In vivo evaluation of different formulation strategies for sustained release injectables of a poorly soluble HIV protease inhibitor

Joke Meeus[1], David J. Scurr[2], Katie Amssoms[3], Pieter Annaert[1], Martyn C. Davies[2], Clive J. Roberts[2], Guy Van den Mooter[1*]

[1] Drug Delivery and Disposition, KU Leuven, Herestraat 49, Leuven, Belgium

[2]Laboratory of Biophysics and Surface Analysis, School of Pharmacy, The University of Nottingham, University Park, Boots Science Building, Nottingham, United Kingdom

[3]Pharmaceutical Companies of Johnson & Johnson, Janssen, Discovery Sciences, PD&S_PDM, Turnhoutseweg 30, Beerse, Belgium

* Corresponding author. Drug Delivery and Disposition, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Campus Gasthuisberg ON2, Herestraat 49 b921, 3000 Leuven, Belgium. Tel.: +32 16 330 304, Fax: +32 16 330 305, e-mail: guy.vandenmooter@pharm.kuleuven.be

ABSTRACT

At present no scientific rationale exists for selecting a particular enabling strategy to formulate a poorly water-soluble drug, although this is crucial as it will influence the *in vivo* performance of the resulting formulation. This study provides an insight into this complicated decision making process for a poorly soluble human immunodeficiency virus (HIV) protease inhibitor based upon *in vivo* test results. A formulation strategy based on the molecular dispersion of this active pharmaceutical ingredient (API) into a biphasic matrix consisting of water-insoluble poly(lactic-co-glycolic acid) (PLGA) and water-soluble polyvinylpyrrolidone (PVP) was evaluated. The long-term *in vivo* performance of this strategy was compared to that of other solubility enhancing approaches by evaluating the exposure in male Beagle dogs. Solid dispersions, based on a PLGA/PVP matrix, were compared to solid dispersions in a pure water-insoluble PLGA matrix. Additionally these solid dispersion strategies were compared to the strategy of particle size reduction by means of an API microsuspension. The *in vivo* performance of the various formulations over a period of 28 days after intramuscular injection was evaluated by the observed initial burst release, plasma concentration-time profiles, time at which maximum plasma levels were reached ($t_{max,obs}$) and the estimated bioavailability. Compared to the other formulation strategies assessed, it was concluded that the addition of PVP in a PLGA matrix resulted *in vivo* in a more sustained release as well as a higher amount of drug released from the polymeric matrix. This was explained based on the structure of these binary PLGA/PVP matrices where the pore network originating from rapidly dissolving PVP plays a crucial role. Moreover, the results suggest that the release of this type of formulations could be delayed by increasing the amount of PLGA in the formulation.

Key words (max 6)

solid dispersions, PLGA, microspheres, controlled release, in vivo

INTRODUCTION

Contemporary drug pipelines contain an increasing number of poorly water-soluble candidates. To overcome this problem, solubility enhancing technologies often focus on impacting aspects of the modified Noyes-Whitney relationship by increasing dissolution rate or drug solubility. Examples of these approaches for solubility enhancement are the solid dispersion of a poorly soluble active pharmaceutical ingredient (API) in an inert matrix, particle size reduction, the use of co-crystals, inclusion complexation with cyclodextrins and lipid based systems [1]. At present no scientific rationale exists for selecting a particular enabling strategy, although this is crucial as it will influence the *in vivo* performance of the resulting formulation. The present study provides an insight into this complicated decision making process for a poorly soluble human immunodeficiency virus (HIV) protease inhibitor (PI) based upon *in vivo* test results.

This poorly soluble model compound needs formulation into an effective long-acting medicine for HIV pre-exposure prophylaxis therapy. Long-acting injectables have been introduced to prevent the transmission of HIV via pre-exposure prophylaxis and could for example be effective in preventing mother-to-child transmission, transmission within serodiscordant couples as well as protecting intravenous drug users [2]. Long-acting formulations are desirable as dosing frequency will be significantly reduced, favouring therapy compliancy. Additionally, they allow sustained release of appropriate amounts of drug resulting in constant low drug plasma concentrations which is sought-after for this HIV pre-exposure prophylaxis approach.

In view of this, we previously reported on the development of spray-dried polymeric microspheres for intramuscular injection for the long-term pre-exposure prophylaxis of

infection with HIV [3,4]. Our formulation strategy was based on the solid dispersion of a poorly soluble API in a polymeric matrix consisting of water-soluble polyvinylpyrrolidone (PVP) and water-insoluble poly(lactic-co-glycolic acid) (PLGA). This combination of materials aimed to secure both solubility enhancement by molecular dispersion of the drug (in PVP) and long-term release (by PLGA) (strategy 1).

In the present study the *in vivo* behaviour of the formulation strategy of a solid dispersion in a binary polymeric matrix combining a water-insoluble polymer (PLGA) with a water-soluble polymer (PVP) (strategy 1) was evaluated in male Beagle dogs. The *in vivo* performance of this formulation strategy was compared to the *in vivo* performance of two other strategies. The second strategy was based on formulating the poorly soluble API as a solid dispersion in a polymeric matrix made up of pure PLGA. PLGA is already well established as a formulation matrix for long-term release as exemplified by commercial products like Trelstar® Depot (Debio RP) [5] and Risperdal®Consta® (Janssen Pharmaceutica) [6]. Hence, the current formulations differed at the level of the polymeric matrix and can be divided in two groups, namely formulations based upon a PLGA/PVP matrix (strategy 1) *vs* formulations made up of a pure PLGA matrix (strategy 2). Consequently the potential benefit of the inclusion of PVP was assessed.

Additionally, these solid dispersion strategies were compared to the strategy of particle size reduction by means of an API microsuspension (strategy 3). Particle size reduction is a well described strategy to increase dissolution rate of poorly soluble compounds. This is exemplified by various publications on micro- and nanoparticles aiming to improve the dissolution performance of a poorly soluble compound [1,7,8]. This strategy has already resulted in the production of successfully marketed formulations such as Invega® Sustenna® [9] (Janssen Pharmaceutica) and Triglide® (Sciele Pharma Inc) [10].

Previously six intramuscularly (IM) injectable sustained release formulations for HIV prophylaxis with a poorly soluble PI were developed and physicochemically characterized [11]. These six formulations (F1-F6, Table 1) represent the three different solubility enhancing strategies selected. F1-F3 exemplify the first strategy, being solid dispersions of the poorly soluble API in a PLGA/PVP-based matrix. A comparison of F1 and F2 demonstrates the influence of an increase in the amount of PLGA (from 25 to 45 wt%). Additionally, F3 was developed to assess the influence of the molecular weight of PVP used. F4 and F5 represent the second formulation strategy which is the solid dispersion of the PI in a pure PLGA matrix. Here, the influence of the manufacturing method was assessed by comparing a spray-dried formulation (F4) to a formulation prepared by the emulsion method (F5). F6 is representative of the third formulation strategy selected, being particle size reduction. Summarized the six formulations differed in composition and manufacturing method and consequently in structural and physicochemical characteristics (Fig. 1) [11]. In the current study the *in vivo* performance of these six formulations, representing three formulation strategies, is evaluated in male Beagle dogs.

EXPERIMENTAL METHODS

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) and polyvinylpyrrolidone K12 PF (PVP K12) (MW 2-3 kDa) were kindly donated by BASF (Ludwigshafen, Germany). The API was a poorly soluble HIV protease inhibitor (PI) provided by Janssen Pharmaceutica (Beerse, Belgium). The structural formula is shown in

Figure 2. Polyvinyl alcohol (PVA) (80% hydrolyzed, MW 9-10 kDa) was obtained from Sigma-Aldrich (Diegem, Belgium). Tocopheryl polyethylene glycol 1000 succinate (TPGS) was supplied by Eastman Chemical Company (Anglesy, UK). Dichloromethane (DCM) was provided by Fisher Scientific (Leicestershire, United Kingdom).

METHODS

Table 1 provides an overview of the composition and manufacturing method of various formulations tested. From here on the formulations will be indicated by their code F1-F6 as shown in Table 1.

Formulation	**Composition (w%)**		**Manufacturing method**
Formulation 1 (F1)	API/PLGA/PVP K30	30/25/45	Spray drying
Formulation 2 (F2)	API/PLGA/PVP K30	30/45/25	Spray drying
Formulation 3 (F3)	API/PLGA/PVP K12	30/45/25	Spray drying
Formulation 4 (F4)	API/PLGA	30/70	Spray drying
Formulation 5 (F5)	API/PLGA	30/70	Emulsion method
Formulation 6 (F6)	API microsuspension		Media milling

Table 1. Overview of formulation composition and manufacturing method.

Formulation manufacturing

Spray drying

F1-F4 were spray dried with a Micro Spray lab scale spray dryer (ProCepT, Zelzate, Belgium) starting from a 5% feed solution in dichloromethane (DCM). The inlet temperature was set to 115°C and the feed rate was 6 mL/min. The co-current drying air had a flow rate of 0.2 m^3/min and the atomizing air was supplied with a pressure of 1.25 bar.

Oil-in-water emulsion method

Microspheres of API/PLGA were produced using an oil-in-water (o/w) emulsion method (F5). 900.0 mg of API was dissolved in 30.0 mL DCM together with 2100.0 mg PLGA. This solution was emulsified in 150.0 mL of a 1.25% PVA solution for 5 minutes using an Ultra Turrax® homogenizer (IKA, Staufen, Germany) at 20,000 rpm to form the o/w emulsion. During homogenization the sample was placed in an ice bath to minimize heating. After addition of 150.0 mL of distilled water, the suspension was stirred overnight with a magnetic stirrer to allow the organic solvent to evaporate as the microparticles hardened. The resulting microparticles were harvested and washed three times with deionized water. As a last step the microparticles were freeze dried and consequently stored in a desiccator at room temperature.

Media milling of the microsuspension

The API microsuspension (F6) was prepared using a roller mill (Peira, Turnhout, Belgium) and glass vials of the appropriate size filled with zirconium oxide grinding beads with a diameter of 1.0 mm (Tosoh Corporation, Tokyo, Japan). All samples were ground for 24 h. Subsequently these beads were replaced by beads of ø 0.5 mm. After 48 h ø 0.3 mm beads were used for the next 16 days. Suspensions consisted of 10% of drug in phosphate buffer of pH containing 3.75% of TPGS.

***In vivo* performance**

Intravenous drug administration

An intravenous (IV) infusion study was performed to determine the area under the curve (AUC) of the plasma concentration-time profile for IV dosing as well as the half-life ($t_{1/2}$) of the API. For this study three male Beagle dogs were fasted approximately 16 h before dosing and food was returned approximately 4 h post dosing. The infused formulation was prepared in a mixture of polyethylene glycol (PEG) and water (PEG/water 70/30 v%) at a final

analyzed concentration of 0.923 mg/mL. The test subjects were infused in a cephalic vein at a dose of 1 mL/kg for 1 h at an infusion rate of 1 mL/h/kg. Hence the obtained final dose was 0.923 mg/kg. Blood samples were collected from a saphenous vein 15 min, 30 min and 1 h after the start of the infusion and 2, 7 and 20 min and 1, 2, 3, 4, 5 and 6 h after the end of the infusion. Immediately after collection, the blood samples were placed on melting ice till centrifugation.

Within 1 h after blood collection, blood samples were centrifuged at 1900 g for 10 minutes. The plasma was separated and stored at -20^{o}C till bioanalysis.

Intramuscular drug administration

The six formulations (F1-F6) were intramuscularly injected, each in four male Beagle dogs. Prior to IM drug administration the test subjects were fasted approximately 16 h before dosing and food was returned approximately 4 h post dosing. Before administration, the microspheres of F1-F5 were suspended in 3.75% TPGS containing phosphate buffer of pH 7. For all formulations the administered drug dose was 23 mg/kg. Blood samples were collected 0.5, 1, 2, 4, 6, 24, 55, 79, 223, 343, 511 and 679 h post dosing from a saphenous vein. Immediately after collection, the blood samples were placed on melting ice till centrifugation. Within 1 h after blood collection, blood samples were centrifuged at 1900 g for 10 minutes. The plasma was separated and stored at -20^{o}C till bioanalysis.

Bioanalysis

Plasma levels of the API were determined using a qualified research liquid chromatography - mass spectrometry/mass spectrometry (LC-MS/MS) method. After protein precipitation (with acetonitrile) plasma samples were quantified on a reversed phase ultra-high performance liquid chromatography (UHPLC) column (Acquity BEH C_{18} 1.7 µm, 2.1 x 50 mm; Waters,

Milford, USA). Mobile phases consisted of 0.1% formic acid (solvent A) and acetonitrile (solvent B). Starting conditions were 60% solvent A and 40% solvent B followed by a linear gradient to 2% solvent A and 98% solvent B over 1.0 min followed by an isocratic hold at a flow rate of 0.6 mL/min.

LC-MS/MS analysis was carried out on an API-4000 MS/MS (Applied Biosystems, Toronto, Canada), which was coupled to an UHPLC-system (Nexera; Shimadzu, Kyoto, Japan). The MS/MS, operated in the positive ion mode using the TurboIonSpray™-interface (electrospray ionization), was optimized for the quantification of the compound. Multiple reaction monitoring (MRM) transition was as follows: 575. > 419.2. Samples were quantified against calibration curves prepared to cover the concentration range of the study samples. The curves were prepared in the same matrix as the study samples. For each analytical batch, independent quality control (QC) samples, prepared in the same matrix as the samples, were analyzed together with the study samples and calibration curve. The limit of quantification in canine plasma was at least 2 ng/mL. The accuracy (intra batch accuracy from independent QC samples) was between 85% and 115% of the nominal value over the entire range for plasma samples.

Pharmacokinetic data analysis

Based on the plasma concentration-time profiles obtained by the IV infusion study the half-life ($t_{1/2}$) of the API was determined via equation 1.

$t_{1/2}$ = 0.693 / (terminal slope * 2.303) (1)

The terminal slope was obtained by linear regression of the logarithm of the plasma concentrations 2, 3, 4, 5 and 6 h after the end of the infusion.

The AUC for both the intravenously and the intramuscularly dosed API (AUC_{IV} and AUC_{IM} respectively) was calculated based on the plasma concentration-time profiles using the linear-up log-down trapezoidal method. After the last time point (h(final)) the plasma concentration-time profile was extrapolated to infinity in order to determine $AUC_{0-\infty}$. Equation 2 was used to estimate $AUC_{h(final)-\infty}$.

$AUC_{h(final)-\infty}$ = plasma concentration $_{h(final)}$ / terminal slope (2)

The terminal slope was obtained by linear regression of the plasma concentrations for the three last time points at which detectable API plasma levels observed.

For each intramuscularly administered formulation, the measured plasma exposure was used to calculate the bioavailability (F) ($0-\infty$) by equation 3.

$F = 100 * (dose_{IV} * AUC_{IM, 0-\infty})/(dose_{IM} * AUC_{IV, 0-\infty})$ (3)

The intravenously administered dose ($dose_{IV}$) of 0.92 mg/kg and an intramuscularly administered dose ($dose_{IV}$) of 23 mg/kg.

The initial burst release of the six formulations was determined based on the observed plasma concentration till 4 h post administration. The percentage of drug released within this timeframe was calculated by equation 4.

$Initial\ burst = 100 * (AUC_{IM,0-4h})/(AUC_{IM, 0-\infty})$ (4)

In this equation $AUC_{IM,0-4h}$ and $AUC_{IM, 0-\infty}$ represent the calculated AUCs based upon the plasma concentration-time profile till 4h post administration and extrapolated to infinity respectively.

Statistical analysis

Statistical differences between the observed time after administration when maximum plasma concentrations were reached ($t_{max,obs}$) for each of the formulations were evaluated via one-way ANOVA as well as the initial burst release (4h post administration) and bioavailability. A Bonferroni post-hoc test was performed at an α level of 0.05. (GraphPad Prism 5 for Windows; GraphPad Software Inc., San Diego, USA).

Mimicking suspension and injection

To investigate the effect of the suspension of the powders of F1-F5 in an aqueous medium and subsequent IM injection this process was mimicked *in vitro*. F1 was selected as a representative for the PLGA/PVP-based formulations (F1-F3) whereas F4 was chosen as an example for the PLGA-based formulations (F4-F5). Powder of both samples was suspended in the same suspension medium and concentration as used for IM injection in the canine test subjects (3.75% TPGS containing phosphate buffer of pH 7, 100 mg API/mL). Injection was mimicked by injecting the suspended powders via a 20 G needle into a glass petri dish. The resulting sample was dried in a vacuum oven C for one week at 25° and subsequently stored in a desiccator at room temperature.

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to gain insight into the morphology of the samples obtained by mimicking suspension and injection for F1 and F4. Samples 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.

RESULTS

Intravenous infusion study

The IV infusion study was performed to determine the pharmacokinetic parameter $t_{1/2}$ of the API which was calculated to be 1.1 (± 0.4) h respectively. Additionally, the IV infusion study resulted in a mean (±SD) AUC for the intravenously dosed API (AUC_{IV}) of 426 (± 135) h*ng/mL.

Plasma concentration-time profiles

Figure 3 shows the plasma concentration-time profiles up to 28 days after IM injection of the six formulations with Figure 4 depicting these profiles up to 5 h after administration. For all formulations except for F4 the mean plasma concentrations presented are based on the observed plasma concentrations of four animal subjects. For F4 the mean plasma concentrations presented are based on the observed plasma concentrations of three animal subjects as the plasma concentrations of the fourth subject 511 h and 679 h post dosing were considered as outliers (as verified by the statistical criterion of Dean and Dixon). Figure 1 of the supplementary information shows the individual plasma concentration-time profiles for the four test subjects (dog 1-4) of F4. These profiles suggest an initial underperforming of F4 in dog 4, illustrated by lower plasma concentrations compared to dogs 1, 2 and 3. However, 511 h post administration a change in exposure is observed which might be attributed to dose dumping and reflects on the plasma concentration observed 679 h post dosing. Because F4 behaves different in dog 4, the results for this test subject were not used to calculate the average plasma concentrations.

Between 0.5 and 5 h post administration the microsuspension F6 and the binary, PLGA-based formulations F4 and F5 demonstrate more rapidly decreasing plasma concentration-time profiles (Fig. 4) which is in contrast to the more constant profiles of the PLGA/PVP based matrices (F1-F3). From roughly two days (55 h) after injection until the end of the study (28 days) the PLGA/PVP-based matrices (F1-F3) show similar plasma concentration-time profiles with F2 having the highest average plasma concentrations followed by F1 and F3 (Fig. 3). Sample F2 shows the highest average plasma concentration from three days after administration (79 h) until the end of the study.

Table 2 represents the observed time after administration when maximum plasma concentrations were reached ($t_{max,obs}$) for each of the formulations. The binary formulations F4 and F5 and the microsuspension F6 have a mean $t_{max,obs}$ of 0.5 h, whereas for the ternary formulations F1, F2 and F3 the average $t_{max,obs}$ is 2.0, 3.0 and 1.5 h respectively. However, for these ternary formulations the $t_{max,obs}$ for the individual test subjects varied between 0.5 h and 4 h, in contrast to F4-F6 where for all test subjects a $t_{max,obs}$ of 0.5 h was observed. Due to the large standard deviations differences between $t_{max,obs}$ were not statistically significant.

Bioavailability

For each formulation, the observed plasma concentrations were used to calculate the bioavailability by equation 3. The results are shown in Figure 5 where the bioavailability values, based upon the observed plasma concentrations until 28 days after administration (calculated based on $AUC_{0-\infty}$), are depicted. For all formulations the bioavailability is considered as a measure for the amount of drug released, assuming linear elimination kinetics. After IV administration, very rapid elimination of the drug was observed, as reflected by the $t_{1/2}$ value, obtained by the IV infusion study, of 1.0 h ± 0.4 h. This suggests that declining

plasma concentrations observed during terminal “elimination phases” for IM administered formulations are very likely limited by the (much slower) absorption rates (flip-flop kinetics).

The amount of drug released from the various formulations was evaluated by comparing the bioavailability (Fig. 5). F2 showed the highest average bioavailability and therefore drug release, followed by the microsuspension F6 with a mean bioavailability for F2 of 101% and of 81% for F6 (Fig. 5). Subsequently the other two ternary PLGA/PVP-based formulations, F1 and F3, released API resulting in an average bioavailability of 72% and 59% respectively. The bioavailability for both F2 and F6 was significantly higher compared to that for the binary formulations, F4 and F5. These PLGA-based formulations had the lowest total drug release with average bioavailability values of 35% and 34% (Fig. 5).

Burst release

Burst release was identified based on the plasma concentrations obtained within 4 h after administration. This time point was selected as by then all plasma concentration-time profiles had reached their maximum plasma concentration. F4 and F6 display a significantly higher initial burst release compared to the other formulations which is illustrated in Figure 4 by higher plasma concentrations already 30 min after IM injection. For each formulation, the observed initial burst release was calculated by equation 4 and the results are shown in Figure 6. The obtained values represent the percentage of drug released, during the first four hours post injection, of the total amount of drug released by the formulation. The results are shown in Figure 6 where the percentage of drug released values after 4 h (as a measure for burst release) are depicted. The average initial burst release of F6 represents 15% of the total amount of drug released, whilst for F4 this is 13%. The average burst release for F1, F2, F3 and F5 is 4% of the total amount of drug administered. Hence, the burst release of F6 is on

average almost four times higher compared to the burst release of F1, F2, F3 and F5. The burst release of F4 is on average three times higher compared to F1, F2, F3 and F5.

Mimicking suspension and injection

To investigate the effect of the suspension of the powders of F1-F5 in an aqueous medium and subsequent IM injection this process was mimicked *in vitro*. F1 was selected as a representative for the PLGA/PVP-based formulations (F1-F3) whereas F4 was chosen as an example for the PLGA-based formulations (F4-F5). SEM images illustrate that before injection and exposure to an aqueous environment both formulations consist of microspheres with a comparable, spherical morphology and a smooth, intact surface (Fig. 8A and 8B). The friction caused by forcing the suspension through the needle did not result in rupture of the shell structured microspheres. However, after exposure to an aqueous environment (the suspension medium) the microspheres of both model formulations show a distinct morphology. The particles of F4 are still spherical with an intact and smooth surface whereas the particles of F1have a more irregular shape (Fig. 8C and 8D).

DISCUSSION

Previously the six formulations F1-F6, representing the three different formulation strategies, have been physicochemically characterized [11]. Physicochemical characterization of these model formulations showed that all ternary formulations (F1-F3) existed as a PLGA-rich surface layer containing small amounts of PVP and an underlying PVP-rich phase containing small amounts of PLGA. The API was molecularly dispersed in the polymeric matrix. Additionally for F2, a separate amorphous drug phase was detected. The binary formulations, F4 and F5, contained a molecular dispersion of the drug as well. Furthermore F5 contained a

crystalline drug fraction and had a higher drug surface coverage. F5 had a larger particle size, with a d_{50} value of 5.62 µm, compared to the d_{50} value of the other formulations which was averagely 2.99 µm. Moreover, the higher surface area of F5 compared to F1-F4 indicates that particles of F5 are more porous. The different manufacturing method of F5 compared to F1-F4 (emulsion method *versus* spray drying) is held responsible for these observed differences in particle characteristics. The polymeric formulations, F1-F5, are schematically represented in Figure 1. The microsuspension F6 existed of both crystalline and amorphous API. Summarized the six formulations differed in composition as well as structural and physicochemical characteristics. In the current study the *in vivo* performance of these formulations was evaluated in male Beagle dogs.

Solid dispersions in a water-insoluble PLGA matrix (F4-F5)

The two binary, PLGA-based formulations differed in that the spray-dried formulation (F4) showed a significant initial burst release *in vivo*, in contrast to the formulation prepared by the emulsion method (F5) (Fig. 4 and 6). This burst release was not only significantly higher compared to the burst release observed for F5, but also compared to the burst observed for the ternary formulations F1-F3. Subsequently this burst was followed by a fast decline in plasma concentration (Fig. 4). This initial burst for F4 represented 13% of the total amount of drug released, in contrast to F5, for which the burst release accounted for 5% of the total amount of drug released. This implies that for F5 the drug is more gradually released compared to F4 and therefore F5 is more suitable for sustained release.

The difference in manufacturing methods between both formulations did not have a significant influence upon their observed mean bioavailability (35% and 34%) and hence the total amount of drug released (Fig. 5). Both binary formulations showed a lower average

bioavailability compared to the ternary formulations (Fig. 5) indicating the advantage of inclusion of PVP in terms of the extent of drug released from these formulations.

The early $t_{max,obs}$ (0.5 h, Table 2) combined with the fast decrease in plasma concentration-time profiles for formulations F4 and F5 suggest that they are less suitable for long-term release compared to the ternary formulations, for which a more constant and prolonged release is observed (Fig. 4). These observations suggest the benefit of inclusion of PVP in the polymeric matrix to obtain a more sustained release.

Noteworthy was the fact that in one of the four test subjects of F4 three weeks after administration a late burst release was observed. This late burst resulted in a total amount of 85% of drug released compared to an average value of 35% for the three other test subjects. This high drug release might be attributed to sudden erosion of the PLGA matrix and consequently disintegration of the microspheres. This divergence in velocity of the erosion of the PLGA matrix and the consequent differences in the observed release profile between the different test subjects is a restriction of this formulation strategy.

Formulation	**t_{max} (h)**
F1	2.0 ± 1.2
F2	3.0 ± 1.0
F3	1.5 ± 1.5
F4	0.5 ± 0
F5	0.5 ± 0
F6	0.5 ± 0

Table 2. Pharmacokinetic parameter t_{max} (mean ± SD, n=4).

Solid dispersions in a biphasic matrix consisting of water-insoluble PLGA and a water-soluble PVP (F1-F3)

Figure 4 demonstrates the more constant and prolonged plasma concentration-time profiles (0.5-5 h after injection) of the PLGA/PVP-based matrices compared to the other formulations which suggests that these PLGA/PVP matrices are more suitable for sustained release. The

higher mean bioavailability of these ternary formulations compared to the binary formulations indicates a higher drug release (Fig. 5). F2 showed the highest bioavailability (101%) suggesting that essentially all drug present in this formulation is released.

Overall, when the binary PLGA-based formulations were compared to the ternary PLGA/PVP-based formulations it can be concluded that the addition of PVP was beneficial for both the amount of drug released and sustained drug release. This can be explained by the structure of the PLGA/PVP-based matrices which are known to consist of a PLGA-rich surface layer containing small amounts of PVP and an underlying PVP-rich phase containing small amounts of PLGA [3,4]. Consequently the release mechanism is dominated by fast dissolution of 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 ingression of aqueous fluids into the particles, followed by fast dissolution of the molecularly dispersed API and diffusion out of the microspheres. Hence, the presence of PVP in the PLGA matrix results in a higher extent of drug release as the resulting pores allow water ingression deeper into the particle compared to into a bulk eroding PLGA matrix. Consequently an increased surface area is exposed to the release medium with an increase in drug dissolution and drug release as a result (Fig. 7A). Additionally the hydrophilic nature of PVP will favour water ingression into the pore network. Moreover, PVP itself is known to increase the solubility of this API [12]. Analogously PVP contributes to the long-term release of these formulations as the pore network originating from dissolved PVP expands deeper into the particles with longer exposure to the aqueous environment. This results in an increasing access to the API dispersed in the polymeric matrix with depth from the surface which acts as a reservoir (Fig. 7B). Altogether, the pore network originating from dissolved PVP controls the observed drug release. A schematic representation is given in Figure 7 where the structural evolution of a

PLGA/PVP-based matrix when exposed to a release environment is compared to that of a PLGA-based matrix. The difference in structural evolution of a PLGA/PVP-based matrix compared to a PLGA-based matrix when exposed to an aqueous milieu is demonstrated by the SEM images in Figure 8. F1 was selected as a representative for the PLGA/PVP-based formulations whereas F4 was chosen as an example for the PLGA-based formulations. It is clear that after exposure to an aqueous environment the microspheres of both model formulations show a distinct morphology. The particles of F4 are still spherical with an intact and smooth surface whereas the particles of F1 have a more irregular shape due to the dissolved PVP. This induces an increased access and ingression of water into these particles via the resulting pores. This enables the exposure of an increased surface area with consequently an increase in drug dissolution and drug release.

From the three ternary formulations F3 appears the least suitable for sustained release which is suggested by its lower averge $t_{max,obs}$ compared to F1 and F2 (Table 2). Additionally the fact that no detectable plasma concentrations are observed after three weeks (511 h) indicates that there was no longer significant drug release (Fig. 3). From the three PLGA/PVP-based formulations F3 has the lowest bioavailability (Fig. 5). These observations indicate an influence of the molecular weight of PVP used as this formulation only differs from F2 by the molecular weight of PVP (PVP K12 *vs* PVP K30). Hence the use of PVP with a lower molecular weight results in less prolonged drug release as well as a lower amount of drug released. This would allow tailoring the desired release profile by changing the molecular weight of the PVP used.

Comparing the plasma concentration-time profiles of F1 and F2 indicate that F2 results in higher average plasma concentrations by the end of the study (Fig. 3). Additionally its higher mean $t_{max,obs}$ suggests that an increase in the amount of PLGA from 25 to 45 wt% results in a

more sustained release (Table 2). This is not surprising as previous research demonstrated that the thickness of the PLGA surface layer increased with a raise in the amount of PLGA in the microspheres [3]. As PLGA is water insoluble it is expected that the thickness of this PLGA surface layer has an influence upon drug release kinetics where the thicker the PLGA layer, the more prolonged the observed release. The prolonging influence of PLGA on the release could be increased by increasing the amount of PLGA present in the formulation. Comparing the bioavailability of F1 and F2 suggests that the amount of PLGA present in the formulations also influences the amount of drug released. This might be attributed to the fact that changing the PLGA/PVP ratio influences the miscibility of the system. This will result in a difference in the PVP pore network and might consequently influence the release. All ternary formulations showed a comparable initial burst (Fig. 6)

Suspension of the powders of the polymeric formulations F1-F5 with a concentration of 100 mg/mL resulted in a viscous suspension. This impeded facile injection of these formulations. Lowering the concentration of the injected formulation could be one approach to facilitate injection of these formulations.

Particle size reduction (F6)

Particle size reduction is a well-known strategy to increase the dissolution rate of a drug via an increase in surface area [1,7,8]. However, for poorly soluble APIs particle size reduction will still result in a relatively low dissolution rate and hence sustained release [2,13,14]. Consequently, for this type of API, particle size reduction is often used as a strategy to develop a sustained release formulation which was the approach investigated here by means of F6.

The microsuspension showed an initial high burst release of the API and reached maximum plasma concentrations 0.5 h after administration (Fig. 4). Four hours post administration 15% of the total amount drug released was already released (Fig. 6). The observed burst release was followed by a fast decline in plasma concentrations between 0.5 and 6 h after administration (Fig. 4). F6 was also the only formulation for which no detectable plasma concentrations could be observed from two weeks (343 h) after injection onwards (Fig. 3). These observations illustrate that of all formulations tested this microsuspension is the least suitable for sustained release applications for this API. The faster drug release can be attributed to the reduction in particle size, used as dissolution rate enhancing strategy for this formulation.

Furthermore, the mean bioavailability of 81% for this formulation indicates that although particle size reduction increased dissolution rate, less drug was released or reached the systemic circulation compared to F2. Therefore, for this drug, particle size reduction was a suitable strategy to increase dissolution rate but it was less applicable for the development of a sustained release formulation.

For the various formulations strategies tested F2 is the most suitable to obtain a long-acting pharmacokinetic (PK) profile which is illustrated by its higher average $t_{max,obs}$ (3.0 h) compared to the other formulations. Additionally this formulation results in the highest plasma concentrations from three days after administration onwards until the end of the study (28 days). Consequently, compared to the other formulations tested, F2 released the highest amount of drug for the same amount of drug dosed, which was illustrated by its higher mean bioavailability value. This is definitely advantageous in terms of total mass of formulation to be suspended and therefore the volume to be administered. For (IM) injectable formulations

the volume injected is preferably as small as possible to avoid pain upon injection, irritation and inflammation.

CONCLUSIONS

In vivo evaluation of the different formulation strategies demonstrated the benefit of combining water-soluble polymer PVP and a water-insoluble PLGA as a matrix for solid dispersions to develop long-term release formulations compared to the other formulation strategies assessed. The benefit is dual and comprises a more sustained release as well as a higher extent of drug release from the polymeric matrix. This was explained based on the structure of these PLGA/PVP-based matrices where the pore network originating from rapidly dissolving PVP results in an increasing access of the aqueous release medium to the API dispersed in the polymeric matrix. This increased access to drug dispersed in the matrix with depth from the surface acts as a reservoir with a higher extent of and more prolonged drug release as a result. Moreover, the results suggest that for the PLGA/PVP-based formulations the release profile can be tailored by changing the molecular weight of PVP and the amount of PLGA in the matrix.

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FIGURES

Figure 1. Schematic representation of the polymeric formulations F1-F5 [11]. PLGA in green, PVP in red and API in yellow.

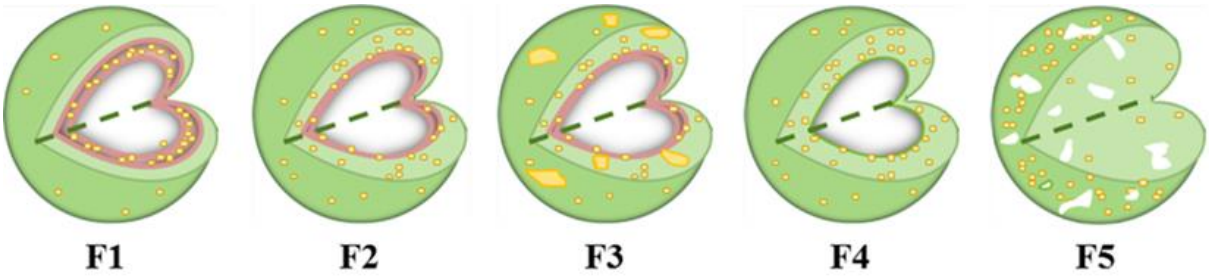


Figure 2. Structural formula of the API, a poorly soluble HIV protease inhibitor.

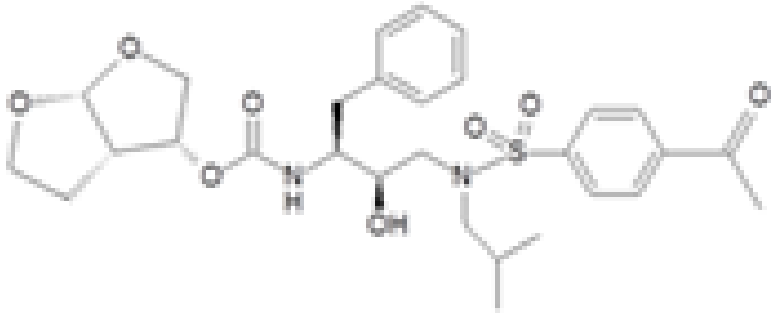


Figure 3. Plasma concentration-time profiles up to 28 days after IM administration of the six formulations F1-F6 (mean ± SD, n=4).

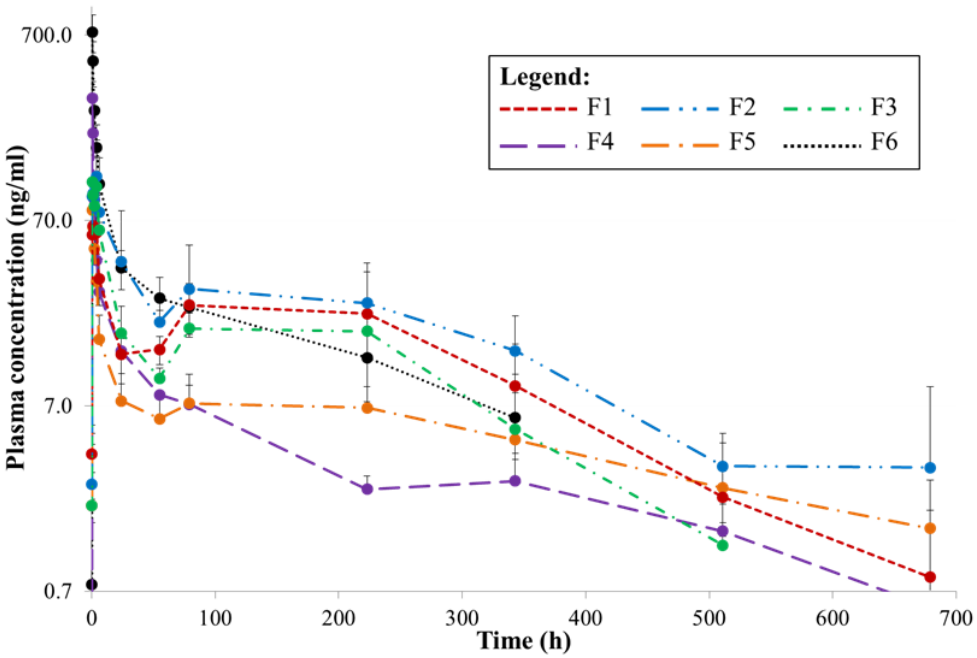


Figure 4. Plasma concentration-time profiles up to five hours after IM administration of the six formulations F1-F6 (mean ± SD, n=4).

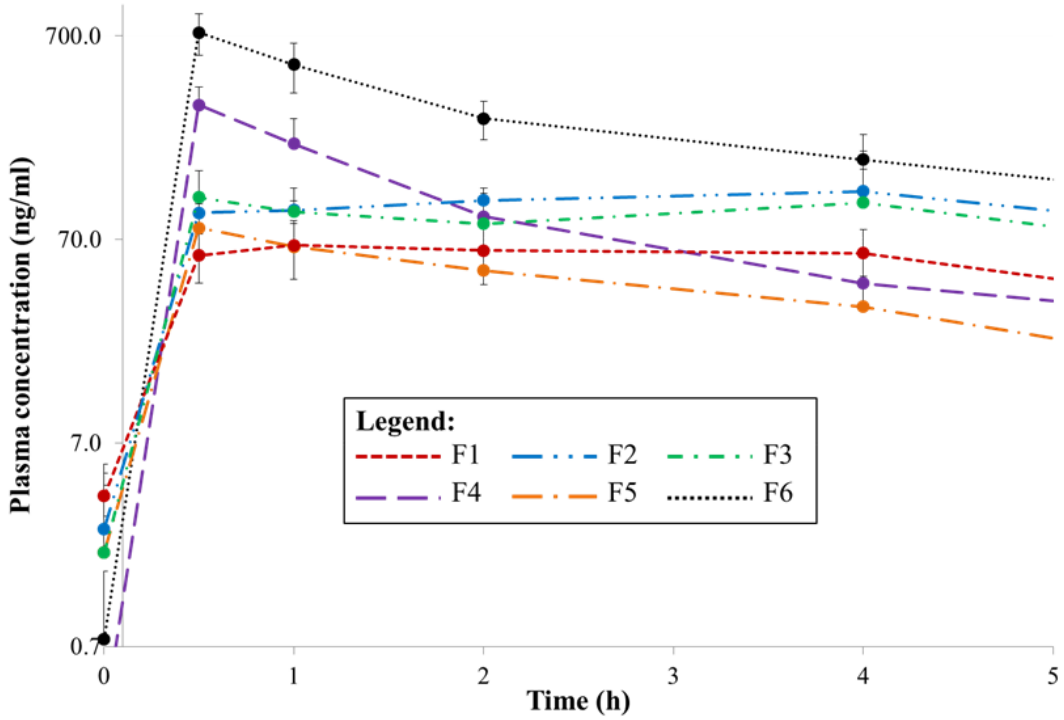


Figure 5. Bioavailability 28 days after IM administration of the six formulations F1-F6 based on total plasma exposure of the API ($AUC_{0-\infty}$) (mean ± SD, n=4).

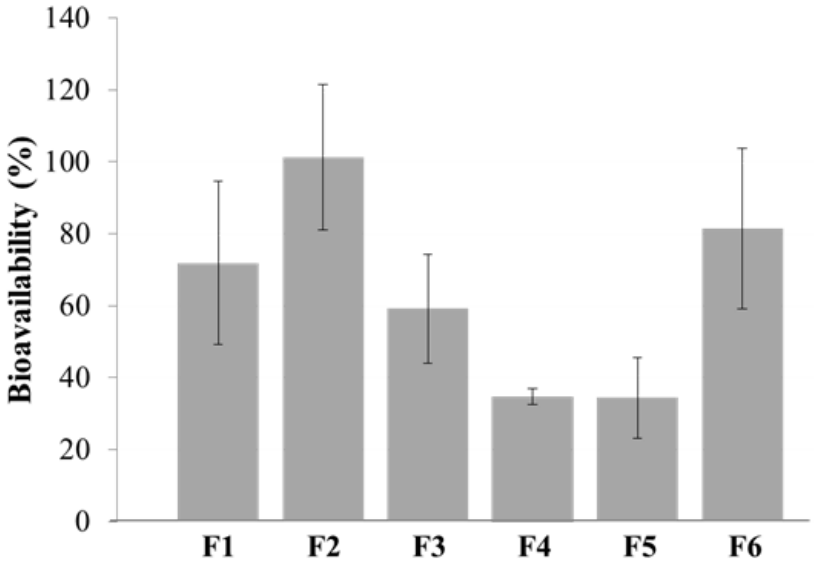


Figure 6. Initial burst release 4 h post administration of the six formulations, expressed as the percentage of drug released (mean ± SD, n=4).

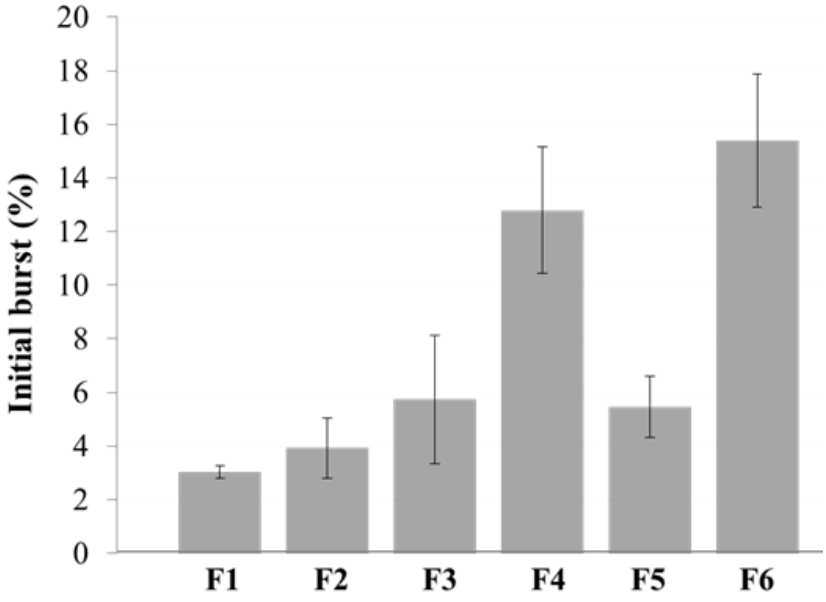


Figure 7. The structural evolution of a PLGA/PVP-based matrix and a PLGA-based matrix when exposed to a release environment, where A = short-term exposure and B = long-term exposure. PLGA in green, PVP in red and API in yellow.

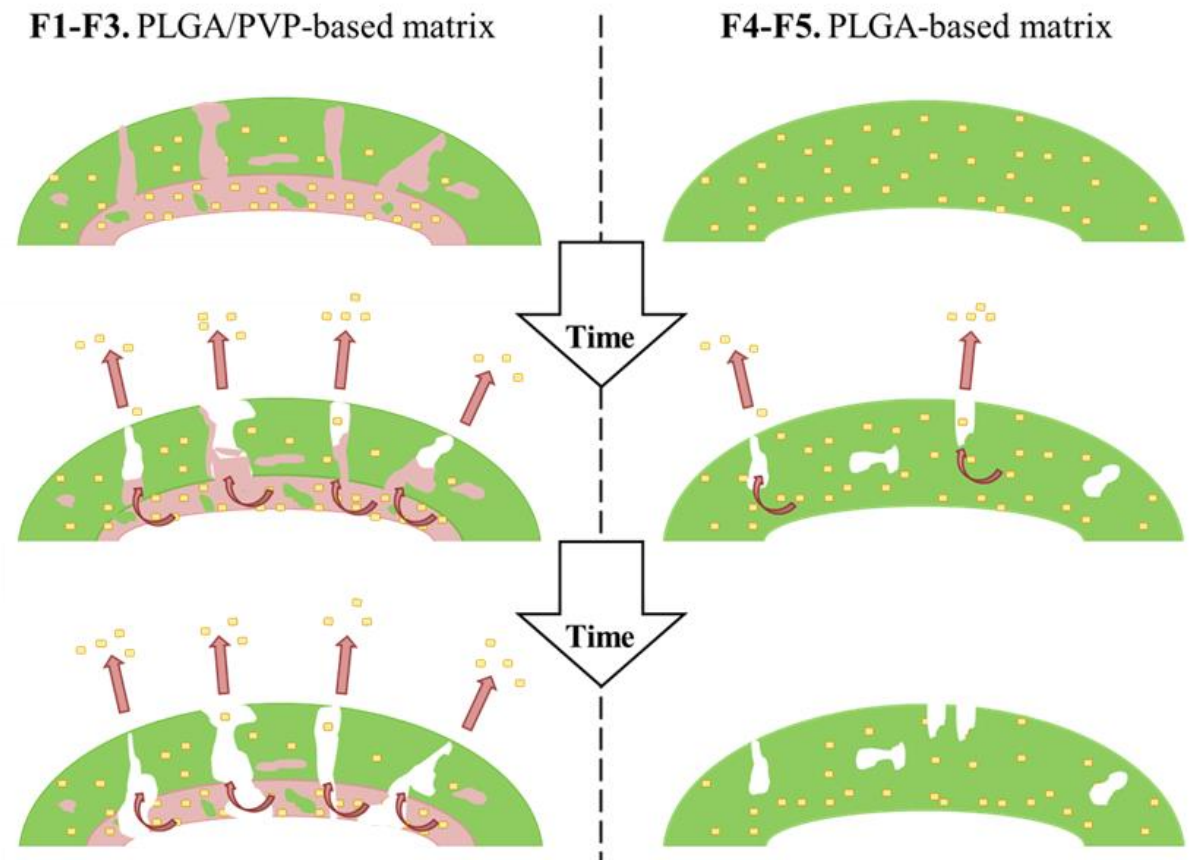
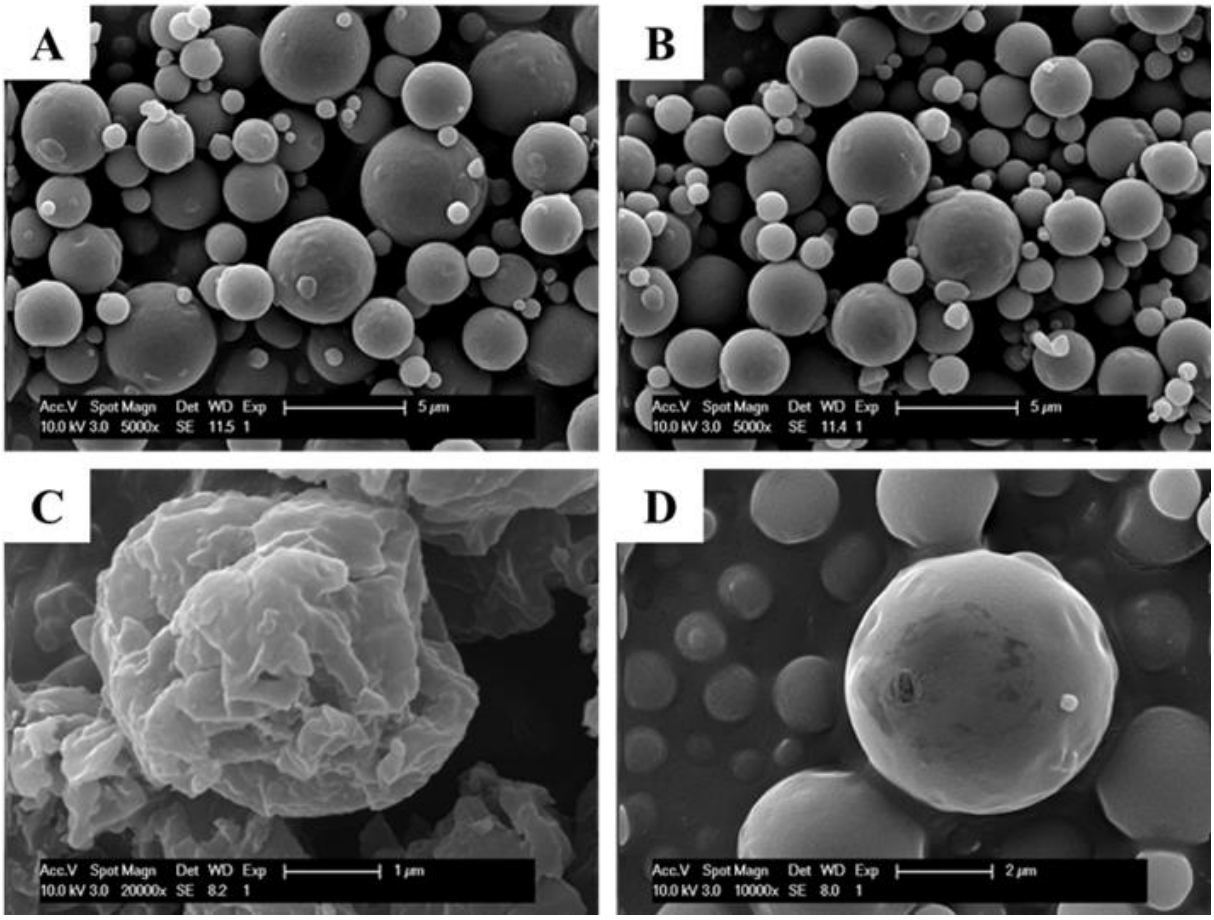


Figure 8. SEM images. where A = F1 before exposure to a release medium, B = F1 before exposure to a release medium, C = F1 after exposure to a release medium, D = F4 after exposure to a release medium. F1 was selected as a representative for the PLGA/PVP-based formulations whereas F4 was chosen as an example for the PLGA-based formulations.



SUPPLEMENTARY INFORMATION

Figure 1. Individual plasma concentration-time profiles up to 28 days after IM administration for the four test subjects (dog 1 - dog 4) of formulation F4.