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Research paper

Improved bioavailability of darunavir by use of κ-carrageenan versus microcrystalline cellulose as pelletisation aid

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ABSTRACT

The aim of this study was to produce pellet formulations containing a high drug load (80%) of the poorly soluble HIV-protease inhibitor darunavir, using wet extrusion/spheronisation with κ-carrageenan or microcrystalline cellulose (MCC) as pelletisation aid. Drug release was assessed *in vitro* by a standardized paddle-dissolution test and *in vivo* by a single-dose pharmacokinetic study in dogs. Mean dissolution time (MDT) was 78.2 ± 3.5 h from MCC pellets (1301 ± 301 μm) and 6.1 ± 0.7 min from κ-carrageenan pellets (966 ± 136 μm). In contrast to κ-carrageenan pellets, MCC pellets did not disintegrate and showed a diffusion-controlled drug release. In line with the *in vitro* findings, the darunavir peak plasma levels and exposure after the administration of a 300 mg dose were more than 60-fold higher when formulated with κ-carrageenan pellets when compared with MCC pellets, and 10-fold higher after co-administration with 10 mg/kg of ritonavir. The relative bioavailability of darunavir versus the reference tablet (F_{rel}) was 155% with κ-carrageenan pellets and 2% with MCC pellets without ritonavir, while 78% and 9%, respectively, in presence of ritonavir. In conclusion, when compared with MCC pellets, the bioavailability of darunavir was substantially improved in κ-carrageenan pellets, likely due to their better disintegration behavior.

1. Introduction

Darunavir (Prezista®) is a protease inhibitor, labelled for use in combination with ritonavir as booster for therapy of HIV-1 [1,2]. When formulated as an oral immediate release form for the treatment of HIV-patients, this drug is taken as twice-daily dosing regimen in order to achieve sufficiently high plasma levels for therapeutic efficacy [3]. Formulations that allow a high drug load in order to establish sufficient plasma levels, while being slowly released over an extended period of time over 24 h, would require less frequent drug dosing and potentially improve patient compliance. The objective of this study was to develop pellets with high darunavir content, suitable for pharmaceutical formulation of an oral dosage form with an improved release profile.

Several techniques can be used to manufacture spherical beads (pellets), such as layering and granulation [4]. Wet extrusion/spheronisation is a well-known process particularly for spherical pellets with a narrow size distribution [5]. Other advantages of the technique are robustness and reproducibility as well as low costs. Using this manufacturing method, the wetted powder material requires special rheological properties like an adequate relationship between brittleness and plasticity [6]. These properties can be obtained by the addition of a pelletisation aid to the dry powder formulation, such as microcrystalline cellulose (MCC) or κ-carrageenan. MCC has been the standard in this manufacturing process for more than 30 years [6,7], lack of pellet disintegration. Recently, κ-carrageenan has been proposed as a suitable alternative, devoid of these shortcomings: the resulting pellet batches are characterised by high yields, spherical pellet shapes, narrow pellet distributions and faster drug release profiles when compared with MCC as pelletisation aid [8–10]. It was therefore tested whether κ-carrageenan can improve the disintegration and release profile of darunavir pellets when compared with MCC as pelletisation agent. Pharmacokinetic behavior of the two formulations was tested in dogs in the absence and presence of ritonavir. The latter protease inhibitor enhances the oral bioavailability of darunavir and other protease inhibitors by hepatic and intestinal inhibition of CYP3A4 metabolism in the liver, as well as by interfering with absorption at the level of P-glycoprotein [11].

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2. Material and methods

2.1. Experimental plan

2.1.1. Pellet preparation and in vitro characterization

The formulations, using MCC or κ-carrageenan, prepared in this study are given in Table 1. The content of pelletisation aid was fixed at 20% and the drug content was targeted at 80% of darunavir (w/w) in all formulations. Additional requirements for the final process were the following: (1) a yield of more than 90% (w/w) in sieve fraction between 700 and 1400 μm, (2) beads with a spherical shape having a median aspect ratio below 1.1, and (3) fast *in vitro* drug release, being defined as at least 80% of darunavir released within 30 min during a standard dissolution test.

2.1.2. In vivo characterization

In order to compare the release profiles of darunavir from MCC and κ-carrageenan pellets, a single-dose pharmacokinetic study was performed in male Beagle dogs, whereby each formulation or a commercial darunavir tablet (reference) was given orally as a 300 mg dose in randomized, cross-over fashion, either alone ($n = 3$) or in combination with 10 mg/kg ritonavir as booster ($n = 3$). Also their relative bioavailability (F_{rel}) versus the reference tablet was determined, in absence or in co-administration with the booster.

2.2. Manufacturing of pellets

2.2.1. Materials for pellet manufacturing

The following materials were used as received: κ-carrageenan (Gelcarin® GP 911 NF, FMC, Philadelphia, PA, USA), darunavir (TMC114 ethanolate, Tibotec bvba, Mechelen, Belgium), microcrystalline cellulose (MCC Sanaq 102 G, Phamatrans Sanaq, Basel, Switzerland).

2.2.2. Extrusion and spheronisation

The dry powders were weighed and blended for 15 min in a laboratory scale blender (LM40, Bohle, Ennigerloh, Germany) and were then transferred to an extruder gravimetric powder feeder (KT 20, K-Tron Soder, Lenzhard, Switzerland). A twin-screw extruder (Mikro 27GL-28D, Leistritz, Nuremberg, Germany) was used, equipped with an axial screen of 23 dies of 1 mm diameter and 2.5 mm length, or of 45 dies 0.7 by 1.25 mm, respectively. During the extrusion process, a constant screw speed of 100 or 125 rpm was applied, at a powder feed rate of 33 g/min and a suitable liquid feed rate (see Section 2.3.2). Deionised water was used as granulation liquid, supplied by a membrane pump (Cerex EP-31, Bran and Luebbe, Norderstedt, Germany): its flow was continuously monitored through a measuring device (Corimass MFC-081/K, Krohne, Duisburg, Germany). Batches of 300 g wet extrudate were collected and spheronised for 5 min at 750 or 1000 rpm in a spheroniser (RM 300, Schlueter, Neustadt/Ruebenberge, Germany) equipped with a cross-hatched rotor plate of 300 mm diameter. The drying step was performed for 10 min in a fluid bed apparatus (GPCG 1.1, Glatt, Dresden, Germany) with an inlet air temperature of 60 °C.

Table 1
Composition and main manufacturing process parameters of pellets with a high-darunavir load, using MCC or κ-carrageenan as pelletisation aid.

	MCC pellets (formulation 1)	κ-Carrageenan pellets (formulation 2)
Darunavir (%)	80	80
MCC (%)	20	–
κ-Carrageenan (%)	–	20
Water content of the extrudate (used during pelletisation) (%)	59–69	59–84
Screw speed (rpm)	125	100
Die plate: diameter × length in mm (n = number of dies)	1 × 2.5 ($n = 23$)	1 × 2.5 ($n = 23$) 0.7 × 1.75 ($n = 45$)
Friction plate speed (rpm)	750	1000
Drying conditions of pellets during manufacturing	10 min, 60 °C	10 min, 60 °C

2.2.3. Assessment of impact of liquid feed rate on pellet quality

The objective for manufacturing was to produce pellets of the best pharmaceutical quality, and therefore achieve an aspect ratio lower than 1.1. This parameter is the most commonly used shape parameter, while a high value of the 10% interval reflects a narrow size distribution (see Section 2.4.2) [10]. For both formulation types, different constant liquid feed rates during the pelletisation process were tested, in order to obtain a water content allowing for the lowest aspect ratio and the highest 10% interval.

2.2.4. Stability and reproducibility of the manufacturing process

Stability and reproducibility of the manufacturing process were assessed by producing several batches ($n = 11$) of the best formulation by continuous wet extrusion and multiple spheronisation processes without interruption for cleaning. After finishing of a spheronising process, the pellets were removed from the apparatus and the process was immediately restarted with new extrudates.

2.3. In vitro characterization

2.3.1. Water content of extrudate

In order to determine the extrudate water content, the loss of water during drying of the extrudate was determined. Three samples of each extrudate batch were taken during extrusion. The MCC containing samples were dried at 105 °C for 24 h in a circulating air oven (UT-6120, Kendo, Hanau, Germany), while carrageenan formulations were dried at 70 °C for 2 weeks in a vacuum oven (Heraeus VT 6060 M, Kendo, Hanau, Germany) [12]. The water content of the extrudates was calculated in % (w/w) based on the remnant dry mass.

2.3.2. Image analysis

Each batch was sieved from 710 to 1400 μm; this fraction was defined as yield of the pelletizing process. Representative samples of the yield fractions for image analysis were obtained by using a rotary cone sample divider (Retschmuehle PT, Retsch, Haan, Germany).

For the image analysis, a computer-aided system was used, consisting of a stereo microscope (Leica MZ 75, Cambridge, UK), a ring-light with cold light source (Leica KL 1500, Cambridge, UK) and a digital camera (Leica CS 300 F, Cambridge, UK) and applying an image-analysing software (Qwin, Leica, Cambridge, UK). Images of 500 pellets of each sample, taken at a suitable magnification (1 pixel = 17.5 μm), were translated into binary images. Contacting pellets were separated by a software algorithm. If the automatic separation failed, pellets were deleted manually. The projected area was determined for each pellet, in order to calculate the equivalent diameter, defined as follows:

$$d_{eq} = \sqrt{\frac{4A}{\pi}} \quad (1)$$

The pellets were further characterised by the aspect ratio, dimensionless particle size and particle size distribution. The aspect ratio was defined as the ratio of the maximum Feret diameter and the Feret diameter perpendicular to the maximum Feret diameter. This parameter allows characterizing the spherical shape, as perfect spheres will have an aspect ratio of 1.0. A mean aspect ratio lower or equal to 1.1 was considered as good, while ratios above 1.1 were rated as insufficient [16].

For the dimensionless particle size, the dimensionless diameter (d) was calculated as follows:

$$d = \frac{d_{eq}}{d_{eq50}} \quad (2)$$

whereby d_{eq} corresponds to the equivalent diameter and d_{eq50} to the median of all equivalent diameters of the batch.

Diameters were determined by their arithmetic mean ($\bar{x}$) and standard deviation (SD); aspect ratios were expressed as $x_{50} \pm$ IQR (median ± interquartile range): as the IQR is not affected by outliers or extreme values of pellet diameters, these data illustrate the distance between the 25th and 75th percentiles, essentially representing the range of the middle 50% of the data.

The 10% interval was used to describe the fraction of pellets within 0.9–1.1 of the dimensionless diameter. This parameter of the particle size distribution should exceed at least 50% for a formulation to be considered of sufficient pharmaceutical quality.

2.3.3. In vitro dissolution and pellet disintegration

In vitro dissolution of darunavir was assessed, using a standardized dissolution test method with a paddle apparatus (USP) at 75 rpm and 2% polysorbat 20 (Cesar & Loretz, Hilden, Germany and Uniqema, Eversberg, Belgium) in 0.05 M sodium phosphate buffer pH 3.0 as a release medium, made with phosphoric acid 85% (Bernd Kraft, Duisburg, Germany) and sodium dihydrogen phosphate monohydrate (Merck, Darmstadt, Germany). The solubility of TMC114 in this medium is 11.1 mg/mL. Six samples of approximately 28 mg of each pellet batch were tested in a randomized order. The concentration of darunavir in the release medium was determined three times per minute by a UV-photometer (267 nm, Lambda 2, Perkin-Elmer, Ueberlingen, Germany), according to the previous calibration of concentrations up to 2.5 mg/mL. Pellet disintegration was visually investigated during the dissolution test. Mean dissolution time was determined as previously described [13]. As about 100% of darunavir was recovered chemically at the end of the dissolution test, the measured release can fully be attributed to pellet disintegration (and not to the formation of metabolites that potentially would interfere with the measurements).

For the *in vitro* release profiles, the% drug dissolved was plotted in function of time. For the MCC pellets, classical Higuchi plots were produced by plotting the percentage of darunavir released from the MCC pellets versus the square root of time [14].

2.3.4. Stability of the κ-carrageenan pellets

As MCC pellets did not result in sufficient dissolution, their stability was not tested. In order to test whether κ-carrageenan pellets maintained their dissolution properties during storage, the stability of the pellets was determined as follows: pellets were stored under regular (25 °C, 60% relative humidity) or accelerated (40 °C, 75% relative humidity) conditions according to ICH [15] for 3 and 6 months in open or closed containers. After 33 days and 6 months, six samples per storage condition were investigated in the *in vitro* dissolution test.

2.4. In vivo release profiles

2.4.1. Test formulations

In the *in vivo* study, the formulations were given in a cross-over fashion, respecting 6 days washout, as follows: (1) two capsules containing κ-carrageenan pellets, each containing 150 mg darunavir per capsule; (2) two capsules containing MCC pellets, each containing 150 mg darunavir per capsule. The formulations were further compared with commercial darunavir tablets that are currently available for clinical use, containing each 300 mg darunavir per tablet (Prezista®, Tiborec bvba, Mechelen, Belgium) and further referred to as reference tablets. For booster, the commercially available oral solution of ritonavir (Norvir®, 80 mg/mL) was used. The solution was diluted 1/10 (v/v) with polyethylene glycol 400, in order to obtain an 8 mg/mL concentration for an oral gavage administration. All formulations were stored at room temperature.

2.4.2. Pharmacokinetic study design

Six male Marshall Beagle dogs (Marshall Farms, Green Hill 2001, Italy) of approximately 1–3 years and weighing 7–11 kg at the start of the experimental phase were used for this study. Dogs were fed 30 min before dosing with 250 g Pedigree® wet food. Three dogs were randomized to receive single oral doses of darunavir without booster in cross-over fashion, given as either one 300 mg tablet (marketed tablet, reference) or two 150 mg capsules containing either κ-carrageenan pellets or MCC pellets. The other three dogs were randomized in the same order to receive the respective formulation in combination with ritonavir, which was given by oral gavage at a dose of 10 mg/kg (8 mg/mL), shortly (5–7 min) prior to the darunavir administration. Administrations were performed on Days 0, 7 and 14 (6 days of washout between treatments). After dosing, the gastric tube used to administer the ritonavir solution was rinsed with approximately 5 mL of water before withdrawal from the dog's stomach.

2.4.3. Blood sampling and plasma preparation

Blood samples (2 mL on EDTA) were collected from the jugular vein at 0 (=pre-dose), 0.5, 1, 2, 4, 7, 24 and 31 h after the administration of the darunavir formulation in presence of ritonavir. As darunavir plasma levels in the absence of ritonavir are low in the dog, plasma sampling in the absence of ritonavir was limited up to 7 h for ethical reasons. For all animals, including those that also received ritonavir, the time of dosing (0 h) corresponds with the time of darunavir administration. Within 2 h of sampling, the blood samples were centrifuged at room temperature at about 1900*g* for approximately 10 min in order to separate blood plasma and within 2 h after the start of centrifugation, plasma was stored in the freezer.

2.4.4. Bioanalysis and data analysis

Plasma concentrations of darunavir (all treatments) and ritonavir (when administered as booster) were determined using a qualified research LC–MS/MS method. Sample preparation included protein precipitation using acetonitrile; 10 μL of the supernatant was subsequently injected into the LC–MS/MS system, equipped with a C-18 Polaris column and using a gradient elution with 0.01 M ammonium acetate solution/acetonitrile/methanol (flow rate 1 mL/min). The calibration curve was linear from 5 to 10,000 ng/mL, and the lower limit of quantification (LLOQ) 5.00 ng/mL.

Individual plasma concentration–time profiles of darunavir and ritonavir were subjected to a non-compartmental pharmacokinetic analysis of the individual or averaged plasma darunavir concentration–time profiles using validated PKAA R1.0a software (WinNonlin, Pharsight). Peak plasma concentrations (C_{max}), corresponding peak times (T_{max}), half-lives ($t_{1/2}$), AUC_{0-last} (AUC until the last time point with measurable plasma concentrations) and $AUC_{0-\infty}$ values were calculated. Descriptive statistics were generated for plasma concentrations and parameters. The relative bioavailability (F_{rel}) of darunavir was calculated based on the $AUC_{0-\infty}$ for the two pelletized formulations relative to the clinical reference tablets. Separate calculations were done for darunavir dosed alone and in combination with ritonavir. The boosting effect of ritonavir was evaluated by comparing the $AUC_{0-\infty}$ values for each darunavir formulation given alone and with ritonavir co-administration.

2.5. Ethics

The dogs were housed with access to water all-day and dry food until late afternoon, and treated according to current ethics, in accordance with the provisions for the protection of vertebrates that are used for experimental and other scientific purposes and for the protection of laboratory animals, as per Belgian laws and European convention (European Council Directives (1986)). The study was approved by the local Ethics Committee on animal experiments and was performed in an AAALAC-accredited laboratory, complying with European and Belgian regulations for animal experiments.

3. Results

3.1. MCC pellets manufacturing and properties

Reproducible pelletisation could be obtained between 59% and 69% of water content in the extrudate. The liquid feed rate of the extruder affected the shape and size distribution of the MCC pellets. An increase in the water content improved their shape and thus also their aspect ratio, which decreased from 1.17 with 59% of water to 1.1 or lower at 63% or higher water. High water contents, such as 69%, however, caused wide pellet size distributions, characterised by small 10% intervals (Fig. 1). It was not possible to manufacture MCC pellets of darunavir having simultaneously an aspect ratio below 1.1 and a narrow size distribution with a 10% interval of at least 50%. The pellet batch with the narrowest size distribution (or highest 10% interval) was manufactured at 59% water content and was selected for further study.

3.2. κ-Carrageenan pellets manufacturing and properties

Fig. 2 shows the process parameters of the extrusion process of the κ-carrageenan pellets. During pelletisation of 80% darunavir with 20% κ-carrageenan, constant power consumption and pressure could be applied after equilibration of the die plate temperature for 30 min of the wet extrusion. However, there was no significant impact of the die temperature to aspect ratio, equivalent diameter, tensile strength and mean dissolution time (Table 3). Using a die plate of 1 mm diameter and 2.5 mm length, the best spherical κ-carrageenan pellets (lowest aspect ratio and highest 10% interval) were achieved with a liquid feed rate resulting in 84% of water content in the pellets (formulation 2.2, Table 2). Median pellet size was 1308 ± 169 μm. Changing the die plate, using holes of 0.7 mm diameter and 1.25 mm length, allowed the production of smaller pellets within the desired yield fraction. The most spherical pellets were obtained at the same water content (84%) (formulation 2.3, Table 2). On testing of the stability and reproducibility of the manufacturing process, no remarkable differences were observed during the process, neither between the various spheronisation processes. Table 3 shows the pellet characteristics from the first, sixth and last batch of formulation 2.3 taken during manufacturing after sieving.

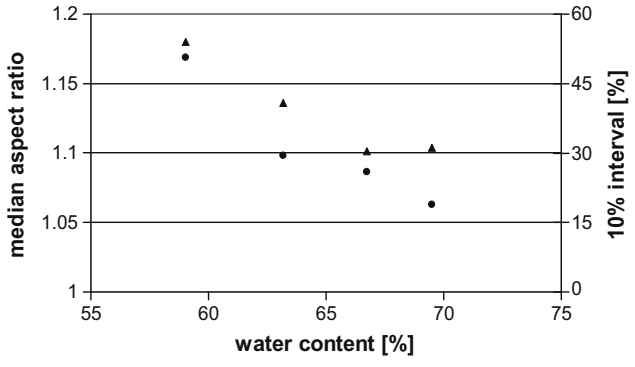

Fig. 1. Impact of the water content on pellet shape and size distribution of MCC pellets containing 80% of darunavir (n = 500): ▲(triangles) median aspect ratio (X_{50}) (Measure of pellet shape: a mean aspect ratio lower or equal to 1.1 is considered as good, while ratios above 1.1 are rated as insufficient (see Section 2.4.2).) and ● (bullets) 10% intervals of the corresponding batch (Measure of pellet distribution: the 10% interval describes the fraction of pellets within the interval 0.9–1.1 of the dimensionless diameter. This value should normally exceed at least 50% for a given particle size distribution, to be considered as sufficiently good for pharmaceutical quality (see Section 2.4.2).) in function of their water content.

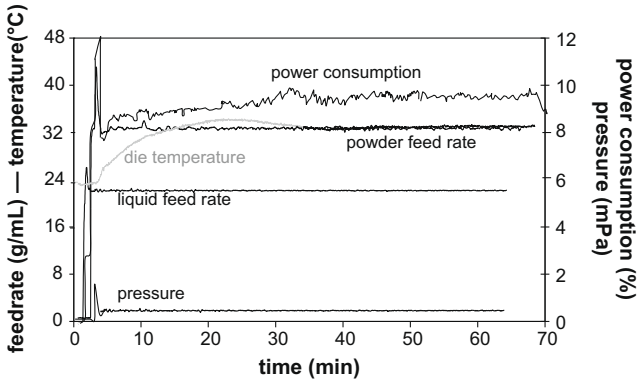

Fig. 2. Extrusion process parameters of darunavir/κ-carrageenan pellets: extrusion performed at constant process parameters.

3.3. In vitro dissolution tests and stability of the formulations

Fig. 3 illustrates the drug release from MCC in the *in vitro* dissolution tests. The release of darunavir from the MCC pellets (batch with 59% water content and narrowest size distribution) was slow, while the pellets appeared not to disintegrate. In the Higuchi plot, a linear correlation was seen up to 60–80% drug release, indicating a matrix-type drug release.

The dissolution profile of darunavir from the κ-carrageenan pellets (formulation 2.3, six batches) and the effect of storage on their dissolution profile using ICH conditions are given in Fig. 4. An immediate darunavir release was observed, which was attributed to fast pellet disintegration. There were no significant ($\alpha = 0.05$) differences in dissolution profiles and mean dissolution times in 6 months of storage.

3.4. In vivo pharmacokinetic results in dogs

Regardless of ritonavir co-administration, plasma concentrations of darunavir were substantially lower after the administration of MCC pellets as compared to κ-carrageenan pellets or the reference tablet. The plasma concentration–time profiles are given in Fig. 5. Mean C_{max} and $AUC_{0-\infty}$ values of darunavir are given in Table 4. Darunavir peak plasma levels and overall exposure (AUC) were more than 60-fold higher with κ-carrageenan than MCC pellets. With co-administration of ritonavir, the difference was somewhat less pronounced, these values being 10-fold higher.

In the absence of ritonavir, maximal concentrations of darunavir were observed at 0.5 h after oral dosing for all formulations. The mean F_{rel} value for darunavir versus the reference tablet was 155% for the κ-carrageenan formulation and 2% for the MCC pellets.

After co-administration with ritonavir, maximal concentrations of darunavir were observed between 1 and 4 h after dosing. The mean F_{rel} values for darunavir versus the reference tablets were 78% for the κ-carrageenan beads and 9% for the MCC beads.

Table 2
Water content and pellet properties of high-load darunavir pellets (80%), using κ-carrageenan as pelletisation aid.

	Formulation 2.1	Formulation 2.2	Formulation 2.3	Formulation 2.4	Formulation 2.5
Batch	e061005aa	e061005ab	e061005ac	e061005ad	e061005ae
Variation in the extrusion/spheronisation process					
Die plate, diameter × length in mm (*n* = number of dies)	1 × 2.5 (23)	1 × 2.5 (23)	0.7 × 1.25 (45)	0.7 × 1.25 (45)	0.7 × 1.25 (45)
Water content of extrudate (%)[a]	99.1 ± 2.5	84.2 ± 1.3	84.3 ± 2.8	68.5 ± 0.7	75.8 ± 0.1
Sieve fraction (m/m)					
>1600 μm (%)	0.1	0.2	0.0	0.0	0.0
>710 μm (%)	99.2	98.2	97.8	98.1	98.3
<710 μm (%)	0.7	1.6	2.2	1.9	1.7
Image analysis					
Equivalent diameter (μm)[a]	1260 ± 178.0	1308 ± 169	944.8 ± 130.8	1042 ± 120.9	972.9 ± 137.4
Aspect ratio[b]	1.09 ± 0.08	1.07 ± 0.06	1.08 ± 0.07	1.20 ± 0.15	1.10 ± 0.08
10% interval (%)	66	70.6	66.6	75.2	65.4

[a,b] Expressed as mean $\bar{x} \pm$ SD.
[b] Expressed as median aspect ratio ± IQR (see Section 2.3.2).

Table 3
Stability and reproducibility of the manufacturing process of pellets with a high-darunavir load, using κ-carrageenan as pelletisation aid: pellet properties of the 1st, 6th and 11th batch generated under continuous pelletisation.[a]

	κ-Carrageenan pellets (formulation 2.3)		
	Batch 1	Batch 6	Batch 11
Die plate	0.7 × 1.25 (45)	0.7 × 1.25 (45)	0.7 × 1.25 (45)
Water content (%)	83.5 ± 0.6	83.5 ± 0.4	83.3 ± 0.9
Sieve fraction (m/m)			
>1600 μm (%)	0.5	0.7	0.7
>1250 μm (%)	0.1	0.2	0.1
>710 μm (%)	97.8	97.4	97.5
<710 μm (%)	1.6	1.7	1.7
Image analysis			
Equivalent diameter (μm)	948.4 ± 117.5	966.2 ± 136.4	947.5 ± 127.6
Aspect ratio	1.08 ± 0.08	1.09 ± 0.08	1.12 ± 0.09
10% interval (%)	71.2	67.6	68

[a] See Table 2 for definitions.

The exposure to darunavir was higher in the presence of ritonavir compared to the administration of darunavir alone: the $AUC_{0-\infty}$ value without ritonavir increased 5-, 2- and 18-fold versus no ritonavir for the reference tablets, κ-carrageenan and MCC pellets, respectively.

4. Discussion

This series of studies showed that the use of κ-carrageenan offers considerable advantages over MCC as pelletisation agent for high drug loads of darunavir (80%), both in terms of manufacturability and drug release *in vitro* and *in vivo*. With regard to manufacturing at the scale used, pelletisation of darunavir by wet extrusion/spheronisation with κ-carrageenan was found to be a reproducible and robust process which resulted in pellets of satisfactory pharmaceutical quality. As evidenced by the analysis of different batches taken during a manufacturing cycle (Table 3), pellet size and size distribution were not affected by the manufacturing process duration. Acceptable aspect ratios and 10% intervals were obtained at water contents of 84% or higher, and the desired equivalent diameters were obtained with small die wholes (0.7 × 1.25 mm). *In vitro* dissolution test evidenced fast release of the drug (occurring within minutes) and adequate stability of the κ-carrageenan pellets on storage. Moreover, the rapid drug release was confirmed by the enhanced bioavailability *in vivo* in dogs when compared with MCC pellets, which moreover fell within the ranges of variability that can be expected for the reference tablets in dogs (+155% without and +78% with ritonavir versus the reference tablet).

In contrast, MCC proved to be inappropriate as pelletisation aid for manufacturing of high-load darunavir pellets, both in terms of formulation properties and darunavir release. The MCC pellets were of poorer quality, while dissolution i*n vitro* took days, the resulting Higuchi plot suggesting a matrix-like slow-release behavior of darunavir. Manipulating the process parameters during extrusion/spheronisation was not successful in improving the slow disintegration. Changes in extrudate stream through the extrusion screen were seen, possibly due to higher plasticity of the extrudates with higher amounts of water, as previously described [17]. Extrudate water contents below 70% had to be used in order to obtain reproducible pelletisation of high-load darunavir pellets, yet acceptable pharmaceutical quality (aspect ratio < 1.1 [16] and a

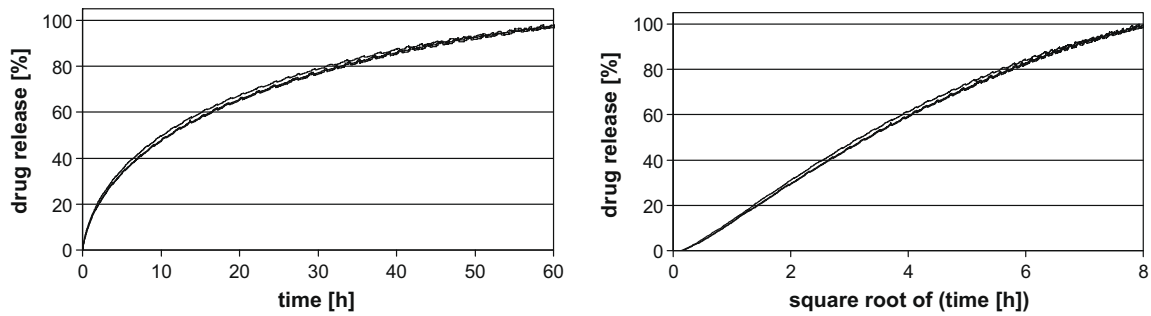

Fig. 3. Dissolution profiles of darunavir from MCC pellets (*n* = 6) (59% water content in pelletisation): dissolution profile (left) and Higuchi plot (right).

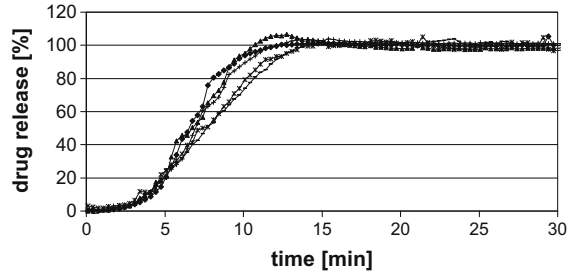

Fig. 4. Dissolution profile of darunavir from κ-carrageenan pellets (84% water content on pelletisation) and the effect of storage on their dissolution after normal or accelerated storage conditions: day 1 (♦); 1 month, 25 °C, 60% relative humidity, open bottles; (▲); 6 months, 25 °C, 60%, open bottles (Ж); 60 °C, 75%, closed bottles; (+) and 60 °C, 75%, closed bottles (–).

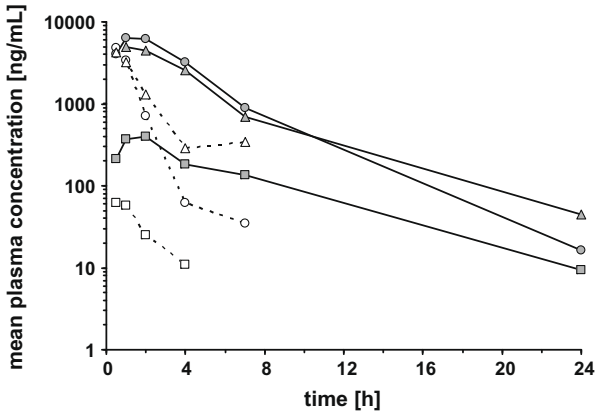

Fig. 5. Mean plasma concentration-versus-time profiles of darunavir (single dose of 300 mg/kg) given as one reference tablet (○---○) or 2 capsules with κ-carrageenan (△---△) or MCC (□---□) pellets, with (full lines, closed symbols) or without (dashed lines, open symbols) ritonavir (10 mg/kg) in dogs.

narrow size distribution as indicated by a 10% interval < 50% [9]) could not be obtained. The water content of the MCC pellets also strongly affected pellet shape and size distributions, as previously described [5,17]: increase in their water content improved pellet shape and decreased the aspect ratio, but adversely increased size distribution. Our observations of slow *in vitro* and *in vivo* darunavir release from the MCC pellets are in line with the earlier findings showing that extruded MCC pellets lack disintegration properties and have a release profile that is highly dependent on the solubility the incorporated drug [14].

The pharmacokinetics of the pellet formulations in dogs were in line with the *in vitro* dissolution findings: a single oral administration of 300 mg of darunavir, alone or in combination with 10 mg/kg ritonavir, resulted in the highest bioavailability of darunavir when κ-carrageenan beads were used (F_{rel} versus reference tablet being 155% with the κ-carrageenan bead formulation versus 2% for the MCC pellet formulation). For the MCC pellets, a slow release profile of darunavir was observed, which is in line with the finding of a matrix-type release on *in vitro* dissolution. In the presence of ritonavir, the bioavailability of darunavir remained high with κ-carrageenan pellets (78%) and low, albeit slightly increased versus without ritonavir, with the MCC pellets (9%). There is no obvious reason for the lower bioavailability of darunavir from the κ-carrageenan pellets relative to the commercial tablet in the presence of ritonavir. With the limited number of animals in this exploratory study, and considering the overall variability in darunavir pharmacokinetics in dogs, it is probable that the bioavailability of the κ-carrageenan pellets is grossly similar to the reference clinical tablet both with and without co-administration of ritonavir.

In this study, also the boosting action of ritonavir was confirmed by the higher exposure to darunavir in the presence of ritonavir for all formulations. A former pharmacokinetic study of darunavir in dogs did not show a substantial effect of ritonavir on darunavir's pharmacokinetics in dogs [18]. However, in that study, ritonavir was given 1 h before the lower darunavir dose and at higher doses (120 mg/kg), while in the current study, ritonavir administration preceded darunavir administration (300 mg, corresponding to less than 30 mg/kg) by less than 10 min. Timing of ritonavir administration is relevant because this booster agent is a very potent inhibitor of hepatic and intestinal CYP3A4 metabolism in the liver and intestinal wall, while it may also reduce absorption via interactions with P-glycoprotein [11]. Typically, the boosting effects are most pronounced in the case of a lower intrinsic bioavailability, as observed with the MCC pellets in the current study, whereas the relative effect of boosting with the high-performing κ-carrageenan pellets remained limited (two-fold). The parallel group design in the evaluation of the ritonavir boosting is a limitation in the current study, calling for caution in the interpretation of the boosting effect.

In conclusion, the *in vitro* and *in vivo* testing of darunavir release supported that the bioavailability of darunavir from κ-carrageenan pellets is much higher than that from MCC pellets, likely due to their better disintegration behavior when compared with MCC pellets.

Table 4
Main pharmacokinetic parameters of oral darunavir (300 mg single dose) in dogs, administered as a commercial reference tablet or two 150 mg pellet capsules with either κ-carrageenan or MCC as pelletisation aid.[a]

Day	0		7		14	
Treatment	Reference tablet	Reference tablet + ritonavir	κ-Carrageenan beads	κ-Carrageenan beads + ritonavir	MCC beads	MCC beads + ritonavir
C_{max} (ng/mL)[b]	4823 ± 1450	6937 ± 1809	4592 ± 3160	5707 ± 3130	68.0 ± 31.3	442 ± 308
$AUC_{0-\infty}$ (h ng/mL)[b]	5793 ± 1648	27,853 ± 8624	8974 ± 1186[e]	21,591 ± 9104	137 ± 43.3[e]	2447 ± 1432
Relative bioavailability versus reference tablet, F_{rel} (%)[c]	–	–	155	78	2	9
Boosting ratio[d]	–	5	–	2	–	18

[a] The single administrations were given in a randomized, cross-over fashion, in absence of ritonavir in three dogs, and in similar randomized order but immediately after ritonavir administration by oral gavage (10 mg/kg) in three other dogs (6 days of washout between treatments).
[b] Mean values in three dogs, unless otherwise specified.
[c] F_{rel} (%) is calculated as the% quotient of the $AUC_{0-\infty}$ values for each of the darunavir pellet formulations versus the reference tablet.
[d] Ratio of the $AUC_{0-\infty}$ values of the respective formulation in the presence versus absence of ritonavir.
[e] $n = 2$.

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