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Spray-dried didanosine-loaded polymeric particles for enhanced oral bioavailability

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ABSTRACT

Didanosine (ddI) is a water-soluble antiretroviral used in the treatment of HIV that undergoes fast gastric degradation to an inactive hypoxanthine. Therefore, its oral bioavailability is relatively low (20–40%). In this work, we investigated for the first time a scalable open-loop spray-drying method with co-current flow for the encapsulation of ddI (model drug) within particles of the biocompatible polyester poly(epsilon-caprolactone). The average diameter of the particles was 36–118 μm and the morphology spherical. The encapsulation efficiency ranged from 60% to 100% with yields of up to 65%. ATR/FT-IR analysis indicated that most of the drug was encapsulated within the particles. *In vitro* release assays showed that the particles released the drug within 120 min. Finally, oral administration to rats led to a statistically significant 2.5-fold increase of the bioavailability with respect to a ddI aqueous solution, highlighting the potential of this technology to encapsulate efficiently other hydrophilic antiretrovirals and, by doing so, to overcome different biopharmaceutical drawbacks associated with the oral administration.

1. Introduction

According to last statistics of the World Health Organization (WHO), 35–40 million people are infected with the Human Immunodeficiency Virus (HIV) worldwide [1–3]. Antiretroviral drugs (ARVs) used for the chronic treatment of the infection are classified according to the target in the virus replicative cycle into different families [4]. The High Activity Antiretroviral Therapy (HAART) combines three to four ARVs from at least two different classes and has been crucial to make the infection manageable [5–7]. Even though HAART has reduced HIV-related mortality, the disease is not curable [7–9]. Moreover, short half-life, first pass metabolism, poor aqueous solubility and gastrointestinal degradation of some ARVs result in reduced and erratic bioavailability [10–12]. Scalable and cost-viable technology interventions are urgently needed to overcome the most crucial biopharmaceutical drawbacks of ARVs [5].

Didanosine (ddI) is a nucleoside reverse transcriptase inhibitor (NRTI) used in adult and pediatric second-line ARV cocktails [13–15]. Regardless of the high aqueous solubility (27.3 mg/mL, 25 °C, pH 6.2), ddI presents relatively low oral bioavailability of 20–40% [13,16–18] due to fast gastric degradation. Thus, it is co-administered with basic salts that neutralize the gastric pH and partially prevent hydrolysis. These excipients could provoke gastrointestinal and renal side effects and alter the absorption of other ARVs co-administered with ddI [17,19]. Different approaches were assessed to develop more suitable dosage forms such as gastro-resistant pellets [14,20]. Owing to toxicity, reduced efficacy and inconvenient dosing requirements, the WHO has recommended the replacement of ddI by another NRTI, lamivudine [21]. At the same time, it still represents a therapeutic alternative in the poorest countries hit by the scourge [22] and an excellent model drug for the investigation of novel drug delivery systems and production processes [23,24].

Spray-drying is a rapid, simple, continuous, easily scalable, cost-effective and reproducible one-step process that comprises the

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transformation of a material from a fluid state into a fine powder of solid dry particles with relatively narrow size distribution by atomization through a nozzle into a hot drying gas medium [25–28]. An additional appeal of this method is that enables the encapsulation of hydrophilic and hydrophobic drugs within polymeric particles [29–31] in relatively short time, without substantial thermal degradation (even of temperature-sensitive products) and with relatively high efficiency and yield [28,32].

The encapsulation of ddI within polymeric particles would reduce its contact with the gastric medium and delay the release to the intestine and the thus increase its chemical stability and oral bioavailability [7,33–35]. Poly(epsilon-caprolactone) (PCL) is one of the most extensively investigated polyesters for drug delivery and it has been approved by the US-FDA for use in human medicine [36–40]. An advantage of PCL over other polyesters is its relatively low cost, that remains crucial for bench-to-bedside translation in poverty-related diseases.

Aiming to explore the potential of this technology to encapsulate water-soluble ARVs, in this work, we investigated for the first time the spray-drying technology for the production of PCL particles loaded with ddI (model ARV) using an open-loop mode with co-current flow and assessed the performance of the particles *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

PCL (40,000 g/mol) was synthesized by microwave-assisted ring-opening polymerization of epsilon-caprolactone [41,42]. ddI was a donation of Richmond Laboratories. Polyvinyl alcohol (PVA, molecular weight = 15,000 g/mol) was obtained from Merck Chemicals. Dichloromethane (DCM) and methanol (MeOH) were of analytical and HPLC grade, respectively. All the other reagents were from Sigma-Aldrich.

2.2. Production of ddI-loaded particles

2.2.1. Solid-in-oil (s/o) suspension method

PCL (1 g) was dissolved in DCM (100 mL, final concentration of 1% w/v) under magnetic stirring (30 min). Subsequently, ddI (50, 100, 150 and 200 mg) was suspended in the organic phase and homogenized using a T25 Ultra-Turrax (IKA®-Werke GmbH & Co. KG) (8,000 RPM, 5 min). Blank particles (without the incorporation of ddI) were prepared and used as control. The different variables used are summarized in Table 1.

2.2.2. Water-in-oil (w/o) emulsion method

PCL (1 g) was dissolved in DCM (100 mL, final concentration of 1% w/v) under moderate magnetic stirring for 30 min and ddI (50, 100, 150 and 200 mg) was dissolved in distilled water (20 mg/mL) containing PVA (10 mg/mL) using an ultrasonic bath. Then, the aqueous phase was added to the organic one and emulsified with a T25 Ultra-Turrax (8,000 RPM, 5 min). The sample named E100 (however without the addition of ddI) was taken as reference for the preparation of blank particles (Table 1).

2.2.3. Spray-drying process

Suspensions and emulsions were fed into a Mini Spray Dryer Büchi B-191 (Büchi Labortechnik AG) through a two-fluid nozzle (0.70 mm inner diameter) using silicone tubing (9 mm inner diameter) and a peristaltic pump in an open-loop mode with compressed air as drying gas. Liquids were maintained under constant stirring (200 RPM) to ensure homogeneity. Air was flown through the drying chamber in the same direction as the sprayed liquid (co-current flow). The operating conditions were the following:

Table 1
Liquid feeds for the production of each batch and properties of the obtained particles by spray-drying. The weight of PCL per batch was always 1 g.

Sample	Liquid phase	ddI (mg)	Distilled water (mL)	PVA (mg)	*D*[v,0.1] (μm) (± S.D.)	*D*[v,0.5] (μm) (± S.D.)	*D*[v,0.9] (μm) (± S.D.)	*D*[4,3] (μm) (± S.D.)	SPAN (± S.D.)	*%LC* (± S.D.)	*%EE* (± S.D.)	*Yield* (%) (± S.D.)
S50	Suspension	50	–	–	32.6 (0.4)	97.6 (3.6)	237.4 (11.3)	118.4 (4.2)	2.10 (0.14)	2.8 (0.1)	59.5 (2.9)	62.3 (6.1)
S100		100			35.8 (0.5)	83.2 (0.1)	173.1 (4.3)	94.9 (1.0)	1.65 (0.05)	5.8 (0.2)	63.8 (2.2)	60.6 (9.0)
S150		150			34.2 (1.5)	101.5 (5.0)	201.6 (7.7)	110.4 (3.9)	1.65 (0.01)	9.0 (0.1)	68.8 (0.7)	49.2 (1.0)
S200		200			33.1 (0.7)	81.6 (2.3)	175.1 (9.6)	93.9 (3.5)	1.74 (0.07)	12.0 (0.2)	72.3 (1.2)	51.6 (5.7)
E50	Emulsion	50	2.5	25	16.8 (0.5)	56.2 (2.0)	123.8 (12.3)	64.8 (4.7)	1.90 (0.14)	4.1 (0.2)	86.4 (4.3)	61.1 (2.3)
E100		100	5	50	10.2 (0.3)	49.8 (1.4)	135.4 (10.7)	63.1 (3.3)	2.52 (0.15)	10.4 (0.5)	99.9 (5.6)	64.9 (1.3)
E150		150	7.5	75	6.0 (0.1)	29.9 (0.6)	111.8 (5.1)	46.8 (1.6)	3.54 (0.09)	13.4 (1.0)	99.7 (7.9)	41.5 (1.2)
E200		200	10	100	4.5 (0.0)	19.2 (0.1)	90.5 (2.1)	35.7 (0.7)	4.48 (0.09)	15.1 (1.6)	98.4 (10.4)	37.7 (2.0)
BS	Suspension	–	–	–	42.9 (0.1)	88.2 (0.4)	165.7 (1.3)	97.2 (0.5)	1.39 (0.01)	–	–	48.4 (1.2)
BE	Emulsion	–	5	50	25.5 (0.1)	61.3 (0.3)	119.7 (1.1)	67.7 (0.5)	1.54 (0.01)	–	–	57.8 (3.8)

air inlet temperature (T_{in}) of 42 °C, air outlet temperature (T_{out}) of 32 °C, atomizing air flow rate (represented as the volume of the air input) of 600 L/h, liquid flow rate of 10 mL/min, carrier gas flow rate (aspirator) of 60 m^3/h and pressure of 5 bar. The dried powder was blown through the cyclone separator, recovered from the glass collection vessel and weighed to determine the yield expressed in % weight (see below). Products were stored in sealed glass vials at room temperature protected from light and moisture until use.

2.3. Characterization of the particles

2.3.1. Size and size distribution

The diameter of the particles expressed as 10%, 50% and 90% volume fractions (D[v,0.1], D[v,0.5] and D[v,0.9], respectively), the equivalent volume mean diameter (D[4,3]) and the width of the size distribution (SPAN) of blank and ddI-loaded particles were determined by Laser Light Scattering (LLS) using a Mastersizer Micro (Malvern Instruments). An amount of particles (determined by the optimum obscuration value between 10 and 30%) was dispersed immediately before measurement in an aqueous solution of 0.1% w/v Tween® 80 and vortexed for 2 min. D[4,3] values were calculated according to Eq. (1)

$$D[4,3] = \frac{\sum d^4}{\sum d^3} \quad (1)$$

Where, d is the diameter of the particle. Surface area has a d^3 dependence and volume or mass has a d^4 dependence.

And SPAN values were calculated according to Eq. (2)

$$SPAN = \frac{D[v,0.9] - D[v,0.1]}{D[v,0.5]} \quad (2)$$

A smaller SPAN value indicates a narrower size distribution or polydispersity. Results of D[v,0.1], D[v,0.5], D[v,0.9], D[4,3] and SPAN are expressed as mean ± S.D. of three independent samples prepared under identical conditions. Data for each single measure was the result of at least three runs.

2.3.2. Loading capacity, encapsulation efficiency and yield

To determine the loading capacity (*%LC*) and the encapsulation efficiency (*%EE*) of the different samples, drug-loaded particles (10 mg) were dissolved in DCM (2 mL). Then, phosphate buffer saline (PBS, pH 7.4, 10 mL) was added to this organic phase. The mixture was vortexed (30 min) and centrifuged (5,000 RPM, 1 h) to isolate the aqueous phase that was diluted in PBS to determine the ddI concentration by UV-visible spectrophotometry (λ = 249 nm, CARY [1E] UV–Visible Spectrophotometer Varian), at 25 °C. ddI concentrations were obtained from a calibration curve with a linearity range between 5 and 30 μg/mL ($R^2 > 0.9997$). Measurements were performed in triplicate and results are expressed as mean ± S.D. The *%LC* of the particles was calculated according to Eq. (3)

$$\%LC = \frac{W_{ddI}}{W_P} \times 100 \quad (3)$$

Where W_{ddI} is the weight of ddI in the particles and W_P is the total weight of particles obtained in each batch.

The *%EE* of the particles was calculated according to Eq. (4)

$$\%EE = \frac{LC_P}{LC_T} \times 100 \quad (4)$$

Where LC_P and LC_T are the experimental and theoretical loading capacity of the particles, respectively.

The *Yield (%)* after process was determined according to Eq. (5)

$$Yield\,(\%) = \frac{W_P}{W_0} \times 100 \quad (5)$$

Where W_P is the total weight of particles obtained after the spray-drying process and W_0 is the total initial amount of polymers and drug employed in their production.

2.3.3. Scanning electron microscopy (SEM)

The external morphology of blank and ddI-loaded particles was visualized by SEM (FEG-SEM, Zeiss Supra 40 TM apparatus Gemini column) operating at an accelerating voltage of 3.0 kV. Prior to examination, samples were coated with gold (thickness of 5–10 nm) using a sputter coating method.

2.3.4. Attenuated total reflectance/Fourier transform-infrared spectroscopy (ATR/FT-IR)

The following samples were analyzed by ATR/FT-IR (Nicolet 380 ATR/FT-IR spectrometer, Avatar Combination Kit), Smart Multi-Bounce HATR with ZnSe crystal 45° reflectance (Thermo Scientific, Inc.): pure ddI, blank particles, PCL:ddI physical mixtures corresponding to the highest and lowest *%LC* (see calculation above) of particles and ddI-loaded particles. Spectra were collected in the wavenumber range between 4000 and 600 cm^{-1} (15 scans, spectral resolution of 4.0 cm^{-1}) using the OMNIC 8 spectrum software (Thermo Scientific, Inc.).

2.3.5. Thermal analysis

The thermal analysis was performed by Differential Scanning Calorimetry (DSC, Mettler-Toledo TA-400 differential scanning calorimeter). Samples (~5 mg) were sealed in 40 μL Al crucible-pans (Mettler) and heated in a simple temperature ramp between 25 and 210 °C at a heating rate of 10 °C/min under dry nitrogen atmosphere. The thermal transitions were compared to those in free ddI and pure PCL. The crystallinity degree (*%C*) of ddI ($\%C_{ddI}$) and PCL ($\%C_{PCL}$) in the different particles obtained was calculated according to Eq. (6) [43]

$$\%C_{ddI} = \frac{\Delta H_{m-ddI}}{\Delta H^c_{m-ddI}} \times 100 \quad (6)$$

And Eq. (7)

$$\%C_{PCL} = \frac{\Delta H_{m-PCL}}{\Delta H^c_{m-PCL}} \times 100 \quad (7)$$

Where $\Delta H_{m\text{-}ddI}$ and $\Delta H_{m\text{-}PCL}$ are the values of melting enthalpy of ddI and PCL in the samples, respectively, and $\Delta H^c_{m\text{-}ddI}$ and $\Delta H^c_{m\text{-}PCL}$ are the values of melting enthalpy of 100% crystalline ddI and PCL, respectively. Values were normalized to the content of each one of the components in the particles.

2.3.6. X-rays diffractometry (XRD)

The crystallinity of ddI and PCL in the particles was analyzed in an X-ray powder diffractometer (Philips X'Pert X-ray diffractometer, PANalytical) equipped with a PW3710 unit, system of soller slits and monochromator reception. An X-ray generator (Philips, type PW1830) with a Cu anode was used at voltage of 35 kV and current of 20 mA. Diffractograms were recorded in the 2θ range of 5–40° with step angle of 2θ 0.02° and count time of 2 s at each step.

2.4. In vitro ddI release

In vitro ddI release assays from the different particles were conducted during 120 min because the gastric transit time in humans is between 15 min and 3 h [44] and in rats is 2–2.6 h [45]. Particles containing 2.5 mg of ddI were placed in a beaker and dispersed in PBS (pH 7.4, 20 mL) as release medium. The volume used ensured sink conditions. The system was maintained at 37 °C ± 2 under magnetic stirring (200 RPM, 120 min). An aliquot of the release medium (1 mL) was withdrawn at predetermined time intervals

and replaced by fresh medium pre-heated at 37 °C. Extracted aliquots of release medium were centrifuged (13,000 RPM, 5 min) and ddI analyzed in the supernatant by UV–visible (see above); results were corrected by the volume of release medium withdrawn. Assays were carried out in triplicate and the results are expressed as mean ± S.D.

2.5. In vitro ddI stability in acid medium

Based on the results of the *in vitro* ddI release assays, ddI-loaded particles that showed slower drug release rates were selected to perform an *in vitro* ddI stability assay under acid conditions. The protocol followed for this test was similar to the *in vitro* release assay (see above) with the difference that to simulate the gastric pH, the PBS release medium was replaced by HCl water solution (pH 1.2). Immediately after extraction, each aliquot (1 mL) was neutralized with 1 N NaOH (150 μL) to prevent further degradation of ddI. Then, ddI was quantified by High Performance Liquid Chromatography (Alliance HPLC, separation module e2695, Waters Corp.) [46] using a Waters 5 μm, C18, 150 mm × 4.6 mm column (Waters) with a dual-wavelength UV detector (λ = 249 and 254 nm, 2998 Photoiodide Array UV/Vis 2D detector, W2998, Waters). For this, a method previously validated for ddI was used with slight modifications [14]. The mobile phase consisted of 0.01 M sodium acetate:MeOH (85:15, pH 6.5). The flow rate of the mobile phase was 1 mL/min. Both ddI and hypoxanthine were detected at different retention times. The apparent first-order degradation rate constant of ddI was determined by plotting the logarithm of remaining ddI as a function of time [46]. All assays were performed in triplicate and results are expressed as the mean ± S.D.

2.6. Oral pharmacokinetics

Oral pharmacokinetics studies were conducted in male Wistar rats (250-300 g) following a protocol approved by the Ethics Committee (Faculty of Pharmacy and Biochemistry, University of Buenos Aires, CICUAL-FFYB, N° 34086/14). Rats were maintained on 12 h light/dark cycles at 22 ± 2 °C with air adequately recycled and received a standard rodent diet and water *ad libitum*; before assays they were fasted overnight (12 h). Oral pharmacokinetics of ddI was systematically compared after administration of a ddI aqueous solution and S100 particles (Table 1). Each group consisted of four animals and the ddI dose and volume administered was 20 mg/kg and 4 mL/kg, respectively. Immediately before the assay, ddI-loaded particles were suspended in the corresponding volume of distilled water, vortexed and the corresponding ddI dose was poured into the stomach of conscious rats through a stomach tube. In the case of the control group, the corresponding amount of pure ddI was solubilized in distilled water and administered as described above; the ddI dose and concentration in solution were identical to those in the particles. After administration, blood samples (70 μL) were collected from the tail vein at predetermined time intervals (5–720 min). The blood sampling time points were established based on a ddI half-life of 1.2 h. The extraction of ddI from blood samples was performed as previously reported with minor modifications [13]. Blood samples were centrifuged (10,000 RPM, 10 min) and plasma (20 μL) was deproteinized with MeOH (20 μL) and 10% w/v zinc sulfate (2 μL) and vortexed for 2 min followed by centrifugation (10,000 RPM, 10 min). The ddI concentration was determined by HPLC using an isocratic regime [14], a UV detector (λ = 249 nm, UVIS 204 Spectrophotometer, Linear Instruments) and a Phenomenex Luna 5 μm, C18, 150 mm × 4.60 mm column (Phenomenex® Gemini-NX). The mobile phase consisted of 0.01 M sodium acetate:MeOH (95:5, pH 6.5) and was pumped at a flow rate of 1.2 mL/min. A calibration curve with a linearity range between 0.16 and 5 μg/mL ($R^2 > 0.9997$) was built for this purpose.

2.7. Evaluation of in vivo data

The following pharmacokinetic parameters were determined: the maximum plasma concentration (C_{max}), the time to reach the C_{max} (t_{max}), the area-under-plasma concentration-time curve between the administration time and 1 h ($AUC_{0\text{-}1}$), the area-under-plasma concentration-time curve between the administration time and infinite ($AUC_{0\text{-}\infty}$) and the elimination rate constant (k_e). The relative oral bioavailability (F_r) was calculated according to Eq. (8) [47]

$$F_r(\%) = \frac{AUC_P}{AUC_R} \times 100 \quad (8)$$

Where AUC_P and AUC_R are the $AUC_{0\text{-}\infty}$ values of the ddI-loaded particles (S100) and the ddI aqueous solution considered as 100% bioavailability (reference), respectively.

Non-compartmental analysis of ddI plasma concentrations was performed using the TOPFIT program (version 2.0, Dr. Karl Thomae Gmbh) that uses a cyclic three-stage optimization routine (one-dimensional direct search; vectorial direct search/Hookee Jeeves modified; Gausse Newton/Marquadt modified). Parameters were compared by unpaired Student's *t*-test. Statistical analysis was performed using GraphPad Prism version 6.01 (GraphPad Software). A value of $p < 0.05$ was considered statistical significance. The inter-individual variability was determined by the coefficient of variation (CV%).

3. Results and discussion

3.1. Preparation and characterization of the particles

We assessed two different liquid feeds, s/o suspension and w/o emulsion [26,29,48,49]. In the first case, ddI was suspended in the organic polymeric solution because its solubility in DCM is less than 1 mg/mL. In the second case, the system was composed of an inner aqueous phase containing ddI solubilized and PVA as stabilizer and an outer organic phase of PCL in DCM. The equipment was operated in an open-loop mode using air as drying gas, which is more stable and cost-effective than a closed-loop mode that demands nitrogen [50]. Evaporation of the organic solvent from the droplets generated by the atomizer is crucial for the formation of particles and involves a heat and mass transfer process [51]. Usually, these droplets enter the drying chamber via the carrier gas flow and undergo evaporation of the solvent and condensation of the solute, resulting in dry solid particles. Therefore, the solvent is a critical parameter of the process because its boiling temperature is one the main variables controlling T_{in} [52]; the T_{in} in the drying chamber must be above the vaporization temperature of the solvent [53]. Moreover, high temperatures can cause melting of the polymer used to produce the particles [28]. PCL has a melting temperature (T_m) between 55–65 °C, so a relatively low T_{in} is required to avoid possible sticking and agglomeration of the formed particles. In this way, DCM was selected as the organic solvent because it has a low boiling point (40 °C) [29,43,54]. Conversely, solvents with higher boiling point require higher T_{in} that could jeopardize the formation of the particles [29]. In addition, the lower the T_{in}, the lower the T_{out} (32 °C). This is advantageous because exposure of the drug to high temperatures could degrade it. At the same time, it should be stressed that, although during the drying process, the droplets are exposed to high temperature, the exposure time is extremely short (in the range of milliseconds or seconds). Thus, drug degradation was not anticipated [28,50,55,56]. This is possible due to the large surface area of very small droplets available for heat and mass transfer process [53]. The droplets are cooled by solvent evaporation, due to the latent heat of vaporization and the product temperature in the co-current dryer is about 10–20 °C below the T_{out}. Therefore,

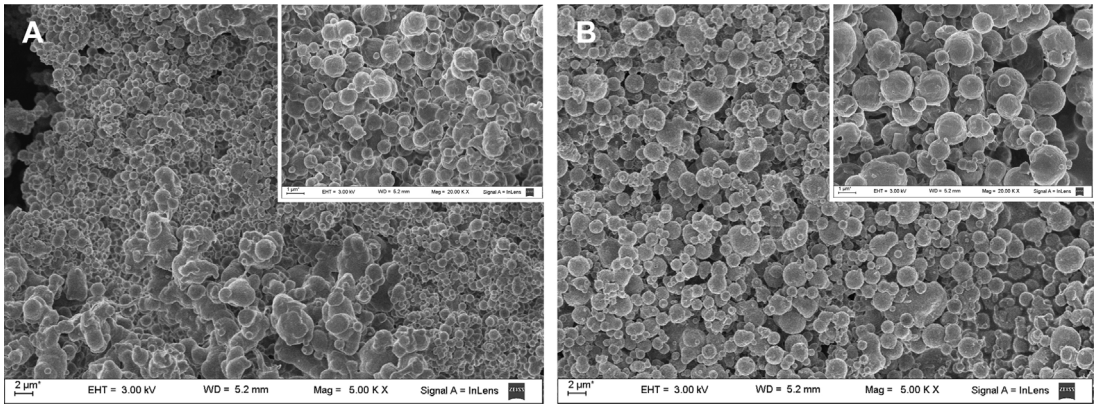

Fig. 1. SEM micrographs of ddI-loaded particles obtained by spray-drying. (A) S100 and (B) E100. Scale bar = 2 μm in main photo and 1 μm in photo insert.

the co-current mode is preferable for drying of thermo-sensitive materials because the final product is in contact with the coolest air [28,53,57,58]. In this work, both the T_{in} and the T_{out} were below the T_m of PCL and were sufficiently low to prevent ddI thermal degradation. In the case of the w/o emulsion, PVA was added to the aqueous phase as stabilizing emulsifier due to its adsorption at the oil/water interface of the droplets [33,56]. In addition, the use of PVA as an excipient has been shown to decrease the coalescence of the particles and allows good re-suspension in aqueous medium after the process [56,58].

3.1.1. Size and size distribution

D[4,3] values varied according to the production method of the liquid phase and the ddI initial payload incorporated in each sample (Table 1). The effect of the ddI payload on *D*[4,3] values was more pronounced in the particles produced by emulsion. In this case, *D*[4,3] values were between 36 μm (for the highest *%LC*) and 65 μm (for the lowest *%LC*). Conversely, in the particles obtained by suspension, sizes were within a narrower range, 94–118 μm (Table 1). The presence of a very small amount of particles of approximately 1 μm suggested the formation of fine powders (data not shown). In addition, SPAN values of particles obtained from suspension ranged between 1.65 and 2.10, while those by emulsion between 1.90 and 4.48. Even though these results suggested the greater polydispersity of the latter, in all cases SPAN values were relatively low and consistent with narrow size distributions. The same trend was observed in the size and SPAN of the blank particles (Table 1).

3.1.2. Loading capacity, encapsulation efficiency and yield

The emulsion technique resulted in higher values of *%LC* and *%EE* than the suspension one (Table 1). For example, in the case of emulsion the values of *%LC* and *%EE* ranged between 4.1 and 15.1% and 86.4 and 99.9%, respectively. Conversely, suspensions resulted in *%LC* of 2.8–12.0% and *%EE* of 59.5–72.3%. These results suggested that emulsions led to the formation of structures that entrapped the drug more efficiently. Regarding the yield, values were more variable for emulsion (37.7%–64.9%) than suspension (49.2%–62.3%) and quite lower than 100% due to the geometry of the laboratory-scale equipment that resulted in the loss of particles on the walls of the drying chamber. On the other hand, yields of up to 80–90% could be achieved with larger scale equipment [59–63].

3.1.3. Morphology

SEM analysis showed that all particles were spherical and with smooth surface, regardless of the liquid feed (Fig. 1). Furthermore, ddI-loaded particles did not showed free ddI crystals on the surface, strongly suggesting that most of the drug was encapsulated.

3.1.4. ATR/FT-IR

To elucidate the presence of free ddI on the surface of the particles, samples were analyzed by ATR/FT-IR spectroscopy. Free ddI showed most of the characteristic bands of hypoxanthine and tetrahydrofurfuryl alcohol [64]. Blank particles showed the band of C=O of PCL with different %C at 1725 cm^{-1} [65,66]. These results indicated that the production process did not substantially alter the crystallinity of the polyester. In addition, PCL:ddI physical mixtures showed the characteristic bands of both the polymer and the drug, confirming that ddI was detectable by this technique, even in particles with the lowest *%LC*. In general, particles obtained from emulsion only showed the bands of PCL with the exception of E200 that also displayed three additional weak bands of ddI. These results indicated that part of the drug was not encapsulated. In contrast, spectra of particles with lower *%LC* (E150, E100 and E50) did not present these bands, suggesting that the drug was majorly encapsulated. Particles produced from suspension showed a similar trend,

Table 2
Thermal analysis of the different ddI-loaded particles obtained by spray-drying.

Sample	T_m (°C)		ΔH_m (J/g)		%C	
	ddI	PCL	ddI	PCL	ddI	PCL
ddI	185	–	116.5	–	100	–
PCL	–	61	–	100.3	–	71.9
S200	183	58	12.0	79.3	85.8	64.6
S150	183	59	5.9	90.7	56.3	71.4
S100	184	59	3.1	84.1	45.9	64.0
S50	–	58	–	92.1	–	67.9
E200	190	58	17.4	68.1	98.9	57.5
E150	190	58	15.4	73.0	98.6	60.4
E100	187	58	10.3	80.0	85.0	64.0
E50	183	59	2.4	84.3	50.2	63.0

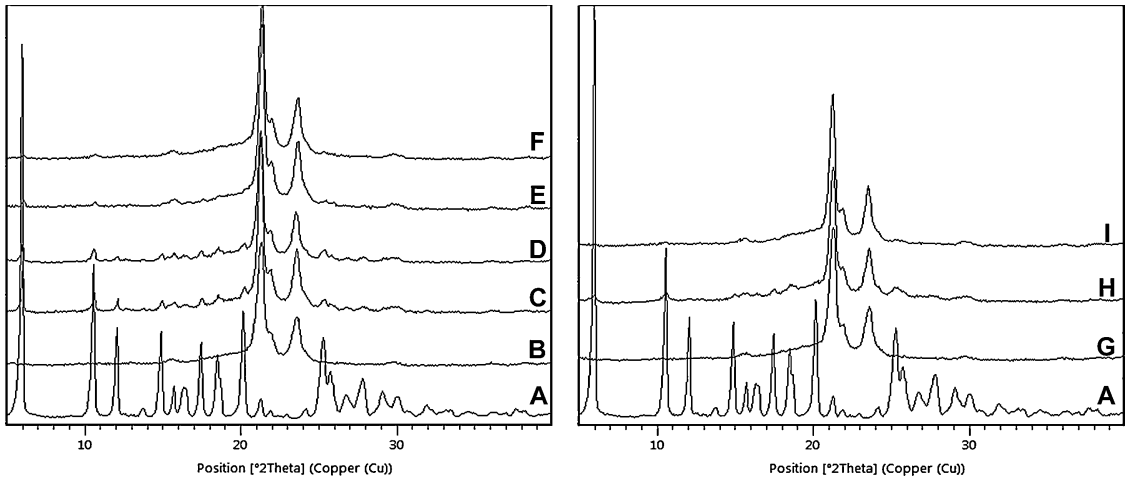

Fig. 2. XRD diffractogram of (A) free ddI, (B) BS, (C) PCL:ddI physical mixture (85:15), (D) S200, (E) PCL:ddI physical mixture (97:3), (F) S50, (G) BE, (H) E200 and (I) E50.

with very weak bands of ddI in S200. Overall, results confirmed the good encapsulation efficiency of both methods.

3.1.5. Thermal analysis

The thermogram of ddI showed a sharp endothermic peak at 185 °C (ΔH_m = 116.5 J/g) that was considered as 100% crystallinity (Table 2) [64]. Pure PCL showed a characteristic broad endotherm at 61 °C (ΔH_m = 100.3 J/g) that, considering a ΔH_m of 100% crystalline PCL of 139.5 J/g [43,65], corresponded to 71.9% crystallinity. Finally, ddI-loaded particles exhibited characteristic melting endotherms (T_m) of PCL at 58–59 °C and of ddI at 183–190 °C (Table 2). Only in S50, the melting of ddI was not detectable. $\%C_{ddI}$ depended on the liquid precursor, values being smaller for suspensions (45.9–85.8%) than for emulsions (50.2–98.9%) (Table 2). These results revealed that in particles obtained from suspensions ddI was amorphized to a higher extent, probably due to a slightly greater interaction with the polymer. Regarding $\%C_{PCL}$ in the particles, values were between 57.5% and 71.4% (Table 2) in good agreement with the behavior of the raw polymer. These results suggested only a minimal effect of the drug on the crystallization of the polymer matrix. It is known that DSC may not be sensitive enough to detect the crystallization energy of compounds present in low proportion (<10%) within a matrix with high crystalline component like PCL [67]. Therefore, we complemented the characterization of the particles with XRD analysis that enables lower detection limits. [67]

3.1.6. X-Rays diffractometry

XRD analysis was used to confirm the crystallinity of ddI and PCL in different samples. Free ddI showed the pattern of a crystalline powder (Fig. 2). Blank particles showed a similar diffraction pattern to that of pure PCL (not shown). This indicated the preservation of relatively high $\%C_{PCL}$ after process (Fig. 2). Furthermore, representative PCL:ddI physical mixtures showed the characteristic peaks of both components. In agreement with DSC analysis, the diffractogram of ddI-loaded particles with the highest *%LC* (S200 and E200) displayed the characteristic peaks of ddI (Fig. 2). As opposed to DSC analysis, in XRD, particles with the lowest *%LC* (S50) also showed very weak diffraction peaks of ddI (Fig. 2). These data discrepancy probably stemmed from the lower sensitivity of the former technique and confirmed that part of the drug was in crystalline form.

3.2. In vitro ddI release

The goal of the encapsulation was to reduce the interaction of the drug with the gastric medium. Since the solubility of ddI in water is high (~30 mg/mL) in a broad pH range, a strong influence of the pH on the release rate was not anticipated. In addition, the degradation in acid medium is extremely fast (in the order of few minutes) [46]. In this framework, assays were conducted under conditions that ensured the chemical integrity of ddI during the assay. Results showed a strong influence of the liquid feed on

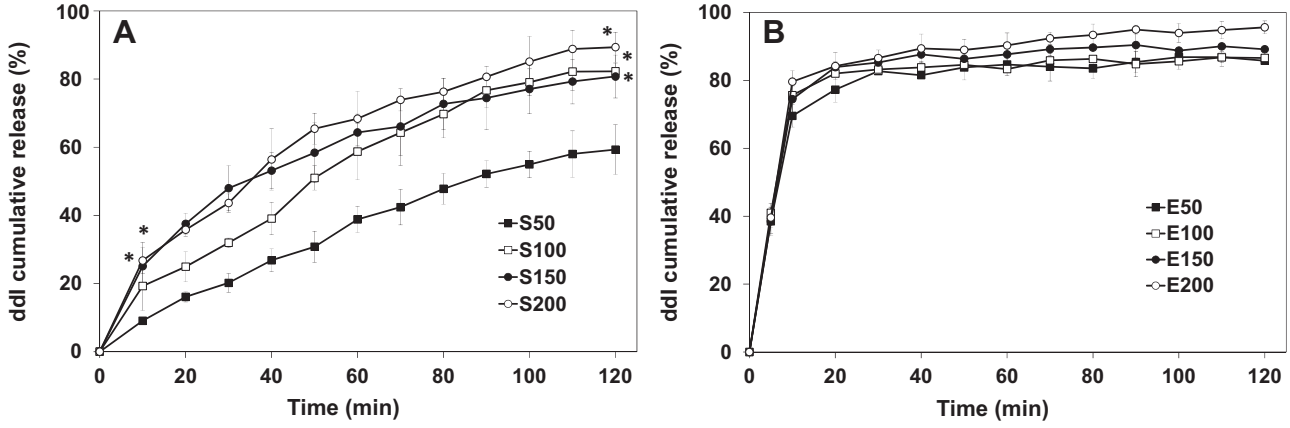

Fig. 3. ddI cumulative release from ddI-loaded particles prepared by spray-drying from (A) suspension and (B) emulsion. Results are expressed as mean ± S.D. (n = 3). *Statistically significant increase with respect to S50 (p < 0.05).

the release profile (Fig. 3). For samples obtained from suspension, some differences were observed. For example, S50 released 9.1% of ddI in the first 10 min while S200 released 26.7%. This trend was also apparent at later time points; release values being between 59.3% (S50) and 89.3% (S200) after 120 min (Fig. 3A). Conversely, samples prepared from emulsion showed faster release rates and differences for growing drug payloads were not statistically significant ($p < 0.05$) at any time point (Fig. 3B). According to ATR/FT-IR and XRD analyses, only a small to negligible amount of free ddI was available on the surface of emulsion-produced particles. Thus, regardless of the fact that ddI was mainly encapsulated within the particles, the presence of PVA probably accelerated the release. These results were in full agreement with the literature showing that the burst release of hydrophilic drugs from PCL delivery systems is more pronounced than for lipophilic ones [68]. Thus, since the released drug is expected to undergo fast acid degradation, only particles obtained from suspension were assessed for *in vitro* stability and oral bioavailability.

3.3. In vitro ddI stability in acid medium

Results of stability showed that the apparent first-order degradation rate constant (K_d) of ddI was 0.0183, 0.0191, 0.0122 and 0.0147 h^{-1} for S50, S100, S150 and S200, respectively. In this context, 80–100% of the encapsulated ddI was degraded within 120 min. Furthermore, ddI degradation percentages in the different samples (Fig. S1) were similar to the amount of released drug (Fig. 3), indicating that the encapsulated ddI was effectively isolated from the medium and that it only underwent degradation upon release. It is interesting to note that the fitting of the degradation rate to the release was irrespective of the fact that the pH conditions used in both assays were different. As described above, this behavior stemmed from the highly aqueous solubility of ddI in a broad spectrum of pH values. When free ddI in solution was exposed to the same acidic conditions, all the drug rapidly degraded within less than 15 min. These results suggested the particles would be able to increase the chemical stability of the drug with respect to the free one.

3.4. Oral pharmacokinetics

Based on these results, the gastric emptying time of particles after oral administration will govern the ability of the particles to protect the drug from the gastric medium. In this framework, the oral bioavailability of encapsulated ddI was compared to that of a solution. For this, one of the samples that showed the slowest release rate *in vitro* (S100) was used. Even though S50 showed the lowest ddI release rate in PBS (pH 7.4), *in vitro* stability assays in acid medium showed that the degradation profile was very similar to that of S100. Therefore, the latter sample was preferred due to a higher *%LC*. Results showed that the C_{max} with S100 increased 1.69-fold with respect to the ddI aqueous solution (Fig. 4, Table 3). Conversely, the t_{max} was similar for both formulations, indicating that the encapsulation process did not change the absorption mechanism and that the release of the cargo from the particles was fast enough. $AUC_{0\text{-}1}$ is a measure of the bioavailability in the time that the C_{max} was achieved (12–15 min), while the $AUC_{0\text{-}\infty}$ represents the ddI total bioavailability. AUC values obtained for S100 were higher than those obtained for the ddI solution; e.g., $AUC_{0\text{-}1}$ and $AUC_{0\text{-}\infty}$ increased 1.61- and 2.46-fold for the particles. In addition, with the particles, ddI was detected in plasma even at time point 360 min. In contrast, the solution resulted in detectable ddI levels not beyond a time point of 240 min (Fig. 4). At 720 min, ddI was not detectable in plasma for any sample. These results suggest that particles may have been retained in the intestinal mucosa, releasing the drug encapsulated over a slightly longer time. Finally, the k_e value was higher for the ddI solution ($k_e = 0.51\ h^{-1}$) than for S100 ($k_e = 0.37\ h^{-1}$) (Table 3), supporting another advantageous feature of the particles.

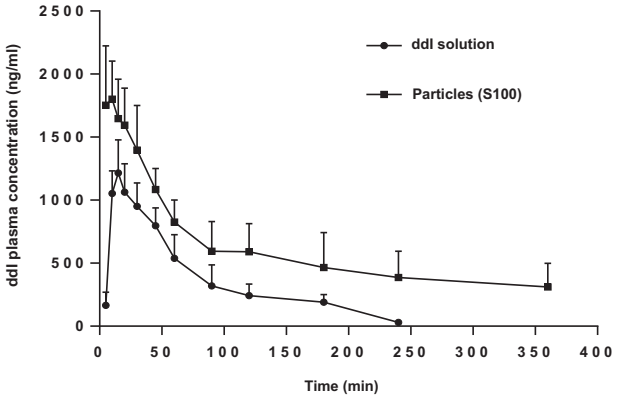

Fig. 4. ddI plasma concentration after the oral administration of the ddI aqueous solution and S100 obtained by spray-drying. ddI dose and concentration were 20 mg/kg and 5 mg/mL, respectively. Results are expressed as mean ($n = 4$).

Table 3
Pharmacokinetic parameters after the oral administration of the ddI aqueous solution and S100 obtained by spray-drying. ddI dose and concentration were 20 mg/kg and 5 mg/mL, respectively, for both cases. Results are expressed as mean ($n = 4$).

Pharmacokinetic parameter	ddI solution		S100	
	Mean	CV%	Mean	CV%
C_{max} (ng/mL)	1279	34.19	2158*	20.89
t_{max} (min)	13.75	18.18	12.50	51.64
$AUC_{0\text{-}1}$ (μg/mL/h)	0.82	39.01	1.32	25.22
$AUC_{0\text{-}\infty}$ (μg/mL/h)	1.91	46.19	4.71	63.93
F_r (%)	100.00	N.D.	246.60	N.D.
k_e (h^{-1})	0.51	22.79	0.37	7.52

$AUC_{0\text{-}1}$: Area-under-the-curve between 0 and 1 h.
$AUC_{0\text{-}\infty}$: Area-under-the-curve between 0 and infinite.
k_e: Elimination rate constant.
ND: Not determined.
* Statistically significant increase of C_{max} of S100 with respect to ddI solution ($p < 0.05$).

4. Conclusions

Spray-drying has increasingly attracted the interest of researchers in both academia and industry to produce polymeric particles of variable sizes loaded with hydrophilic and hydrophobic drugs. To explore the potential of this technique, herein we encapsulated the highly water soluble ARV ddI within PCL particles as a strategy to reduce its acid degradation in the stomach. Particles showed size at the microscale with variable encapsulation efficiency (60–100%) depending on the liquid feed. On one hand, *in vitro* release assays showed that the drug was release at a relatively fast rate. On the other, a delay in the release had a strong effect on the oral bioavailability that increased 2.5 times with respect to a free ddI aqueous solution. These findings were counterpoised to some extent to those of the *in vitro* experiments, and suggested that the gastric transit time was shorter than previously envisioned; a mild prolongation of the release playing a relevant role in preventing its degradation. Overall results support the extension of this scalable and cost-viable technology to the production of innovative products of state-of-the-art water-soluble ARVs as a strategy to overcome the most relevant biopharmaceutical drawbacks.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2014.09.055.

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