The influence of spray drying parameters on phase behaviour, drug distribution and *in vitro* release of injectable microspheres for sustained release

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ABSTRACT
For ternary solid dispersions it is indispensable to characterize their structure, phase behaviour and the spatial distribution of the dispersed drug as this might influence the release profile and/or stability of these formulations. This study shows how formulation (feed concentration) and process (feed rate, inlet air temperature and atomizing air pressure) parameters can influence the characteristics of ternary spray-dried solid dispersions. The microspheres considered here consist of a poly(lactic-co-glycolic acid) (PLGA) surface layer and an underlying polyvinylpyrrolidone (PVP) phase. A poorly soluble active pharmaceutical ingredient (API) was molecularly dispersed in this matrix. Differences were observed in component miscibility, phase heterogeneity, particle size, morphology as well as API surface coverage for selected spray drying parameters. Observed differences are likely due to changes in the droplet generation, evaporation and thus particle formation processes. However, varying particle characteristics did not influence the drug release of the formulations studied, indicating the robustness of this approach to produce particles of consistent drug release characteristics. This is likely due to the fact that the release is dominated by diffusion from the PVP layer through pores in the PLGA surface layer and that observed differences in the latter have no influence on the release.
INTRODUCTION

Injectable sustained release formulations offer multiple advantages for the treatment of chronic diseases. Side effects of drugs with a narrow therapeutic window can be limited and importantly the administration frequency can be reduced to significantly improve patient compliance. This is expedient in the treatment of chronic diseases and crucial for the therapy of viral infections like those with the human immunodeficiency virus (HIV). In this case controlled release of the drug assures minimal inhibitory drug concentrations and thereby avoids the development of viral resistance. Additionally the sustained release of appropriate amounts of drug resulting in constant low drug plasma concentrations is sought-after for HIV pre-exposure prophylaxis. Different injectable sustained release formulations are already marketed. For example Trelstar® Depot (Debio RP),¹ Sandostatin LAR® (Novartis Pharmaceuticals),² and Risperdal®Consta® (Janssen)³ which are based on the biodegradable polymer poly(lactic-co-glycolic acid) (PLGA) as a carrier.

We previously reported on the development of spray-dried polymeric microspheres for intramuscular injection for the long-term prophylaxis of infection with HIV.⁴,⁵ These shell structured microspheres consist of two biocompatible polymers, water-insoluble poly(lactic-co-glycolic acid) (PLGA), and water-soluble polyvinylpyrrolidone (PVP). It was hypothesized that the function of the PLGA in the formulation is to form a phase separated surface layer so as to provide the required slow release characteristics of the formulation. The underlying PVP phase was used to increase the solubility and dissolution rate of a poorly soluble active pharmaceutical ingredient (API) by forming a solid dispersion. A model formulation was prepared by spray drying where the resulting microspheres consisted of a ternary solid dispersion API/PLGA/PVP 30/25/45 wt%. The model drug used was a poorly soluble HIV protease inhibitor.
Various studies and reviews have already discussed the influence of spray drying parameters on the resulting pharmaceutical product. The majority of these studies describe how various spray drying parameters influence particle size and morphology and/or investigate binary systems. In contrast to previous work, the present study focusses on how spray drying parameters (both process and formulation) effect phase behaviour and spatial drug distribution of ternary solid dispersions and the consequences for in vitro release behaviour of these systems. Phase behaviour and spatial drug distribution can be decisive for the release characteristics of a formulation. For example, phase behaviour might influence the release, as particles containing a poorly soluble drug in the form of amorphous precipitates in the polymeric matrix are likely to have a slower release compared to ideal glass solutions where the drug is molecularly dispersed within a matrix. Spatial drug distribution might also influence the observed release. For instance, when a drug-rich phase containing a poorly soluble drug is exposed to a dissolution medium, the dissolution will be drug-controlled, characterized by slow dissolving of the drug, compared to a faster dissolving polymer. In contrast in a glass solution the release will be controlled by the dissolution of the polymer.

For the current study feed concentration was selected as a formulation parameter, whereas feed rate, inlet air temperature and atomizing air pressure were the process parameters tested. The particle size and morphology, phase behaviour and API surface coverage of the resulting microspheres were then characterized. The drug surface coverage is defined by the API distribution throughout the microspheres and hence can be used as an indicator for the latter.

In this study a variety of complementary techniques were used to characterize the spray-dried samples. Firstly the phase behaviour of three model formulations was studied by means of modulated differential scanning calorimetry (MDSC). Secondly we examined the chemical
composition of the sample surface by time-of-flight secondary ion mass spectrometry (ToF-SIMS). Other factors which might influence the release, such as particle size\textsuperscript{17-19} and surface morphology (e.g. smoothness, porosity)\textsuperscript{19} were studied via scanning electron microscopy (SEM). Release experiments were performed in a surfactant containing phosphate buffer at pH 7.

In these complex ternary systems (API/PLGA/PVP) it is indispensable to have an insight into the structure of the binary polymeric matrix and the spatial distribution of the API as this might influence the release profile and/or stability of these formulations. Additionally, the findings of the present study were used to elucidate the underlying release mechanism of these ternary API/PLGA/PVP microspheres.

**EXPERIMENTAL SECTION**

**Materials**

Poly(lactic-co-glycolic acid) (PLGA) (lactide:glycolide molar ratio of 75:25, inherent viscosity of 0.2 dl/g) was purchased from PURAC Biomaterials (Gorinchem, The Netherlands). Polyvinylpyrrolidone K30 (PVP K30) (MW 44-54 kDa) was kindly donated by BASF (Ludwigshafen, Germany). The API was a poorly soluble investigational compound provided by Janssen (Beerse, Belgium). Disodium hydrogenphosphate dodecahydrate (Na\textsubscript{2}HPO\textsubscript{4}.12H\textsubscript{2}O) and formic acid were provided by Chemlab (Zedelgem, Belgium). Sodium hydrogenphosphate monohydrate (NaH\textsubscript{2}PO\textsubscript{4}.H\textsubscript{2}O) was supplied by Merck (Darmstadt, Germany). Acros Organics (Geel, Belgium) supplied dimethylformamide (DMF) and ammonium formiate. Polysorbate 80 (Tween 80) was obtained from Alfa Aesar GmbH (Karlsruhe, Germany). Polyethyleneglycol 400 (PEG) was purchased from Fagron (Waregem,
Belgium). Dichloromethane (DCM) and acetonitrile (ACN) were provided by Fisher Scientific (Leicestershire, United Kingdom). All solvents used were of HPLC or analytical grade. Ultrapure water was produced with an Elga Maxima system (Elga Ltd. High Wycombe Bucks, United Kingdom).

Methods

Spray drying

All samples were spray dried with a Micro Spray lab scale spray dryer (ProCepT, Zelzate, Belgium) starting from a feed solution in dichloromethane (DCM) and with a constant co-current drying air with a flow rate of 0.2 m³/min.

The model formulation, API/PLGA/PVP 30/25/45 wt%, was spray dried with varying formulation and process parameters. Feed concentration was selected as a formulation parameter, whereas feed rate, inlet air temperature and atomizing air pressure were the process parameters tested. Each parameter studied was evaluated at a low and a high level with all other parameters at the middle level (reference). Samples were compared to a reference sample (middle level) (Table 1). Three reference samples were independently spray-dried (Reference A, B and C).

The binary samples API/PLGA and API/PVP were spray dried under the same conditions as the reference samples.

Physical mixtures

Physical mixtures of amorphous spray-dried PVP K30 and API were prepared according to the rules of geometrical blending using a mortar and pestle.

In vitro drug release
Release experiments were performed at room temperature in rotating test tubes (17 rpm) containing an amount of the spray dried powders corresponding to an API dose of 0.6 mg in 40.0 mL release medium. In this way sink conditions were assured throughout the experiment. The dissolution medium consisted of phosphate buffer at pH 7 containing 0.25% Tween 80 and 2.5% PEG 400. Samples were collected at 5, 15, 30, 60 and 240 minutes, filtered over a Chromafil RC-20/15 cellulose acetate filter with a pore size of 0.2 µm (Macherey-Nagel, Düren, Germany) and subsequently analysed by high performance liquid chromatography (HPLC) with UV detection. Experiments were performed in triplicate.

**Solubility determination**

The solubility of the API in an aqueous solution without PVP as well as in presence of 0.5, 1, 2 and 5 % PVP K30 was determined. Therefore 10 mL of the polymer solution was transferred into a test tube and an excess amount of API was added. These tests were performed at room temperature and test tubes were rotated for 48 hours at 17 rpm. Subsequently these samples were filtered over a Chromafil RC-20/15 cellulose acetate filter with a pore size of 0.2 µm and analyzed by HPLC with UV detection. Experiments were performed in triplicate.

**High performance liquid chromatography (HPLC) assay**

A Waters HPLC system (Milford, USA) consisting of a Waters 1525 Binary HPLC pump, a Waters 717plus Autosampler, set at a 10 µl injection volume, and a Waters 2487 Dual Lambda Absorbance detector, set at a detection wavelength of 255 nm, were used to quantify drug release. A Waters Sunfire C18 3.5 µm (4.6 mm × 100 mm) column was utilised. Samples were injected in duplicate and analyzed using a 1.0 mL/min flow rate at room temperature. The average result of both injections was reported. The mobile phase consisted
of a 50 mM formiate buffer (pH 4) and acetonitrile (57/43, v/v). The pH was measured using a WTW 330i pH meter (Weinheim, Germany). The buffer was filtered over a 0.45 µm polytetrafluoroethylene (PTFE) filter and the mobile phase was degassed prior to use. Standard curves were made in dimethylformamide (DMF) and were linear over the concentration range used (1.56-100 µM). Data were analyzed using Breeze software Version 3.30 (Waters).

**Statistical analysis**

For each time point (5, 15, 30, 60, 120 and 240 min) statistical differences between the observed *in vitro* release were evaluated for all samples via one-way ANOVA. A Bonferroni post-hoc test was performed at an \( \alpha \) level of 0.05. (GraphPad Prism 5 for Windows; GraphPad Software Inc., San Diego, USA).

**Modulated differential scanning calorimetry**

The bulk phase behaviour of the spray-dried microspheres was determined by MDSC (Q2000, TA Instruments, Leatherhead, UK). Thermal Analysis Software (Version 4.4A) was used to analyze the obtained data. Crimped aluminum pans (TA Instruments, Brussels, Belgium) were selected for the analysis of the samples. An empty pan was used as a reference and the masses of the reference pan and of the sample pans were taken into account. The DSC cell was purged with a nitrogen flow rate of 50 mL/min. Indium and n-octadecane were used for temperature calibration. The enthalpic response was calibrated with indium. The modulation parameters used were: a heating rate of 1°C/min, a period of 40 s and an amplitude of 1°C. Calibration of the heat capacity was done using sapphire. Samples were analyzed from -20°C to 220°C. Glass transitions were analyzed in the reversing heat flow signals.
Scanning electron microscopy

SEM was used to gain insight into the morphology and particle size of the samples which were prepared by fixing an amount of powder on an aluminum stub using double-sided carbon tape. The samples were coated with a gold-palladium mixture by sputtering for 45 s at 20 mA. Field emission gun scanning electron micrographs (FEG-SEM) were taken by using a Philips XL30 ESEM-FEG instrument (Philips, Eindhoven, The Netherlands) at an acceleration voltage of 10 kV.

Time-of-flight secondary ion mass spectrometry

Spray-dried samples were adhered to double-sided adhesive tape in order to produce an immobile surface suitable for ToF-SIMS analysis. The data were acquired using a ToF-SIMS IV instrument (ION-TOF GmbH) equipped with a bismuth liquid metal ion gun and a single-stage reflectron analyzer. Typical operating conditions utilised a 25 kV Bi$_3^+$ primary ion source with a pulsed target current of approximately 0.3 pA. A flood gun producing low energy electrons (20 eV) was used to compensate for surface charging caused by the positively charged primary ion beam on the insulating sample surface. A 4 mm × 4 mm area of each sample was raster scanned at a resolution of 100 pixels per mm. PLGA and PVP were identified using C$_6$H$_7$O$_4^-$ (m/z = 143), and C$_5$H$_8$O$^-$ (m/z = 84) respectively. The API was characterized by C$_5$H$_{11}$SO$_5^-$ (m/z = 183). Prior to sample analysis, reference materials were analyzed and the characteristic ion peaks C$_6$H$_7$O$_4^-$, C$_5$H$_8$O$^-$ and C$_5$H$_{11}$SO$_5^-$ were selected and only present in PLGA, PVP and API respectively. Negative polarity ToF-SIMS spectra showing the markers for API, PLGA and PVP are shown in Figure 1 of the Supporting Information. Static conditions were ensured by keeping the total primary ion beam dose for every analyzed area below $1 \times 10^{12}$ions/cm$^2$ throughout the analysis. Data in the negative
secondary ion polarities were collected and analyzed using SurfaceLab 6 software (IONTOF). The data were acquired using the 'high current bunched’ setting on the instrument to achieve high mass resolution. For any given sample, the measured secondary ion intensity for each polymer marker peak was normalized to the total intensity count to enable a semi-quantitative comparison of the different samples.

RESULTS

1. Influence of spray drying parameters on particle characteristics and in vitro release

Morphological characterization

Particle size and morphology of samples produced by varying spray drying parameters was evaluated by SEM. Although a statistically significant number of particles were not analyzed, SEM could be used as an indicator for both particle size and morphology. Both parameters demonstrated to be comparable for all samples with the exception of the sample prepared from a feed solution with a concentration of 1% (Fig. 1). Particles spray-dried from a 1% feed concentration (the low level for this parameter, Table 1) appeared to be smaller and had a ‘shriveled’ morphology. This was in contrast to samples spray-dried under all other conditions (Table 1) which exhibited spherical particles, with a smooth, intact surface and an estimated diameter approximately between 2 µm and 7 µm (Fig. 1).

Miscibility

MDSC was used to thermally characterize the pure compounds as well as the miscibility behaviour of the spray-dried microspheres. The spray-dried pure compounds were identified by their glass transition temperature ($T_g$) being approximately 38°C, 56°C and 174°C for the
PLGA, API and PVP respectively under the given experimental conditions. MDSC was utilized to study the bulk miscibility of the spray-dried samples through their $T_g$s, which were observed in the reversing heat flow curves (Fig. 2 and Table 2). For each sample two mixing $T_g$s were observed, the first one approximating to the $T_g$ of PLGA and the second one shifting towards the $T_g$ of pure PVP (Fig. 2). The $T_g$ of the API (around 56°C) was not observed in the thermograms.

In order to study the influence of various spray drying conditions not only the observed temperature values for the $T_g$s were evaluated but also the width of the $T_g$ range was evaluated. The width of the $T_g$ range provides information regarding the heterogeneity of the amorphous phase whereby the broader the $T_g$ range, the more heterogeneous the amorphous.20-22 Differences in $T_g$ values and the width of the $T_g$ ranges were observed for various samples (Table 2). As preliminary studies demonstrated the high reproducibility of the MDSC measurements only one measurement per sample was carried out. The formulations spray-dried with a feed concentration of 1% or an atomizing air pressure of 1.5 bar resulted in a broader $T_g$ range for the PLGA-rich phase ($T_g1$). The formulation spray-dried with a feed concentration of 1% showed a higher $T_g$ of the PVP-rich phase ($T_g2$). Samples prepared with a feed rate of 10 mL/min or an atomising air pressure of 1.5 bar showed a broader $T_g$ range for the PVP-rich ($T_g2$). In contrast, the sample spray-dried with a feed concentration of 10% resulted in a narrower $T_g$ range for the PVP-rich phase (Table 2).

**API surface coverage**

The API surface coverage of the samples was compared by ToF-SIMS. The spatial distribution of the API and PLGA at the sample surface of a reference formulation and the formulation prepared with a feed concentration of 1% is represented in Figure 3. A negligible amount of PVP is detected at the particle surface, which corresponds with previous studies.4-5
At the micron-scale spatial resolution of the ToF-SIMS data acquired the drug appears to be homogeneously distributed at the surface of the three model formulations, illustrated by the absence of separate bright red (API) spots. Figure 4 shows the measured API intensity (normalized to total counts) at the sample surface and hence depicts a relative measure of API surface coverage for the various formulations. It is clear that one sample has a higher presence of API at the microsphere surface, namely the sample spray-dried with a low feed concentration (1%).

**Release behaviour**

*In vitro* release testing showed no significant differences for formulations spray-dried under the selected formulation and process parameters. Release profiles of samples produced under these varying conditions (Table 2) were compared to those of the three independently spray-dried reference samples. The release behaviour of selected formulations is depicted in Figure 5. Statistical differences between the observed release of the various samples were evaluated via ANOVA for each time point (5, 15, 30, 60, 120 and 240 min). The only statistical significant difference observed was at the first time point (5 min) for the sample spray-dried with a low feed rate (2 mL/min). This sample had at this point a lower release compared to all other samples.

2. **Elucidation of the release mechanism**

**Function of PLGA**

To confirm the role of PLGA in the formulation (API/PLGA/PVP 30/25/45 wt%), binary formulations consisting of API/PLGA 10/90, 20/80 and 30/70 wt% were spray dried. MDSC analysis showed one mixing $T_g$ for each of the formulations. The value of this $T_g$ increased with increasing API content (Fig. 6A). *In vitro* release testing gave comparable results for the
three formulations (Fig. 7A). After five minutes averagely 4.2% (± 0.3%) of the API was released. This percentage did not significantly increase in the further course of the experiment with a total average drug release of ± 4.9% (± 0.8%) after four hours.

**Function of PVP**

To assess the role of PVP in the formulation, binary formulations consisting of API/PVP 10/90, 20/80 and 30/70 wt% were spray dried. Again, MDSC analysis showed one mixing $T_g$ for each of the formulations with a decreasing value of the observed $T_g$ as API content increases (Fig. 6B). Formulations API/PVP 20/80 and 30/70 wt% showed a release comparable to the ternary formulation (API/PLGA/PVP 30/25/45 wt%) (Fig. 7B). Formulation API/PVP 10/90 wt% demonstrated a higher initial release but had a comparable cumulative drug release after four hours.

Additionally the influence of the presence of PVP was investigated by performing release tests on physical mixtures of spray-dried API and PVP. The following mixtures were tested, API/PVP 10/90, 20/80 and 30/70 wt%. After four hours these physical mixtures had released a similar amount of drug compared to the spray-dried ternary formulation (API/PLGA/PVP 30/25/45 wt%) (Fig. 7C). The observed difference in release is the absence of a burst release for the physical mixtures. Furthermore the influence of the presence of PVP upon the solubility of the API was assessed. These results are shown in Figure 2 of the Supporting information, where drug solubility is depicted as a function of the percentage of PVP present. This graph illustrates the linear increase in API solubility with an increasing amount of PVP present. The initial aqueous solubility of this drug was 0.018 mg/mL. This value increased up to 0.045 mg/mL after addition of 5% of PVP K30.
DISCUSSION

1. Influence of spray drying parameters on particle characteristics and *in vitro* release

The influence of various spray drying parameters on particle morphology and size was investigated. These are important sample characteristics as particle morphology influences decisive powder characteristics such as flowability and powder density.\(^{23-25}\) Particle size has already repeatedly been reported as having an influence upon release behaviour of microspheres.\(^{17-19}\)

Microspheres spray-dried from a feed concentration of 1% differed from all other samples, with a smaller particle size and ‘shriveled’ morphology. The reduction in particle size may originate from a decreased viscosity of the feed solution compared to the other samples as well as from a later solidification from droplet into particle.

Viscosity of a solution is determined by the identity and concentration of compounds dissolved. For a given solute-solvent system a lower concentration results in a lower viscosity of that solution. When spray drying, viscosity of the feed solution will determine droplet size during the atomization process and therefore the particle size of the final particles. The lower the viscosity the smaller the droplets and resulting particles.\(^{8,26,27}\)

Additionally, the lower the concentration of a compound in the feed solution, the further it will be from its solubility limit and the longer it will take to reach the solidification point during the particle formation process. This results in a longer evaporation time with smaller droplets and hence smaller particles as a potential result.\(^8\)

MDSC was used to thermally study the phase behaviour of all samples. Preceding work has already shown that spray drying a formulation composed of API/PLGA/PVP 30/25/45 wt% results in hollow spheres with a PLGA-rich surface layer (containing small amounts of PVP),
an underlying PVP-rich layer (containing small amounts of PLGA) and a molecular dispersion of the API in these polymeric layers.\textsuperscript{4,5}

The present study confirms these findings by the observation of two mixing $T_g$s for all samples, the first one approximating the $T_g$ of PLGA and the second one shifting towards the $T_g$ of pure PVP (Fig. 2 and Table 2). In addition, the absence of a $T_g$ around 56°C ($T_g$ of the API) indicates that the API is molecularly dispersed in a phase separated system made up of a PLGA-rich phase and a PVP-rich phase. We want to remark that the expression “molecular dispersion” based on DSC data does only take into account heterogeneity/homogeneity above ca. 30 nm domain size.

MDSC demonstrated that changing spray drying parameters not only affects the glass transition temperature ($T_g$) but also the width of the $T_g$ range ($\Delta T_g$) (Fig. 2, Table 2). The formulations spray-dried with a feed concentration of 1\% or an atomising air pressure of 1.5 bar resulted in a broader $T_g$ range for the PLGA-rich phase indicating a more heterogeneous PLGA phase for these samples compared to the reference samples. Moreover, the sample spray-dried with a feed concentration of 1\% showed a higher $T_g$ of the PVP-rich phase. This might indicate a lower presence of PLGA and/or API in this phase as these compounds have a plasticizing effect on the PVP phase due to their lower $T_g$. Samples prepared with a feed rate of 10 mL/min or an atomising air pressure of 1.5 bar showed a broader $T_g$ range for the PVP-rich phase indicating a more heterogeneous PVP phase for these samples compared to the reference samples. In contrast, the narrower $T_g$ range for the PVP-rich phase of the sample spray-dried with a feed concentration of 10\% implies a more homogeneous PVP phase for this sample. These observations clearly indicate that varying spray drying parameters influences component miscibility and phase heterogeneity of the
samples. This might have consequences for release behaviour and stability performance of these formulations.

Surface characteristics such as API surface coverage might also significantly influence the behaviour of the formulation, both in terms of release characteristics and stability. Therefore, ToF-SIMS was used to analyse the surface of the different spray-dried samples. The results indicated that only the sample spray-dried with a feed concentration of 1% contained a significantly higher amount of API at the microsphere surface compared to the reference.

It was already noted that the viscosity of the feed solution influences particle size. Additionally feed viscosity can influence the distribution of a compound throughout the particle and hence surface coverage. The Stokes-Einstein equation (equation 2) shows the influence of viscosity on the diffusion coefficient of a compound and hence on its distribution in the spray-dried particle.\(^8\)

\[
D = \frac{k_B T}{6\pi\eta r}
\] (2)

In this equation D is the diffusion coefficient, \(r\) the globular radius, \(T\) the absolute temperature and \(\eta\) the viscosity of the solution. \(k_B\) is the Boltzmann constant.

The lower the concentration of a compound in the feed solution the further it will be from its solubility limit and the longer it will take to reach the solidification point during the particle formation process.\(^8\) This possible difference in solute deposition time might also influence the spatial distribution of the compounds and potentially causes the observed increase in API surface coverage for the sample spray-dried with a lower feed concentration.

Another hypothesis is that migration of the API to the sample surface might be more pronounced for this sample because of the slower droplet evaporation and hence particle

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formation. Evidence of preferential migration of a compound towards the droplet-air interface exists, where studies have reported surface segregation of apolar side groups at a polymer/air interface, including non-polar methyl side groups as present in PLGA.\cite{4,28-31} The same mechanism of surface restructuring might occur in the spray-dried particles and account for the increased presence of API at the particle surface.

These findings are also complementary with the MDSC data where it was observed that the sample spray-dried from a 1% feed concentration possessed a higher $T_g$ for the PVP-rich phase compared to the reference sample. It can now be confirmed that this is most likely due to the lower amount of drug present in the PVP phase of this sample. Analogously the more heterogeneous PLGA phase, characterized by a broader $T_g$ range, can be ascribed to the increased presence of API in the PLGA layer of this sample.

For the investigated spray drying parameters it is observed that feed concentration is the most determinative parameter and must therefore be controlled most rigorously. A low feed concentration resulted in smaller, differently shaped particles with divergent phase behaviour and enrichment of the drug at their surface. These different characteristics might have consequences for the behaviour of the formulation, with respect to both release and stability.

**Release behaviour**

Besides the influence of varying spray drying parameters on particle characteristics, the influence upon release behaviour of the resulting formulations was investigated and compared to that of three independently spray-dried reference samples. *In vitro* release testing showed that even when microsphere characteristics were influenced by varying the spray drying parameters this was overall not reflected in significant differences in their release profiles.
(Fig. 5). In order to explain these observations the underlying release mechanism was investigated. To do so additional release testing of binary API/PVP and API/PLGA formulations, as well as of physical mixtures of API and PVP was performed to gain insight in to role of PLGA and PVP in these formulations.

2. **Elucidation of the release mechanism**

**Function of PLGA**

For the three spray-dried API/PLGA samples the presence of one mixing $T_g$ confirmed the presence of drug in the PLGA matrix as a glass solution. The observed mixing $T_g$ increased with increasing API content. This is in agreement with the Gordon-Taylor equation which states that if the weight percentage of this compound increases, the mixing $T_g$ shifts towards the $T_g$ of the compound having the highest $T_g$. Therefore an increase in the amount of API ($T_g$ 56°C) versus the amount of PLGA ($T_g$ 38°C) results in an augmented value for the observed mixing $T_g$.

These samples showed a very restricted drug release (4.9% after four hours), which is not surprising because this water-insoluble polymer is well established as a matrix for sustained release, formulations.¹⁻³

**Function of PVP**

For the three spray-dried API/PVP samples the presence of one mixing $T_g$ confirmed that the drug was present in the PVP matrix as a glass solution. The observed mixing $T_g$ decreased with increasing API content. This again correlates with the Gordon-Taylor equation which predicts a lowered value for the observed mixing $T_g$ if the amount of API ($T_g$ 56°C) increases. The molecular dispersion of the drug increases its solubility and consequently increases its release.
The initial increase in drug release was the highest for the samples API/PVP 10/90 wt%. This can be explained by the fact that this formulation has the highest polymer to drug ratio which is known to have a beneficial influence upon drug solubility, and here consequently upon drug release. Solubility testing in the presence of PVP proved that API solubility increased with increasing PVP concentration and therefore the role of PVP in the ternary formulations is dual. Firstly PVP increases drug release via an increased drug solubility due to the molecular drug dispersion in this matrix. Secondly the presence of PVP has a beneficial effect on the drug solubility (Fig. 2, Supporting Information).

A noteworthy difference between the release profiles of the spray-dried API/PVP and API/PLGA/PVP formulations on the one hand and the physical mixtures of API/PVP on the other hand is the absence of a burst release of the latter in contrast to the spray-dried samples. Hence, the observed burst release can be ascribed to the additional solubility enhancement from processing the drug as a solid dispersion.

**Release mechanism**

In a previous study we described how the spray-dried polymeric matrix evolves upon exposure to increased humidity. When exposed to increased humidity a rearrangement occurs whereby a decrease in PLGA present at the surface is observed, coupled with an increased presence of PVP. This knowledge combined with our current findings and additional release testing of binary API/PVP and API/PLGA formulations resulted in a hypothesis for the underlying release mechanism as illustrated schematically in Figure 8.

It is postulated that in the investigated time frame (4 h) the release mechanism is dominated by fast PVP leaching from the small domains of PVP present in the PLGA layer due to the high solubility of PVP. The resulting pores in the PLGA surface layer allow further ingress of release medium followed by rapid dissolution of the molecularly dispersed API followed
by diffusion out of the microspheres. The observed differences in the PLGA surface layer, such as API surface enrichment, will therefore have no significant influence in this stage of the release.

In general terms, these injectable formulations comprising of a combination of a water-soluble polymer (PVP) and a water-insoluble polymer (PLGA) was used. The water-soluble polymer increased the solubility of a poorly soluble API whereas the water-insoluble polymer assured the sustained release behaviour of the formulation.

Combinations of water-soluble and water-insoluble polymers have already been reported for oral formulations. For example the combination of ethyl cellulose (EC), water-insoluble, and hydroxypropyl cellulose, water-soluble, for oral administration has been described with pore formation due to HPC leaching after immersion in aqueous media as driving force of drug release.33 These findings are in agreement with the release mechanism proposed in the present study of an injectable matrix for a poorly soluble drug.

**CONCLUSIONS**

This study demonstrated how formulation and process parameters influence the characteristics of spray-dried microspheres. Differences were observed for miscibility and heterogeneity of the samples, particle size and morphology as well as API surface coverage. Observed differences are likely due to changes in the droplet evaporation and the subsequent particle formation process. For the investigated spray drying parameters at their assessed levels, feed concentration is the parameter to be controlled most rigorously as a low feed concentration resulted in divergent particle size, particle shape, compound miscibility, phase heterogeneity and API surface coverage. Despite the observed differences, varying particle characteristics did not influence the release behaviour of the formulation studied. This is likely due to the
proposed release mechanism where the release is dominated by diffusion from the PVP layer through pores in the PLGA surface layer. Hence, for the timeframe tested, spray drying resulted in a formulation with robust drug release characteristics. Moreover, it was confirmed that the function of the PLGA in these formulations is to form a phase separated surface layer so as to assure the required slow release characteristics of the formulation, whereas the underlying PVP phase increases the solubility and hence dissolution rate of a poorly soluble drug by forming a solid dispersion.

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Figure 1. Scanning electron micrographs of selected formulations spray-dried under various conditions, where a = Reference and b = Feed concentration of 1%.
Figure 2. MDSC of selected formulations spray-dried under various conditions, where a = Reference, b = Feed concentration 1%, c = Feed rate 10 mL/min, d = Inlet air temperature 135°C. $T_g$ values and width of the $T_g$ range for these samples are reported in Table 2.
Figure 3. ToF-SIMS ion intensity maps of selected formulations spray-dried under various conditions, where A = Reference and B = Feed concentration 1% samples (4 × 4 mm² scan size). Panel a shows results of the total intensity signal. Panels b and c show negative polarity images of respectively API (m/z = 183, C₅H₁₁SO₅⁻) and PLGA (m/z = 143, C₆H₇O₄⁻). Panels d show the negative polarity overlay images (API in red, PLGA in green).
Figure 4. I. Histogram representing the intensity at m/z 183 (secondary ion indicative of API) for all formulations spray-dried under various conditions, where A = Reference A, B = Reference B, C = Reference C, D = Feed concentration 1%, E = Feed concentration 10%, F = Inlet air temperature 95°C, E = Inlet air temperature 135°C, F = Atomising air pressure 1.0 bar, G = Atomising air pressure 1.5 bar, H = Feed rate 2 mL/min, I = Feed rate 10 mL/min (1 × 1 mm² scan size, n=4)

II. Negative ToF-SIMS spectra at m/z 183 of selected samples.

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Figure 5. Release behaviour of selected spray-dried API/PLGA/PVP formulations.
Figure 6. MDSC of spray-dried binary API/polymer combinations.

I. API/PLGA, II. API/PVP

a. 10/90 wt%, b. 20/80 wt%, c. 30/70 wt%
Figure 7. Release behaviour of binary API/polymer combinations.

I. Spray-dried API/PLGA, II. Spray-dried API/PVP, III. Physical mixture API/PVP.
Figure 8. Hypothesized release mechanism. PLGA in green, PVP in red and API in yellow. Left, before exposure to an aqueous release medium. Right, after exposure to an aqueous release medium.
Table 1. Overview of the spray drying parameters studied and the levels at which these parameters were evaluated.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inlet air temperature (°C)</th>
<th>Feed concentration (%)</th>
<th>Feed rate (mL/min)</th>
<th>Atomising air pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low level</td>
<td>95</td>
<td>1</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>Middle level</td>
<td>115</td>
<td>5</td>
<td>6</td>
<td>1.25</td>
</tr>
<tr>
<td>High level</td>
<td>135</td>
<td>10</td>
<td>10</td>
<td>1.50</td>
</tr>
</tbody>
</table>
Table 2. $T_g$ values and width of the $T_g$ range for samples spray-dried with varying process and formulation parameters.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_g1$ (°C)</th>
<th>Width $T_g1$ (°C)</th>
<th>$T_g2$ (°C)</th>
<th>Width $T_g2$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference A</td>
<td>44.5</td>
<td>3.2</td>
<td>104.6</td>
<td>27.8</td>
</tr>
<tr>
<td>Reference B</td>
<td>44.9</td>
<td>3.3</td>
<td>106.0</td>
<td>29.3</td>
</tr>
<tr>
<td>Reference C</td>
<td>44.7</td>
<td>3.8</td>
<td>104.4</td>
<td>27.7</td>
</tr>
<tr>
<td>Feed concentration 1%</td>
<td>44.3</td>
<td>7.6</td>
<td>125.9</td>
<td>29.1</td>
</tr>
<tr>
<td>Feed concentration 10%</td>
<td>45.4</td>
<td>2.8</td>
<td>104.1</td>
<td>21.3</td>
</tr>
<tr>
<td>Feed rate 2 mL/min</td>
<td>44.2</td>
<td>4.3</td>
<td>103.9</td>
<td>29.6</td>
</tr>
<tr>
<td>Feed rate 10 mL/min</td>
<td>43.5</td>
<td>4.6</td>
<td>106.1</td>
<td>36.5</td>
</tr>
<tr>
<td>Inlet air temperature 95°C</td>
<td>44.2</td>
<td>4.0</td>
<td>103.6</td>
<td>28.6</td>
</tr>
<tr>
<td>Inlet air temperature 135°C</td>
<td>44.9</td>
<td>3.4</td>
<td>105.7</td>
<td>28.3</td>
</tr>
<tr>
<td>Atomising air pressure 1.0 bar</td>
<td>45.3</td>
<td>2.8</td>
<td>107.0</td>
<td>27.9</td>
</tr>
<tr>
<td>Atomising air pressure 1.5 bar</td>
<td>43.3</td>
<td>6.0</td>
<td>106.0</td>
<td>36.6</td>
</tr>
</tbody>
</table>
Figure 1. Negative ToF-SIMS spectra showing the markers for API (m/z = 183, C₅H₁₁SO₅⁻), PLGA (m/z = 143, C₆H₇O₄⁻), and PVP (m/z = 84, C₅H₈O⁻)
Figure 2. Solubility of the API in the presence of PVP K30.