>) (see Fig. 1 for C numbering). For the spray dried materials, these peaks have shifted to a higher wavenumber by  $\sim 5$  cm $^{-1}$  (Fig. 4a) and there is also a clear difference between PROG-SD and PROG Form I in the fingerprint region, where the Form I polymorph shows a peak at 871 cm $^{-1}$  compared to PROG-SD which shows a peak at 862 cm $^{-1}$  (Fig. 4b). These changes indicate that PROG-SD is indeed Form II [26] and so FTIR can be used to differentiate between the formulations regardless of excipients present.

The presence of PROG form II following spray drying fits with the current theory that PROG Form II is encouraged through fast evaporation of solvent, as proposed by Sarkar et al. [27] and by Tripathi et al. [28] in their studies of polymorphism of PROG. It is interesting to note that the difference in feedstock solvent boiling points does not affect which polymorph is present in the formulations.

#### 3.2.2. Progesterone – Excipient interactions

PXRD was also used to understand the interactions between PROG and formulation excipients and confirmed that PROG-LEU and PROG-LEU-HPMCAS formulations are physical mixtures of PROG Form II and excipients. Physical mixtures can be identified through comparison with PROG and LEU reference patterns which confirm the presence of two crystalline phases in the final product rather than an amorphous solid dispersion (Fig. S2). It is seen that crystalline material is produced





**Fig. 6.** Solubility data comparing the solubility of PROG-LEU formulation with PROG-LEU-HPMCAS over the course of 24-hours at 20 °C (Mean ± SD). **A)** Solubility data in H<sub>2</sub>O, **B)** Solubility data at 24 h in SLF. One-way ANOVA Significance levels: \*\*\* =  $p < 0.001$ , \*\* =  $p < 0.01$ , \* =  $p < 0.05$ , NS =  $p > 0.05$ .

through the spray drying process regardless of feedstock composition and there is no significant difference in crystallinity between equivalent PROG Form II peaks in PROG-LEU and PROG-LEU-HPMCAS (full width at half maximum values, one-way ANOVA, NS =  $p > 0.05$ ). The presence of LEU as a separate phase to PROG is most clearly identified by peaks at  $\sim 6^\circ 2\theta$  and  $\sim 19^\circ 2\theta$ . For the PROG-LEU-HPMCAS formulation, no additional diffraction peaks are seen (Figs. S3, S4).

The PXRD observations were confirmed using FTIR as shown in Fig. 5. FTIR data for PROG-SD, PROG-LEU and PROG-LEU-HPMCAS compared LEU references show clear composites of PROG Form II and LEU. There is no difference in the carbonyl region where peaks are present for PROG Form II at  $\sim 1705\text{ cm}^{-1}$  and  $\sim 1670\text{ cm}^{-1}$  and for LEU at  $\sim 1575\text{ cm}^{-1}$ . There would likely be shifting in these peaks if there was hydrogen bonding between PROG and LEU through the carbonyl regions. This composite spectrum is true for all the other PROG-LEU formulations, showing their uniformity in intermolecular interactions despite the use of different solvent systems (Fig. S5). An additional peak at  $\sim 1740\text{ cm}^{-1}$  is attributable to the amorphous HPMCAS in the PROG-LEU-HPMCAS formulation.

### 3.3. Effect of spray drying process on physical properties

#### 3.3.1. In vitro solubility study

The effect of spray drying on the solubility of PROG was investigated. Fig. 6A shows the mean solubility values for the PROG-LEU formulations, compared to the solubility data for PROG-LEU-HPMCAS in water. There was no significant difference between the solubilities of PROG-LEU formulations produced from different solvent systems, so a mean value of all nine samples was used as the PROG-LEU solubility value.

When comparing PROG-LEU and PROG-LEU-HPMCAS formulations there is a significant difference in solubility at the 3-hour time point (One-way ANOVA,  $p < 0.05$ ). PROG-LEU-HPMCAS shows an  $\sim 7\text{ }\mu\text{gml}^{-1}$  increase in solubility which then reverts to the starting solubility by the 24-hour time point. This result indicates that the addition of polymer to the system was able to facilitate a temporary increase in PROG solubility during the experiment.

A further 24-hour solubility experiment was conducted using simulated lung fluid (SLF) to compare PROG-LEU and PROG-LEU-HPMCAS to the PROG Form I reference (Fig. 6B). For the spray dried formulations, there is a significant increase in PROG solubility compared to the reference but no difference with the addition of HPMCAS. Therefore, differences in saturated solubility can be attributed to the different PROG polymorphs. This finding is in line with literature which reports

**Table 2**

Aerosol performance for PROG-LEU formulations.

Solvent	Ratio	MMAD ( $\mu\text{m}$ )	GSD ( $\mu\text{m}$ )	FPF (%)	FPD (mg)	ED (%)
Ethanol	50:50	5.81 $\pm$ 0.21 (NS)	2.01 $\pm$ 0.21 (***)	42.47 $\pm$ 1.57 (NS)	3.16 $\pm$ 0.25 (*)	37.10 $\pm$ 1.79 (**)
	60:40	5.41 $\pm$ 0.31 (NS)	1.90 $\pm$ 0.02 (***)	45.62 $\pm$ 3.06 (NS)	4.10 $\pm$ 0.45 (*)	44.75 $\pm$ 2.06 (*)
	70:30	5.52 $\pm$ 0.22 (NS)	1.40 $\pm$ 0.02 (***)	44.14 $\pm$ 2.00 (NS)	2.78 $\pm$ 0.30 (*)	31.43 $\pm$ 2.09 (*)
Propan-2-ol	50:50	5.83 $\pm$ 0.18 (**)	1.40 $\pm$ 0.02 (***)	41.77 $\pm$ 0.95 (**)	3.44 $\pm$ 0.31 (*)	41.16 $\pm$ 2.73 (*)
	60:40	4.50 $\pm$ 0.08 (**)	1.76 $\pm$ 0.01 (***)	56.83 $\pm$ 0.73 (**)	4.99 $\pm$ 0.53 (*)	44.01 $\pm$ 5.19 (*)
	70:30	4.58 $\pm$ 0.27 (**)	1.78 $\pm$ 0.02 (***)	55.69 $\pm$ 3.68 (**)	6.27 $\pm$ 0.62 (*)	56.16 $\pm$ 2.20 (*)
Acetone	50:50	5.49 $\pm$ 0.12 (NS)	1.85 $\pm$ 0.01 (**)	44.53 $\pm$ 1.15 (NS)	7.28 $\pm$ 0.20 (***)	81.73 $\pm$ 1.32 (***)
	60:40	5.72 $\pm$ 0.25 (NS)	1.99 $\pm$ 0.02 (***)	43.53 $\pm$ 2.21 (NS)	4.45 $\pm$ 0.53 (***)	50.95 $\pm$ 3.53 (***)
	70:30	5.59 $\pm$ 0.23 (NS)	1.89 $\pm$ 0.01 (***)	43.74 $\pm$ 2.58 (NS)	4.64 $\pm$ 0.47 (***)	52.91 $\pm$ 2.33 (***)

One-way ANOVA (i.e. within solvent groupings). NS  $p > 0.05$ , \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation,

FPF = Fine Particle Fraction (%  $< 5 \mu\text{m}$  of ED), FPD = Fine Particle Dose (mg  $< 5 \mu\text{m}$ ),

ED = Emitted Dose (%).

PROG Form I to have a lower aqueous solubility than Form II (Table S1).

A variety of solubility values for PROG Form I and II have been reported in literature depending on temperature and media composition (Table S1). In general, the results presented here are comparable with previous literature values which show Form II to have a higher solubility than Form I. It is suggested that increased solubility will result in a higher bioavailability through the pulmonary route and therefore Form II may be beneficial for inhaled PROG formulations [18].

### 3.3.2. Cascade impaction – In vitro lung deposition study

The effect of spray drying on aerodynamic properties was investigated through cascade impaction studies; the statistical analysis of these results is summarised in Table 2.

With respect to mass median aerodynamic diameter (MMAD), all PROG-LEU formulations showed a value close to  $5 \mu\text{m}$  which is a minimum requirement for good aerosol performance and lung deposition in vivo. Ideally, an MMAD of  $< 5 \mu\text{m}$  is preferred for alveolar deposition, which is the case for IPA 60:40 and 70:30 formulations. The influence of changing the solvent ratio is only significant for the IPA formulations where the 50:50 ratio has a significantly higher MMAD than the 60:40 and 70:30 ratios (5.83  $\mu\text{m}$  compared to 4.5  $\mu\text{m}$  and 4.58  $\mu\text{m}$ ). These 60:40 and 70:30 formulations from IPA showed the smallest MMAD of all the solvent systems which suggests that their deposition will occur deeper in the lung. Ragab et al. showed that PROG crystals generated from antisolvent solutions with high proportions of IPA produced smaller MMAD values [6]. Although these formulations were not generated through spray drying, these data may confirm that IPA with smaller proportions of water is an advantageous solvent to generate inhalable powders of PROG.

When considering geometric standard deviation (GSD) values each formulation showed a GSD value greater than 1.2  $\mu\text{m}$  and so was

classified as being aerodynamically heterodisperse. A monodisperse aerosol is advantageous for clinical administration of API since it leads to much more precise dosing. Fine tuning of spray drying parameters and the addition of excipients would be required to produce a more monodisperse aerosol. There were significant differences in GSD within each formulation solvent type and of the formulations, EtOH 70:30 and IPA 50:50 show the smallest GSDs.

Viewing scanning electron microscopy (SEM) images of the formulations may give some insight into the MMAD and GSD findings. It was noted that PROG-LEU formulations show different surface morphologies especially for the IPA formulation (Fig. 7). The IPA formulation shows a more “wrinkled surface” compared to the formulations from ACE and EtOH which are seen especially when LEU has dried on the surface of the particles [16]. The presence of LEU on the surface is believed to improve inhalation properties [16].

Fine particle fraction (FPF) values range from 42% to 57% across the formulations and the formulations from IPA show the highest values. In the case of the IPA formulations the 50:50 ratio shows a significantly lower FPF than the 60:40 and 70:30 ratios (42% compared to 57% and 56%). This trend in FPF mirrors the trends seen in the MMAD data. In general, the FPF corresponds to particles which will deposit deeper in the lung and as such, this value should be as high as possible. Further study to increase the FPF in these carrier free DPI formulations is required. The low FPF of these formulations is further understood when considering the emitted dose (ED; % Dose delivered to the impactor). For most formulations, the dose of PROG delivered to the impactor is low which shows that much of the formulation is impacting in the oropharynx region and therefore by default, the amount reaching the lower airways is less. However, significant differences do exist with respect to ED and fine particle dose (FPD; mg PROG  $< 5 \mu\text{m}$ ) between the ratios for all solvents. Since ED and FPD describe the amount of drug delivered to the lungs, these values were used to compare the inhalation performance between the solvent ratios.

**3.3.2.1. Cascade impaction – Comparison of ED and FPD.** For EtOH formulations, the 60:40 ratio shows the highest FPD values at 4.1 mg and 45% which is an increase of  $\sim 20\%$  from the 50:50 ratio and an  $\sim 30\%$  increase from the 70:30 ratio. These increases are due to an increase in ED fraction. For EtOH, the 60:40 ratio shows the best aerodynamic performance and was selected for further analysis.

For IPA formulations, the 70:30 ratio shows the highest values at 6.27 mg and 56% which for the FPD correspond to an  $\sim 45\%$  increase from 50:50 ratio and  $\sim 20\%$  increase from the 60:40 ratio. This increase is also due to the increase in ED fraction. Therefore, the IPA 70:30 ratio shows the best aerodynamic performance and was selected for further analysis.

For ACE, the 50:50 ratio shows the highest values at 7.28 mg and 81.73% which corresponds to an  $\sim 35\%$  increase from the 70:30 ratio and  $\sim 40\%$  increase from the 60:40 ratio with respect to FPD. For ED, the increase from 60:40 and 70:30 to 50:50 is similar at  $\sim 35\%$ . This could indicate that for the 60:40 formulation a much larger proportion of the ED is deposited in the upper airways compared to the 50:50 and 70:30 formulations. Therefore, the ACE 50:50 formulation shows the best aerodynamic performance and was selected for further analysis.

From these data there is not an obvious trend between solvent ratio and inhalation characteristics for each solvent. For IPA, it seems that increasing the proportion of organic solvent in the feedstock consistently improves the inhalation properties. However, this is not the case for ACE and EtOH where no relationships between solvent ratio and inhalation properties exist. Changes in the organic solvent used resulted in significant differences to inhalation properties which may be related to the proportion of residual solvent in the powders following spray drying [29]. There is evidence to support that the extent of drying is directed by the solvent boiling points and presence of excipients [29], which points to the need for higher sensitivity residual solvent analysis for these

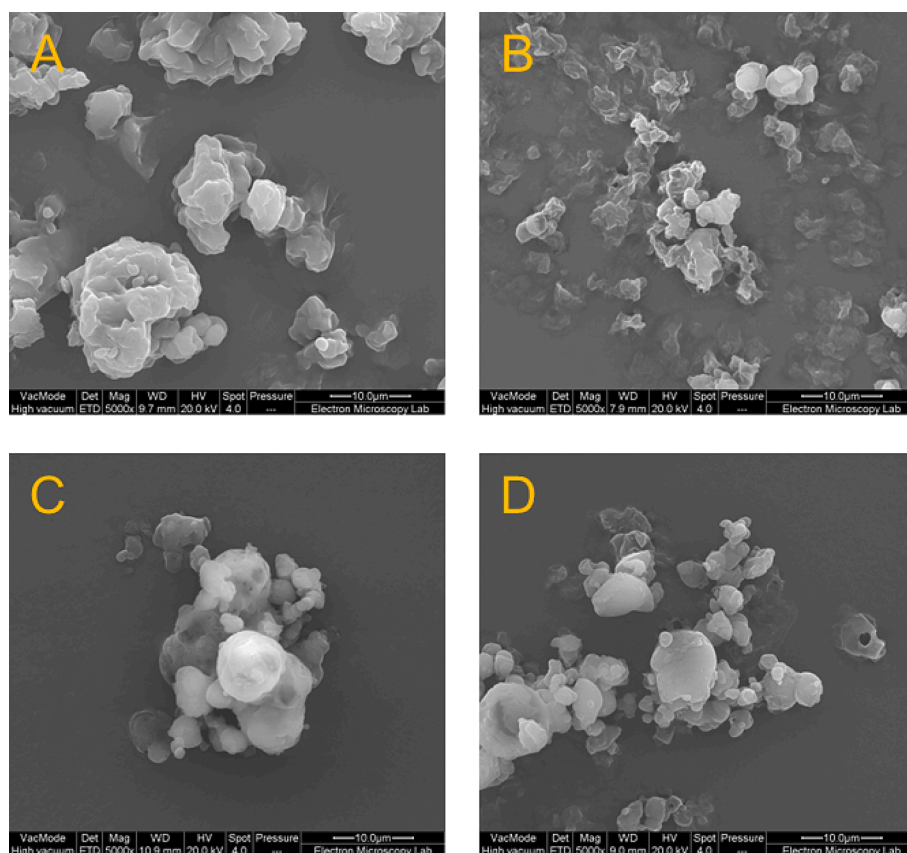


Fig. 7. SEM images showing A) PROG-LEU-HPMCAS, B) PROG-LEU from IPA, C) PROG-LEU from ACE, D) PROG-LEU from EtOH.

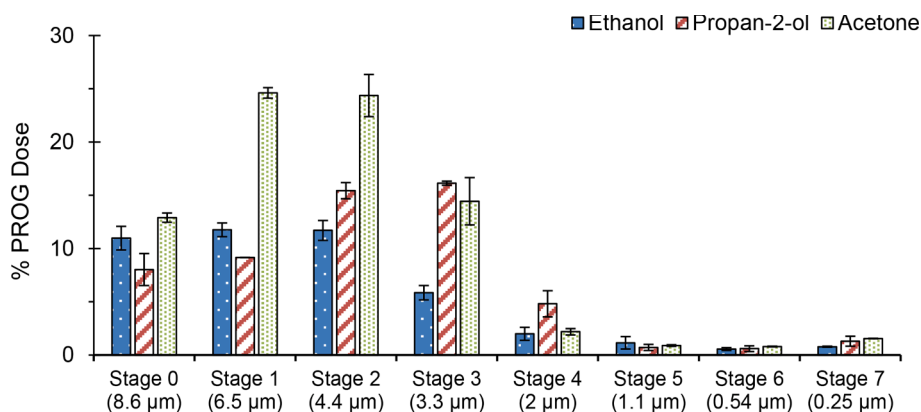


Fig. 8. Andersen Cascade Impactor data comparing the best ratio of each solvent based on FPD (mg) and ED (%).

powders as commented on previously.

### 3.3.2.2. Cascade impaction – Comparison of best organic solvent ratios.

The deposition profile for the solvent ratio which showed the best aerodynamic properties for each solvent was compared. Fig. 8 shows there to be clear differences between the deposition of these three formulations. IPA and ACE formulations appear to show a normal distribution like curve with maximum deposition in stages 1—2 and stages 2—3 respectively. By contrast the ethanol formulation gives steady deposition in stages 0—2 then shows a gradual decline throughout the impactor.

The ACE formulation shows a higher ED of PROG than IPA and EtOH (82% compared to 56% and 45% respectively). However, much of this is delivered into the upper airways (stage 0—2) meaning that the IPA

formulation delivers a higher percentage of PROG into the lower lung (24% compared to 20%). This is mirrored in the MMAD values where IPA (2.80 μm) shows a significantly lower value than ACE (3.47 μm) (One-way ANOVA,  $p < 0.001$ ). However, it is worth noting that the majority of the dose deposition is still found in the oropharyngeal region of the cascade impactor which is not favourable for alveolar deposition and therefore systemic absorption of PROG. In addition to this, all formulations show a comparatively low ED [30] which, although acceptable for an experimental DPI formulation, will need significant improvement in further formulation development.

**3.3.2.3. Reformulation with HPMCAS.** Since the ACE 60:40 system was shown to be a high yielding, low deposition formulation it was reformulated with additional HPMCAS with the aim of increasing the ED and

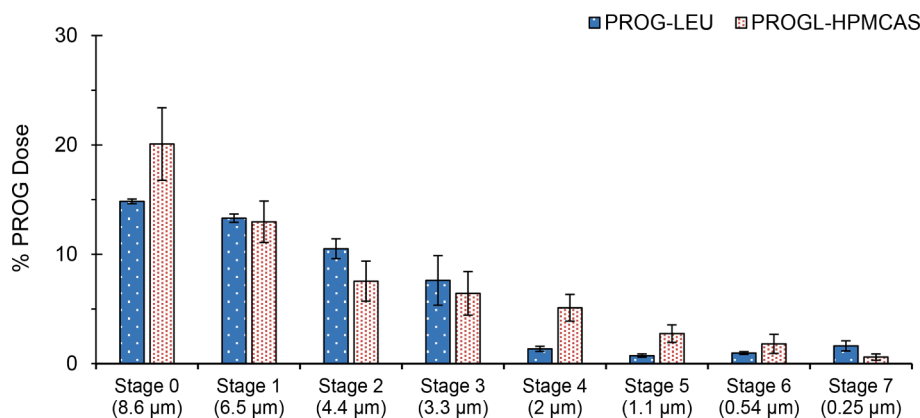


Fig. 9. ACI data comparing PROG-LEU formulation with PROG-LEU-HPMCAS.

Table 3

Aerosol performance of PROG-LEU and PROG-LEU-HPMCAS.

Solvent	Ratio	MMAD (μm)	GSD (μm)	FPF (%)	FPD (mg)	ED (%)
Acetone 60:40	PROG-LEU	5.72 ± 0.25 (**)	1.99 ± 0.03 (NS)	43.53 ± 2.70 (**)	4.45 ± 0.65 (NS)	50.95 ± 4.32 (NS)
		4.87 ± 0.22 (**)	1.96 ± 0.16 (NS)	51.57 ± 3.32 (**)	5.90 ± 1.08 (NS)	57.29 ± 10.73 (NS)
	PROG-LEU-HPMCAS	4.87 ± 0.22 (**)	1.96 ± 0.16 (NS)	51.57 ± 3.32 (**)	5.90 ± 1.08 (NS)	57.29 ± 10.73 (NS)
		4.87 ± 0.22 (**)	1.96 ± 0.16 (NS)	51.57 ± 3.32 (**)	5.90 ± 1.08 (NS)	57.29 ± 10.73 (NS)

One-way ANOVA: NS  $p > 0.05$ , \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation,

FPF = Fine Particle Fraction (%  $< 5 \mu\text{m}$  of ED), FPD = Fine Particle Dose (mg  $< 5 \mu\text{m}$ ),

ED = Emitted Dose (%).

Table 4

DSC results for all experiments showing melting endotherm onset and heat of fusion values.

Solvent	Ratio	Peak Onset (°C)	$\Delta H_{\text{fus}}$ (Jg <sup>-1</sup> )	Peak Onset (°C)	$\Delta H_{\text{fus}}$ (Jg <sup>-1</sup> )
Acetone	50:50	121.41	40.74	128.82	23.68
	60:40	121.66	46.49	129.07	9.985
	70:30	121.7	46.58	129.17	15.74
Ethanol	50:50	121.35	62.49	129.02	4.512
	60:40	121.23	57.79	128.9	12.81
	70:30	121.83	53.78	129.45	2.212
Propan-2-ol	50:50	121.53	49.71	129.1	15.71
	60:40	120.53	48.59	128.9	8.457
	70:30	121.14	50.53	128.89	12.66
Acetone (+HPMCAS)	60:40	120.27	41.53	n/a	n/a

alveolar deposition of PROG. ACE 60:40 was chosen on a balance of spray drying yield and low FPF. The inhalation performance of the PROG-LEU-HPMCAS powder was then assessed through ACI (Fig. 9, Table 3). There is no significant difference between the GSD, FPD or ED values for these two formulations. However, there is a significant difference between the MMAD and FPF values. The addition of HPMCAS has increased the FPF from 44% to 52% ( $p < 0.05$ , One-way ANOVA) and reduced the MMAD by 15 % to less than  $5 \mu\text{m}$ . These changes can be seen when comparing the deposition profiles (Fig. 9).

While the formulations have a similar looking deposition profile, the PROG-LEU-HPMCAS formulation appears to show much higher deposition in from stages 4 onwards (corresponding to  $< 2 \mu\text{m}$  aerodynamic diameter). This equals 10% PROG deposition in the lower airways compared to 5% for the PROG-LEU formulation. Therefore, with correct

optimisation, the addition of HPMCAS may generate a formulation with more advantageous alveolar deposition.

It is worth commenting that for many successful dry powder inhaler formulations an MMAD of  $3 \mu\text{m}$  and emitted dose of  $> 90\%$  is desirable [31]. Therefore, further optimisation of the spray dried method is required to achieve PROG formulations with better inhalation properties.

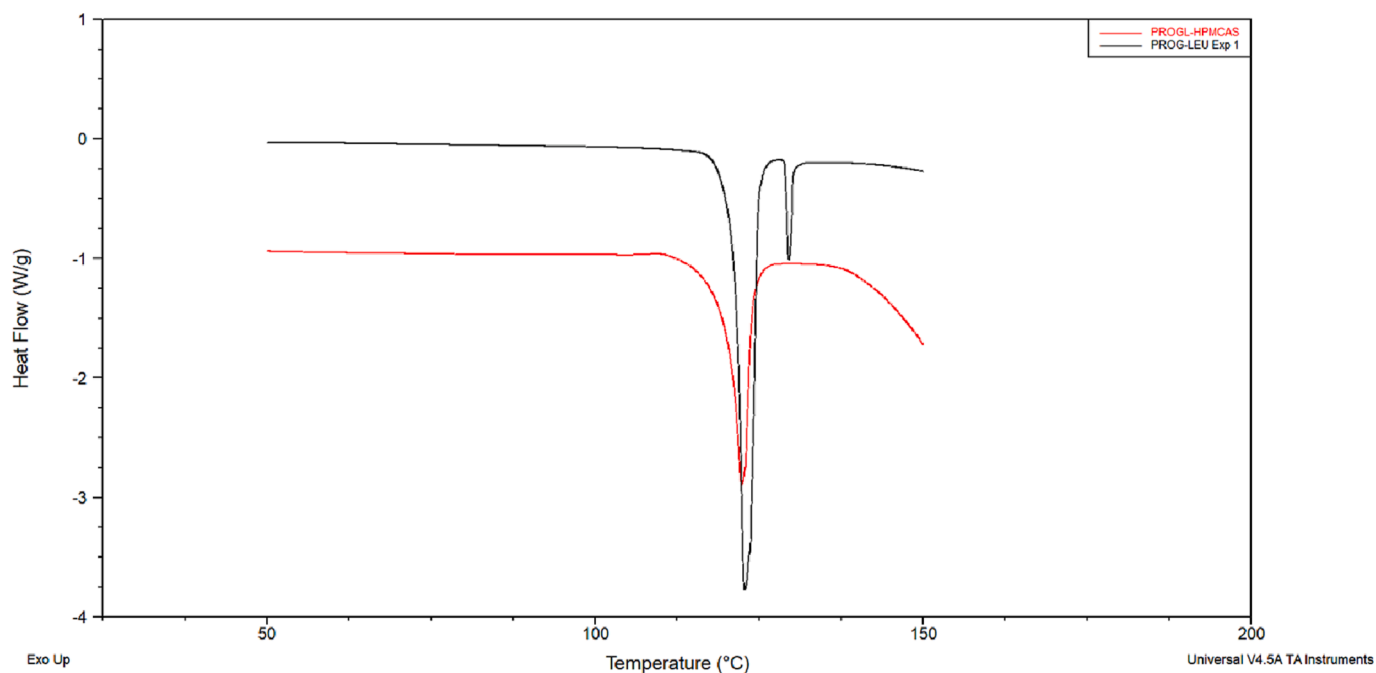
### 3.4. Polymorphic transformation studies

To further understand the stability of PROG Form II within these spray dried formulations, DSC analysis was carried out. Firstly, the DSC data for all PROG-LEU formulations show two endothermic peaks present at  $\sim 121^\circ\text{C}$  and  $\sim 129^\circ\text{C}$  (Table 4, Fig. S6). These temperatures correspond to the reported melting points for PROG Form II and I respectively. Comparison with PXRD data shows that a temperature-induced polymorphic transition has occurred during the DSC experiment, since all formulations are exclusively Form II rather than a physical mixture of the two (section 3.2.1).

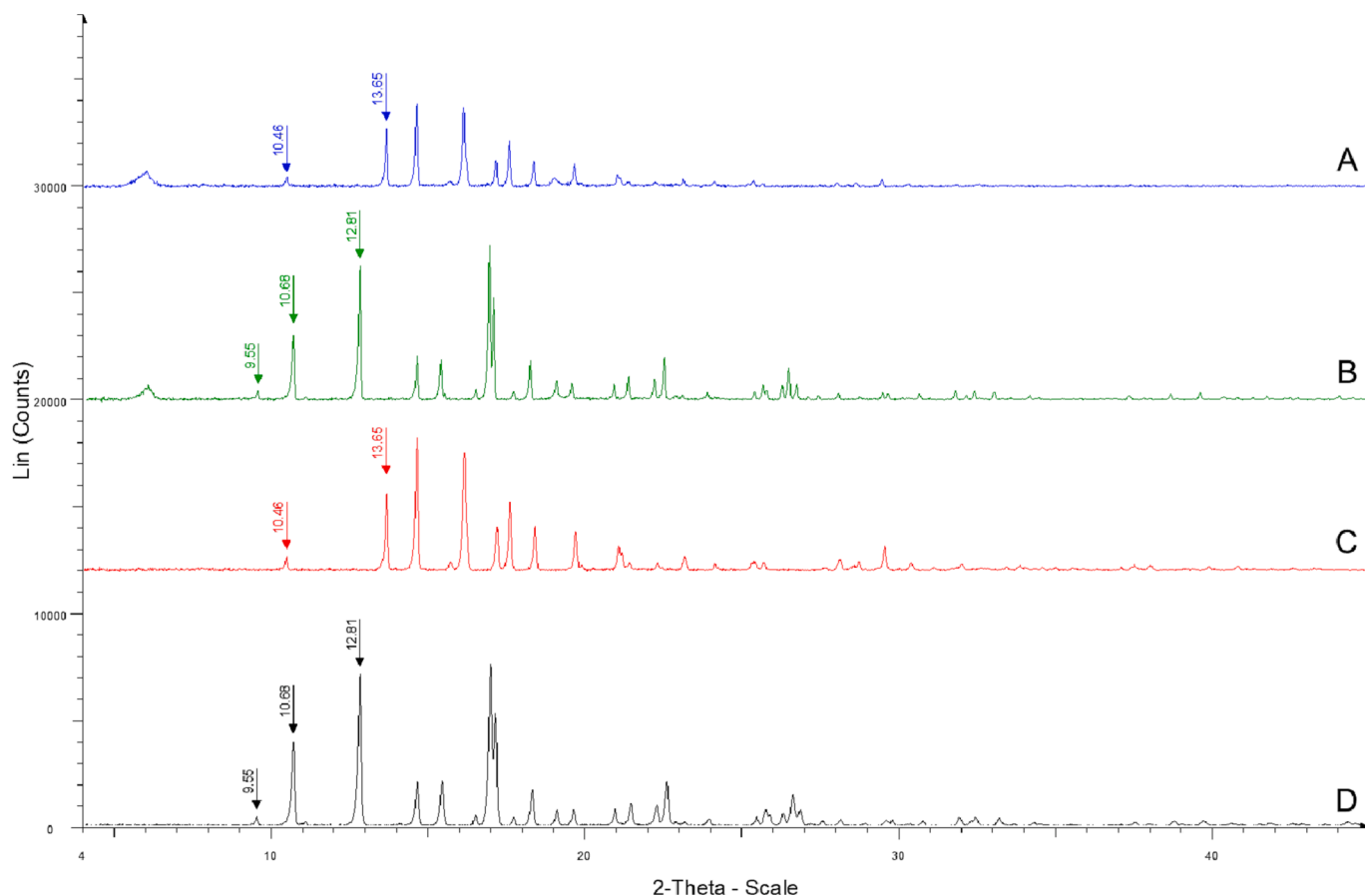
In comparison, there is only one thermal event seen for PROG-LEU-HPMCAS over the range  $100\text{--}150^\circ\text{C}$ . This event has a slightly lower onset temperature than the other formulations but results in the same peak temperature as shown in Fig. 10. This event is most likely attributed to Form II melting and in the absence of any thermal event at  $\sim 129^\circ\text{C}$  indicates that no polymorphic transition has taken place. It is most likely that following melting of Form II, HPMCAS has prevented the recrystallisation of PROG to Form I.

To further test hypotheses with respect to polymorphic transformation, PROG-SD, PROG-LEU and PROG-LEU-HPMCAS formulations were investigated using a VT-PXRD method. A series of PXRD measurements were made over the temperature range identified in DSC experiments i.e.  $100\text{--}130^\circ\text{C}$ . Through this experiment, PROG-SD clearly showed a transition from Form II to Form I over the temperature range, proceeding via a physical mixture of the two solid forms (Fig. S7). When the temperature reached  $130^\circ\text{C}$ , all Form II peaks disappeared from the diffraction pattern, an observation which agrees with the DSC data.

Repeating the same experiment with the PROG-LEU formulations showed the thermal transformation to take place at a lower temperature than for PROG-SD ( $\sim 110^\circ\text{C}$  vs  $\sim 120^\circ\text{C}$ ) with full transformation to Form I before the melting point (Fig. S8). However, when heating the PROG-LEU-HPMCAS formulation there is no evidence of any change to Form I before the formulation melts (Fig. S9). This confirms that the presence of polymer is preventing recrystallisation of Form I on heating and a solid dispersion with PROG and HPMCAS is formed on melting. Fig. 11 compares PROG-LEU and PROG-LEU-HPMCAS at  $120^\circ\text{C}$  where the difference in polymorph can be clearly seen.



**Fig. 10.** DSC data comparing a typical PROG-LEU thermogram (Black, Experiment 1) against PROG-LEU-HPMCAS (Red). Note the two melting points in PROG-LEU corresponding to Form II (~121 °C) and Form I (~129 °C) respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 11.** VT-PXRD data at 120 °C for A) PROG-LEU-HPMCAS (Blue) and B) PROG-LEU (Green). Compared to C) PROG Form II reference (Red) and D) PROG Form I Reference (Black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



## 4. Conclusion

Inhalable formulations of PROG have been generated through a spray drying method which caused the complete transformation of PROG to the Form II polymorph regardless of the solvent system used. The effect of solvent and organic:aqueous solvent ratio in the spray drying feedstock was investigated showing these to influence the aerodynamic properties of the formulations. Spray drying with the addition of HPMCAS and LEU showed a beneficial effect on the solubility of the PROG and changed the lung deposition profile of PROG with a higher proportion depositing in the lower airways. The addition of HPMCAS to the formulation was also shown to prevent PROG Form II polymorph from recrystallisation to Form I polymorph on heating. The generation of these formulations may assist in the generation of a PROG DPI as an alternative to traditional oral formulations. Further studies are required to focus on generating a more uniform formulation with respect to GSD through adjusting spray drying parameters or the addition of excipients. Furthermore, understanding the mechanism for preventing Form II to Form I transformation and the temporary increase in solubility with the addition of HPMCAS will need future study.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejpb.2023.06.018>.

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