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PRILLING OF FATTY ACIDS AS A CONTINUOUS PROCESS FOR THE DEVELOPMENT OF CONTROLLED RELEASE MULTIPARTICULATE DOSAGE FORMS

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Abstract

In this study, prilling was evaluated as a technique for the development of multiparticulate dosage forms using the fatty acids stearic acid and behenic acid as potential matrix formers to control the release of metoprolol tartrate (MPT), a highly water soluble drug. The in vitro drug release was dependent on the drug load, type of fatty acid and pH of the dissolution medium. Higher drug loads resulted in faster release with behenic acid releasing drug over longer periods relative to stearic acid. The in vitro drug release was pH-dependent at low drug load with the release being slower at lower pH. Due to ionization of the fatty acid at pH 7.4, drug release was susceptible to the ionic strength at this pH value. Solid state characterization indicated that the crystalline state of the fatty acids was not affected by thermal processing via prilling, while the crystallinity of MPT was decreased. During storage, the amorphous MPT fraction recrystallized in the entire matrix. Drug release from behenic acid matrices was increased during storage at 40 °C, however no polymorphism of behenic acid was detected. The bioavailability of MPT, after oral administration to dogs as prills containing 30 % and 40 % MPT using behenic acid as matrix former, was not significantly different from a commercial sustained release reference formulation, although the 40 % MPT prills showed a burst release.

Keywords: Prilling, fatty acids, metoprolol tartrate, controlled release, multiparticulate dosage forms, stability

1. INTRODUCTION

The need for alternative excipients that allow for continuously producing solid dispersions to improve the dissolution of poorly water soluble drugs or to create sustained release dosage forms has been reported by different authors [1-4]. The application of oral sustained release formulations has improved patient compliance due to a lower dosing frequency and a reduced incidence of adverse side effects [5]. Moreover, the production of sustained release multiparticulate dosage forms is advantageous since their transport in the gastro-intestinal tract is independent of gastric emptying and they exhibit a reduced risk of dose accumulation and local irritation when compared to single-unit dosage forms [6].

Lipid-based excipients such as triglycerides and fatty acids have been used for the development of solid dosage forms as matrices for controlled drug release [7-10], as taste-masking agents [11], to enhance drug solubility [12-14] and for the manufacturing of floating dosage forms [15, 16]. Compared to polymers [17], triglycerides exhibit several advantages, including low cost, non-toxicity and biodegradability. Nevertheless, their physical instability during storage (reflected in changes in the melting enthalpy and melting range which modify drug release [10]), remains the main barrier to overcome when applying these excipients for drug formulation [7, 18].

While lipid-based solid dosage forms have been processed using techniques such as solid lipid extrusion [19], extrusion/spheronisation [20], melt granulation [21], melt pelletization [7, 9] and spray-congealing [13], prilling has received limited attention in the pharmaceutical industry as a technique to efficiently incorporate drugs in a multiparticulate lipid-based solid dosage form. The prilling process consists of pumping a mixture of drug and lipid through calibrated nozzles, creating a liquid jet. By applying vibrational energy, the liquid jet breaks up into droplets which are then cooled by falling through a temperature-controlled prilling tower [22]. This technique offers the advantage of obtaining in a continuous fashion spherically shaped particles with a narrow particle size distribution. Having excellent flow properties, the particles can be easily filled into gelatin capsules [23], creating a

multiparticulate formulation. Additionally, no solvent is involved, resulting in a shorter and environmentally friendly pharmaceutical process. However, the major disadvantage of the prilling process is the need of a high prilling tower, linked to higher costs and difficulties in operation and maintenance [24].

The aim of this study was to evaluate the use of prilling for the manufacturing of multiparticulate dosage forms using fatty acids as potential matrix formers to control the release of highly water soluble drugs. For this purpose, stearic acid and behenic acid, C18 and C22 fatty acids, respectively, were combined with metoprolol tartrate (MPT) as model drug and the in vitro performance was assessed. The solid state of the formulations, termed 'prills' further on in this study, was characterized using modulated differential scanning calorimetry (MDSC), X-ray diffraction (XRD), Raman spectroscopy, Raman microscopic mapping and attenuated total reflection Fourier-transform (ATR FT-IR) spectroscopy. Furthermore, the physical stability of the prills during 6 months storage at 25 and 40 °C was monitored. Finally, the bioavailability of the different formulations was evaluated after oral administration to dogs and compared to a commercial sustained release formulation.

2. MATERIALS AND METHODS

2.1 Materials

Metoprolol tartrate (MPT) (Esteve Quimica, Barcelona, Spain) was selected as a model drug. Behenic acid (Radiacid 0560) was purchased from Oleon (Ertvelde, Belgium) and had a C22 purity of 89 %. Stearic acid, with a C18 purity of 98.7 %, was purchased from Mosselman (Ghlin, Belgium). All other chemicals were of analytical grade.

2.2 Methods

2.2.1   Prilling

Prilling was carried out with a custom-made prilling equipment developed by Peira (Turnhout, Belgium). After melting the fatty acid and heating the melt to 90 °C (stearic acid) or 100 °C (behenic acid), MPT was added to the melt under stirring. Droplet formation was started after complete dissolution of the drug in the molten matrix. By applying air pressure, the mixture was fed towards the thermostated nozzle (90 °C) equipped with a valve and a needle (inner diameter: 0.33 mm). To manufacture solid particles containing 10 % MPT and using behenic acid as matrix former, a drop time (i.e. period during which the valve is open) of 0.04 s and an air pressure of 0.5 bar were applied, whereas the drop time and air pressure were set at 0.07 s and 0.5 bar for the 20 % MPT and 30 % MPT formulation. When the drug load was increased to 40 %, a drop time of 0.07 s and a pressure of 1 bar were used. A drop time of 0.04 s and an air pressure of 0.5 bar were needed for all MPT/stearic acid combinations. Droplets formed at the needle end, were quench cooled in liquid nitrogen in order to obtain solid spherical particles.

2.2.2   Particle size and shape

The particle size and shape were determined using an image analysis system. Photomicrographs of the prills were taken with a digital camera (Camedia® C-3030 Zoom, Olympus, Tokyo, Japan), linked with a stereomicroscope system (SZX9 DF PL 1.5x, Olympus, Tokyo, Japan). A cold light source (Highlight 2100, Olympus, Germany) and a ring light guide (LG-R66, Olympus, Germany) were used to obtain top illumination of the prills

against a dark surface. The images were analyzed by an image analysis software (AnalySIS®, Soft Imaging System, Münster, Germany). At least 20 particles were analyzed from each batch. Each individual particle was characterized by the mean Feret diameter (FD) (average of 180 calliper measurements with an angle of rotation of 1°). An average value for all prills has been calculated as the mean particle size (mean FD). To evaluate sphericity, particles were characterized by the aspect ratio (AR) (ratio of the longest Feret diameter and its longest perpendicular diameter).

2.2.3   In vitro drug release

In vitro dissolution was performed using USP dissolution apparatus 1 (baskets). The equipment consisted of a VK 7010 dissolution system coupled with a VK 8000 automatic sampling station (Vankel, New Jersey, USA). An amount of prills corresponding to 30 mg MPT was inserted into the baskets. The basket rotational speed was set at 100 rpm and the temperature of the dissolution medium was maintained at 37 ± 0.5 °C. Samples of 5 ml were withdrawn after 0.5, 1, 2, 4, 6, 8, 12, 16, 20 and 24 h and analyzed spectrophotometrically at 222 nm using a double beam spectrophotometer (UV-1650PC, Shimadzu, Antwerp, Belgium). MPT concentrations were calculated from a calibration curve between 0 and 33 µg/ml. Demineralized water, 0.1N HCl (pH 1) and a phosphate buffer (USP, pH 7.4) were used as dissolution media. The influence of the ionic strength µ on MPT release was studied in diluted phosphate buffer with µ = 0.0089 (10-fold dilution), µ = 0.018 (5-fold dilution) and µ = 0.045 (2-fold dilution), phosphate buffer (µ = 0.089), phosphate buffer with increasing NaCl concentrations (µ = 0.14 and µ = 0.20), 0.1N HCl and 0.1N HCl with µ = 0.20. Each experiment was performed in triplicate. The similarity between dissolution profiles was evaluated using the similarity factor f_2, according to Shah *et al.* (1998), and was calculated using the following equation (Eq. 1):

$$f_2 = 50 \log_{10}\left(\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2 \right]^{-0.5} * 100 \right) \tag{1}$$

where R_t and T_r represent the cumulative drug release at each sample point of the reference and the test sample with n equal to the number of sample points. As f_2 is sensitive to the number of sample points leading to bias, only one sample point exceeding a drug release of 85 % was considered in the calculation. f_2 values higher than 50 indicate similarity between 2 dissolution profiles based on an average difference of less than 10 %, while f_2 values below 50 represent significant differences [25].

2.2.4 Modulated differential scanning calorimetry

The thermal behavior of the pure compounds (MPT, stearic acid and behenic acid), the physical mixtures and the corresponding formulations was evaluated using a differential scanning calorimeter Q2000 (TA Instruments, Zellik, Belgium) equipped with a refrigerated cooling system. The DSC was calibrated for temperature and enthalpy using an indium standard. Tzero calibration was performed in 2 steps; baseline calibration (without samples or pans) and sapphire calibration (using large sapphire disks on both the sample and reference positions). Small sapphire disks, placed in a Tzero pan, were used for the heat capacity (MDSC) calibration. Samples (± 5 mg) were run in Tzero pans (TA Instruments, Zellik, Belgium) with an underlying heating rate of 2 °C/min. The modulation period and amplitude were set at 60 s and 0.318 °C, respectively (heat-iso method). Dry nitrogen was used as a purge gas through the DSC cell at a flow rate of 50 ml/min. MDSC data were analyzed using the Universal Analysis software (TA Instruments). Melting enthalpies were determined in the total heat flow signal. Melting temperatures were reported as onset temperatures.

2.2.5 X-ray diffraction

Crystallinity was analyzed using X-ray diffraction on the pure compounds, the physical mixtures and the corresponding formulations. X-ray diffraction was performed with a D5000 Cu Kα diffractor (λ = 1.54 Å) (Siemens, Karlsruhe, Germany) with a voltage of 40 mV in the angular range of 10° < 2θ < 60° using a step scan mode (step size = 0.02°, counting time = 1s/step).

2.2.6 FT-IR analysis

Attenuated total reflection Fourier-transform infrared (ATR FT-IR) spectroscopy was performed on the pure substances, physical mixtures and prills to identify the interactions that were formed between MPT and the fatty acids during prilling. Spectra were recorded using a Bruker Vertex 70 FT-IR spectrometer equipped with a Hyperion FT-IR microscope and MCT detector. A Ge ATR crystal was pressed against the prills in order to obtain the ATR FT-IR spectra (4 cm^{-1} resolution, 50 scans).

2.2.7 Raman spectroscopy

Raman spectra were collected with a Raman Rxn1 spectrometer (Kaiser Optical Systems, Ann Arbor, MI, USA), equipped with an air-cooled CCD detector. The laser wavelength was the 785 nm line from a 785 nm Invictus NIR diode laser. All spectra were recorded over the 0 – 1800 cm^{-1} range with a resolution of 4 cm^{-1} and an exposure time of 5 s, using a laser power of 400 mW. Data collection and data transfer were automated using the HoloGRAMS^{TM} data collection software, the HoloREACT^{TM} reaction analysis and profiling software and the Matlab software (version 7.1, The MathWorks Inc., Natick, MA). Data analysis was performed using SIMCA P+ (Version 12.0.1.0, Umetrics, Umeå, Sweden). All spectra were SNV-preprocessed. Raman spectroscopy was performed on the pure compounds, the physical mixtures and the corresponding formulations.

The solid state distribution of MPT in the prills was evaluated by Raman microscopic mapping using a Raman Rxn1 Microprobe. Cross sections of prills were scanned by a 10 x long working distance objective lens (spot size 50 µm) in area mapping mode using an exposure time of 4 s and a step size of 50 µm in both the x and y directions (= 234 spectra per mapping). The resulting images provide information about the distribution of the solid state of MPT in the prills.

2.2.8 Storage

The formulation containing 30 % MPT and 70 % behenic acid was selected for a stability study. Immediately after prilling, the formulation was filled in hermetically sealed bags under controlled circumstances (35% RH) and stored at 25 °C and 40 °C. To investigate the influence of storage on solid state and drug release, the prills were studied immediately after manufacturing, after 1 week, 1 month, 3 months and 6 months storage. The solid state was analyzed using MDSC, XRD, Raman spectroscopy and Raman microscopic mapping.

2.2.9   In vivo evaluation

All procedures were performed in accordance with the guidelines and after approval by the Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO) (Merelbeke, Belgium). To study the influence of MPT concentration, the following formulations were administrated to 6 dogs:

Formulation 1 (F1): prills containing 30 % MPT and 70 % behenic acid

Formulation 2 (F2): prills containing 40 % MPT and 60 % behenic acid

Formulation 3 (F3): Slow-Lopresor® 200 Divitabs® (Sankyo, Louvain-la-Neuve, Belgium), a commercial sustained release formulation consisting of matrix tablets containing 200 mg MPT.

The test formulations F1 and F2 were filled in hard-gelatin capsules, corresponding to 200 mg MPT. Slow-Lopresor® 200 Divitabs® (1 tablet) was used as a reference formulation (F3). All formulations were administrated to 6 male mixed-breed dogs (23 - 41.5 kg) in a cross-over study with a wash-out period of at least 8 days. The dogs were fasted 12 h prior to the administration and 12 h after administration, although water was available ad libitum. Before the administration, an intravenous cannula was placed in the lateral saphenous and a blank blood sample was collected. The formulations were administrated with 20 ml water and blood samples were collected in dry heparinized tubes at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h after administration. The obtained blood samples were centrifuged at 1500 g during 5 min.

2.2.9.1 Metoprolol tartrate assay

A validated HPLC method with fluorescence detection was used for the determination of MPT in dog plasma. Plasma samples (300 µl) were mixed with 20 µl of a 2.75 µg/ml aqueous bisoprolol hemifumarate (internal standard, IS) solution and 320 µl of a 4 % (v/v) aqueous phosphoric acid solution. The mixtures were vortexed during 30 s. Drug and internal standard were extracted using solid phase extraction (SPE) cartridges (Oasis® MCX 1 cc (30 mg), Waters, Brussels, Belgium) and a 10-port vacuum extraction manifold. After conditioning the SPE columns with 1 ml methanol and 1 ml water, the plasma samples were transferred to the columns. The columns were rinsed with 1 ml of a 2 % (v/v) aqueous formic acid solution, followed by 1 ml methanol. 1 ml of a 5 % (v/v) solution of ammonium hydroxide in methanol was used to elute MPT and IS. Samples were evaporated to dryness under a N_2-flow and reconstituted in 150 µl distilled water. 20 µl was injected into the HPLC system. MPT plasma concentrations were calculated from a calibration curve, determined by adding 20 µl of a MPT standard (0.375, 0.5625, 0.75, 1.5, 2.25, 3.75 and 5.25 µg/ml), 20 µl of the IS solution and 320 µl of an aqueous phosphoric acid solution (4% (v/v)) to 280 µl of blank plasma. The mixtures were then treated as described previously. The method validation indicated a linear relationship between MPT plasma concentration and response (range: 0 – 353.5 ng/ml; R^2 = 0.999 ± 0.001 (n = 8)). The limit of detection and limit of quantification were 10.1 and 30.6 ng/ml, respectively. MPT showed a retention time of 14 min, while the IS eluted after 18 min.

The HPLC system consisted of an isocratic solvent pump (L-7100, Merck, Hitachi LaChrom, Tokyo, Japan), an automatic autosampler (L-2200, Merck, Elite LaChrom, Tokyo, Japan), a guard column (LiChroCart® 4-4, LiChrospher® 100 CN (5 µm), Merck, Darmstadt, Germany) followed by a reversed phase CN column (LiChroCart® 250-4, LiChrospher® 100 CN (5 µm), Merck, Darmstadt, Germany) and a variable wavelength fluorescence detector (L-7480, Merck, Hitachi LaChrom, Tokyo, Japan). Peak integration was performed using the software package D-7000 HSM Chromatography Data Station (Hitachi Instruments, San

Jose, CA, USA). The mobile phase consisted of a phosphate buffer solution (2M sodium phosphate monobasic dihydrate), acetonitrile and water (0.5/3.5/96; v/v/v) adjusted to pH 3 with phosphoric acid. The pump flow was set at 1.1 ml/min and the excitation and emission wavelength were 275 nm and 300 nm, respectively.

2.2.9.2 Data analysis

The peak plasma concentration (C_{max}), the extent of absorption (AUC_{0-12h}) and the time needed to reach the highest plasma concentration (t_{max}) were determined. The controlled release characteristics of the formulations were evaluated by means of the $HVD_{t50\%Cmax}$ (half-value duration) defined by the period during which the plasma concentration exceeds 50 % of the C_{max} [26, 27]. The effect of the formulation on the bioavailability was statistically evaluated by repeated-measures ANOVA (univariate analysis) using SPSS 17 (SPSS, Chicago, USA). To compare the effects of the different treatments on the pharmaco-kinetic parameters, a multiple comparison among pairs of means was performed using a Bonferroni post-hoc test with $p < 0.05$ as significance level.

3. RESULTS AND DISCUSSION

3.1 In vitro drug release

Prilling was performed using a process temperature ranging between 90 °C and 100 °C. Thermogravimetric analysis (TGA), performed on MPT, stearic acid and behenic acid, indicated thermal stability of these compounds at the process temperature (data not shown). In a first step, the fatty acids were molten and MPT was added to the melt. Next, droplet formation was started after complete dissolution of the drug in the molten carrier. Process parameters were chosen in order to obtain a fast droplet formation at the needle-end and to limit the residence time of the drug/lipid mixture in the reservoir. Drug load influenced the processability as a higher air pressure was required to process mixtures with a higher melt viscosity. The mean particle size and shape of the prills, determined by measuring the mean Feret diameter and aspect ratio (AR), are shown in Table 1 for prills containing 10 %, 20 %, 30 % and 40 % MPT in a behenic acid matrix and for prills containing 30 % MPT in a stearic acid matrix. Only minor variations in particle size were noticed for the 10 %, 20 % and 30 % MPT formulations, however the 40 % MPT prills had a remarkable smaller particle size possibly attributed to the higher air pressure needed to process this mixture. As all aspect ratio's approached the ideal sphericity value of 1, these data indicated that prilling resulted in spherically shaped particles with a narrow particle size distribution. All particles exhibited a smooth surface.

The influence on drug release using behenic acid or stearic acid as matrix formers, in combination with 30 % MPT, is illustrated in Fig. 1A. Drug release in water was found to be faster using stearic acid as matrix former since 94 % of the total drug load was released after 4 h, while only 62 % was released when MPT was incorporated in a behenic acid matrix. The slower drug release using behenic acid could be attributed to its longer fatty acid chain relative to stearic acid. In a study performed by Desai *et al.* (2010), a similar influence of chain length on drug release was reported [28]. Since behenic acid released MPT over

longer periods, only the combination of MPT and behenic acid is discussed further on in this study.

Fig. 1B represents the dissolution profiles in water for prills containing 10 %, 20 %, 30 % and 40 % MPT in a behenic acid matrix. At low drug load (10 %), only 38 % of the MPT was released after 24 h. The release rate was enhanced by increasing the drug load, although only minor differences between prills containing 20 % MPT and 30 % MPT were observed, reflected by a similarity factor of 53. The influence of pH on drug release of the 10 % and 30 % MPT formulations was evaluated in two dissolution media: 0.1N HCl (pH 1) and phosphate buffer (USP, pH 7.4) to mimic the pH conditions in the stomach and the small intestine, respectively. Drug release was pH-dependent at low drug concentration (10 %) (Fig. 1C). At pH 7.4, 91 % of MPT was released after 20 h compared to 62 % after 24 h at pH 1. This effect was attributed to (partial) ionization of the carboxyl group of behenic acid at higher pH, resulting in a less hydrophobic matrix. When the drug load was increased to 30 %, the differences between the 2 dissolution media were smaller and MPT was released faster: at pH 1, 93 % of MPT was released after 12 h (data not shown). Due to the higher drug load, an extended channel system was possibly generated in the prills during immersion, consequently the matrix effect of behenic acid was less pronounced. Since the morphology of the prills was unchanged after dissolution, MPT release from the lipid matrices was diffusion-controlled.

Next, the influence of the ionic strength of the dissolution medium on the drug release was investigated. Fig. 1D demonstrates that at pH 7.4, for prills containing 30 % MPT and 70 % behenic acid, the slowest drug release was obtained in the dissolution medium with the highest ionic strength. Only 62 % MPT was released after 8 h in a medium of μ = 0.20, whereas 88 % and 97 % were released after 8 h in the media of μ = 0.089 and μ = 0.018, respectively. Further dilution (μ = 0.009) of the medium had no influence anymore on the release profiles. Increasing the ionic strength at pH 1 did not alter the release profile in this medium (data not shown). At higher pH, the ionized carboxyl group of behenic acid interacts

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with positively charged ions from the dissolution medium, resulting in slower drug release. A similar influence of the ionic strength on drug release was noticed for the 10 % MPT formulation (data not shown). Johnson *et al.* (1992) reported that the electrolyte composition of the gastrointestinal fluids ranged from 0.010 to 0.166 and hence, the drug release from behenic acid matrices will likely be affected during the transit in the gastrointestinal tract [29]. The susceptibility of lipid formulations to ionic strength was also reported by Chang *et al.* (1997). The release of propranolol hydrochloride from monoglyceride matrices showed only a minor susceptibility to the ionic strength at pH 7.4 as monoglycerides are non-ionic amphiphilic molecules [30].

3.2 Thermal behavior and crystallinity

Fig. 2 shows the MDSC thermograms for MPT, stearic acid, behenic acid, the 30/70 MPT/fatty acid physical mixtures and the corresponding formulations. Metoprolol tartrate showed an onset melting temperature at 120.7 °C, while stearic acid and behenic acid started melting at 68.2 °C and 74.7 °C, respectively, indicating the crystalline state of these compounds. Even-numbered saturated fatty acids can crystallize in at least four polymorphs, named A, B, C and E. As form C is thermodynamically stable at high temperature, all other polymorphs irreversibly transform into form C upon heating [31]. However, as no solid-solid transformations were detected prior to the melting endotherms of stearic and behenic acid (i.e. 68 and 75 °C, corresponding to the melting point of the C polymorph), it indicates that only the polymorph C was present in the formulations [32]. These findings were in agreement with Kobayashi (1988), who stated that only the thermostable C-form could be formed from a melt [31]. In contrast, Corvis *et al.* (2011) reported recrystallization of stearic acid in the E-form from a ibuprofen/stearic acid melt. However, upon heating, this polymorph converted to the thermostable C-form at about 45 °C [32]. Thermal analysis of the physical mixtures and the formulations only revealed a melting endotherm of the fatty acids due to dissolution of the MPT crystals in molten stearic acid or behenic acid. Addition of MPT lowered the melting temperature of the fatty acids with a few degrees. A glass transition temperature of MPT

could not be detected in the reversed heat flow signal of the formulations. In the physical mixtures and the formulations, the melting enthalpy of fatty acids was higher than expected, suggesting that the excess of energy was needed to dissolve the MPT crystals.

The solid state was also characterized using XRD and Raman spectroscopy. The X-ray diffraction patterns of the pure compounds, the 30/70 MPT/behenic acid physical mixture and the corresponding formulation are shown in Fig. 3. MPT showed representative peaks for 2θ at 10.7°, 16.0°, 19.6° and 23.3°. The highly crystalline state of behenic acid was indicated by sharp diffraction peaks at 21.7° and 24.3°. As these peaks also showed up in the formulation, it could be concluded that the crystallinity of the fatty acid was not affected by thermal processing via prilling. The X-ray diffraction pattern for the formulation also revealed diffraction peaks of MPT, demonstrating that the crystalline state of MPT was at least partially maintained in the prills. These results were in agreement with the Raman spectra shown in Fig. 4. However, the representative Raman peaks for MPT in the region 800 – 875 cm^{-1}, representing the out-of-plane O-H vibration of the carbonyl group [33], had broadened in the formulation (Fig. 4A) in comparison with the physical mixture. In the 920 – 980 cm^{-1} region, depicting the O-H deformation [33], MPT bands in the formulation had almost disappeared, indicating a decrease in crystallinity and the presence of amorphous MPT. Moreover, the MPT band at 1210 cm^{-1}, corresponding to the C-N stretching vibration [33], was shifted to a lower wavenumber (Fig. 4B), revealing interactions between the drug and the fatty acid. During processing at higher temperature, MPT dissolved in the molten behenic acid phase and the interactions between drug and carrier were partially retained after cooling as indicated by changes in the Raman spectrum. Based on the crystalline nature of the fatty acid matrix, it is unlikely that the MPT fraction interacting with the carrier is molecularly dispersed throughout the lipid carrier, hence it is suggested that an amorphous MPT fraction is distributed as separate phases throughout the entire matrix. The combination of MPT with stearic acid via prilling resulted in similar changing Raman bands of MPT (data not shown). ATR FT-IR spectroscopy (Fig. 5) confirmed that the prilling process generated hydrogen

bonds and Van der Waals interactions between MPT and the fatty acids and that the complete MPT molecule was involved in these interactions. Fig. 5 illustrates that the ratios of the vibration peaks of MPT and fatty acids in the prills were significantly different from the physical mixture with the characteristic vibration peaks of MPT (1584 cm^{-1} and 1512 cm^{-1}, 1249 cm^{-1}, 1109 cm^{-1} and 821 cm^{-1} for the $\nu_{C=C}$ stretch vibrations of the aromatic ring, δ_β in-plane bending of the hydroxyl groups, ν_{C-OH} stretching of the hydroxyl groups and δ_γ out-of-plane bendings of the aromatic C-H bonds, respectively) appearing less intense in the prills. Since no additional carboxyl vibrations appeared in the prills, electrostatic interactions between MPT and the fatty acids could be excluded. These observations were confirmed by solid state ^{13}C – NMR (data shown in supporting information).

3.3 Physical stability

Stability issues when using lipid excipients have been reported by different authors [7, 10, 18]. Influences of storage parameters on drug release were observed by Hamdani *et al.* (2002). The prolonged release of phenylephrine hydrochloride from fatty binder mixtures (Precirol® and Compritol®) was significantly affected by storage after 6 weeks at 40 °C and 75 % relative humidity (RH) [7]. In a study performed by San Vicente *et al.* (2000), salbutamol hydrochloride release was altered after storage for 1 year at room temperature, depending on the type of Gelucire used [18].

To investigate the physical stability, the prills containing 30 % MPT and 70 % behenic acid were stored, immediately after manufacturing, in hermetically sealed bags at 25 °C and 40 °C during 6 months. The influence of storage on drug release is shown in Fig. 6. Drug release profiles remained similar during storage at 25 °C as indicated by the f_2 values, ranging between 73 and 85, whereas the release rate increased during storage at 40 °C. After one week of storage at 40 °C, 73 % MPT was released after 4 h, compared to 62 % immediately after manufacturing. The drug release after 4h was increased to 80 % after one month of storage. Drug release profiles obtained after one month, 3 months and 6 months of

storage were not significantly different ($f_2 > 50$), but differed significantly from the freshly prepared formulation ($f_2 < 50$).

X-ray patterns of the 30 % MPT formulation stored during 6 months at 25 °C and 40 °C are compared with the X-ray pattern of the formulation immediately after processing in Fig. 3. MPT diffraction peaks show similarity between the 3 formulations. The sharp diffraction peaks of behenic acid at 21.7° and 24.3° are unchanged during storage at both conditions, indicating the stability of the crystalline structure. These results were confirmed by MDSC measurements (Table 2). Only small fluctuations in onset melting temperature and peak melting temperature (T_m) were detected during storage at 25 °C and 40 °C. Moreover, the melting enthalpy of behenic acid showed a similar trend at both storage conditions. Differences in MPT solid state during storage were observed with Raman spectroscopy (Fig. 7). At both storage conditions, Raman absorption bands for MPT (800 – 875 cm^{-1}) had sharpened after 1 week compared to the formulation immediately after prilling and MPT bands that were absent in the 920 – 980 cm^{-1} region due to the prilling process, became visible again. The MPT band at 1206 cm^{-1} was shifted back to 1210 cm^{-1} after 1 week. This indicated a transition of MPT from its amorphous state to its crystalline state during the first week of storage. The Raman spectra collected after 1 month, 3 months and 6 months storage were not significantly different. The influences of storage on Raman bands of behenic acid are shown in Fig. 7G and 7H, respectively. No changes or shifts were detected at 25 °C, neither at 40 °C, indicating no polymorphism of behenic acid during both production and storage of the prills.

To evaluate the distribution of the solid state during storage, Raman microscopic mapping was performed and compared with the formulation immediately after processing. The peak width of the Raman band of MPT in the 810 - 830 cm^{-1} region was monitored to map the solid state of MPT in the matrix since there was no interference of behenic acid in this region. Fig. 8 shows the distribution of MPT in the inner and outer layers immediately after prilling and after 2 days storage, a red color corresponds to a broad peak width, while a

blue color corresponds to a smaller peak width. The MPT band in the 810 – 830 cm⁻¹ region showed a broad peak width in the inner (Fig. 8A) and outer layers (Fig. 8B) confirming the initial presence of amorphous MPT which can be attributed to the fast cooling rate applied to the droplets. Raman microscopic mapping after 2 days of storage at 25 °C and 40 °C (Fig. 8C - 8F) indicated that the amorphous MPT fraction was recrystallized in the entire matrix. Although difficult to conclude for the inner layers in the stored prills (Fig. 8D and 8F), the mean Raman spectra collected in this region showed increased MPT crystallinity. In comparison with the freshly prepared formulation, the Raman peaks in the 810 - 875 cm⁻¹ had sharpened and the peak at 1206 cm⁻¹ was shifted to 1210 cm⁻¹. Raman microscopic mapping performed after 6 months storage did not show significant differences with the images obtained after 2 days. MPT crystals remained homogenously distributed in the fatty acid matrix.

Although the faster dissolution rate of MPT during the first month of storage at 40 °C could not be clarified by means of solid state characterization, a higher mobilization of the fatty acid chains at higher storage temperature might be a contributing factor, thereby inhibiting crystallization of MPT or yielding smaller drug crystals, thus affecting the release rate.

3.4 In vivo evaluation

To study the MPT bioavailability, the prills containing 30 % MPT (Formulation 1, F1) and 40 % MPT (Formulation 2, F2) in a behenic acid matrix, were selected for in vivo evaluation in dogs. The bioavailability was compared with a commercially available controlled release reference formulation (Formulation 3, F3): Slow-Lopresor® 200 mg Divitabs®. The in vitro drug release from the reference, with a complete drug release after 12 h, was faster compared to F1 and slower compared to F2 (data not shown). The mean plasma concentration-time profiles (n = 6) after oral administration of F1, F2 and F3 are illustrated in Fig. 9, while the mean pharmacokinetic parameters (AUC, C_{max}, t_{max} and $HVD_{t50\%Cmax}$) are reported in Table 3. The fast in vitro drug release from F2 was reflected in the in vivo study

since this formulation showed a burst release with a mean C_{max} of 6.75 (µg/ml)*kg obtained after 1.67 h compared with a C_{max} of 4.20 and 3.44 (µg/ml)*kg obtained after 2.33 h and 3.83 h after administration of F1 and F3, respectively. Moreover, F2 was characterized by a lower $HVD_{t50\%Cmax}$, illustrating less controlled release characteristics. Prills containing 30 % MPT (F1) showed a similar plasma concentration-time profile compared with the reference (F3). Statistical analysis of the pharmacokinetic parameters (AUC, C_{max}, t_{max} and $HVD_{t50\%Cmax}$) revealed no significant differences between the 3 formulations (p > 0.05). Remnants of prills, which still contained 4.39 % MPT, were found in the faeces of the dogs after administration of F1. Although the prills containing 40 % MPT (F2) were intact after in vitro dissolution, no prills were recovered from the faeces after oral administration of these multiparticulates to dogs, indicating that the composition of gastro-intestinal fluid and motility play an important role in the disintegration of these solid lipid dosage forms.

4. CONCLUSION

In this study we have demonstrated that prilling is a promising technique for the production of multiparticulate dosage forms. Using the fatty acids stearic acid and behenic acid, sustained release of the hydrophilic drug MPT could be achieved. In vitro drug release was dependent on the drug load, type of fatty acid, pH and ionic strength of the dissolution medium. Solid state characterization indicated that the crystalline state of the fatty acids was not affected by thermal processing via prilling, while the crystallinity of MPT was decreased. During storage, the amorphous MPT fraction recrystallized in the entire matrix but no polymorphism of behenic acid was detected. The in vivo bioavailability of MPT, after oral administration of the test formulations to dogs, did not significantly differ from a commercial sustained release reference formulation.

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Figures

Fig. 1. Drug release in function of (A) fatty acid: 30 % MPT in combination with stearic acid (●) and behenic acid (▲); (B) drug load: 10 % (●), 20 % (■), 30 % (▲) and 40 % MPT (▼); (C) pH of the dissolution medium: 10 % MPT in pH 1 (●) and pH 7.4 (■); (D) ionic strength of the dissolution medium: μ = 0.009 (●), 0.018 (■), 0.044 (▲), 0.089 (▼), 0.1445 (♦) and 0.20 (○).

Fig. 2. MDSC thermograms of MPT (A), stearic acid (B), behenic acid (C), physical mixture of 30/70 MPT/stearic acid (D), physical mixture of 30/70 MPT/behenic acid (E), prills containing 30 % MPT and 70 % stearic acid (F) and prills containing 30 % MPT and 70 % behenic acid (G).

Fig. 3. X-ray diffraction patterns of MPT (A), behenic acid (B), physical mixture of 30/70 MPT/behenic acid (C), prills containing 30 % MPT and 70 % behenic acid immediately after manufacturing (D), after 6 months storage at 25 °C (E), after 6 months storage at 40 °C (F).

Fig. 4. Raman spectra of MPT (green), behenic acid (blue), 30/70 MPT/behenic acid physical mixture (black) and prills containing 30 % MPT and 70 % behenic acid (red).

Fig. 5. ATR FT-IR spectra of MPT (A), prills containing 30 % MPT and 70 % behenic acid (B), 30/70 MPT/behenic acid physical mixture (C) and behenic acid (D).

Fig. 6. Influence of storage at 25 °C (A) and 40 °C (B) on drug release. Mean dissolution profiles (± S.D.) of prills containing 30 % MPT and 70 % behenic acid immediately after manufacturing (●), after 1 week (■), 1 month (▲) and 6 months (♦) of storage.

Fig. 7. Raman bands for MPT (A – F) and behenic acid (G – H) in prills containing 30 % MPT and 70 % behenic acid immediately after manufacturing (black) and after 1 week (red), 1 month (green), 3 months (blue) and 6 months (orange) storage at 25 °C (A, C, E and G) and at 40 °C (B, D, F and H).

Fig. 8. Solid state mapping of MPT in prills containing 30 % MPT and 70 % behenic acid immediately after prilling (A-B), after 2 days storage at 25 °C (C-D) and 40 °C (E-F). Figures A, C and E represent the outer layers, whereas the inner layers are represented in figures B, D and F. A red color corresponds to a broad peak width, blue corresponds to a smaller peak width.

Fig. 9. Mean plasma-concentration time profiles (± S.D.) after oral administration of 200 mg metoprolol tartrate to dogs (n = 6) as prills containing 30 % MPT and 70 % behenic acid (●), as prills containing 40 % MPT and 60 % behenic acid (■), and as Slow-Lopresor® 200 Divitabs®(▲).

Tables

Table 1. Mean particle size (± S.D.) and aspect ratio (± S.D.) for prills containing different amounts of MPT in combination with stearic acid or behenic acid.

Table 2. Thermal behavior of the prills during 6 months storage at 25 °C and 40 °C. The melt onset, peak melting temperature (T_m) and the melting enthalpy of the prills immediately after manufacturing were 69.8 ± 0.5 °C; 73.7 ± 0.1 °C and 144.8 ± 4.2 J/g, respectively.

Table 3. Mean pharmacokinetic parameters (± S.D.) after oral administration of 200 mg metoprolol tartrate to dogs (n = 6) as prills containing 30 % MPT and 70 % behenic acid (F1), as prills containing 40 % MPT and 60 % behenic acid (F2) and as Slow-Lopresor® 200 mg Divitabs® (F3).

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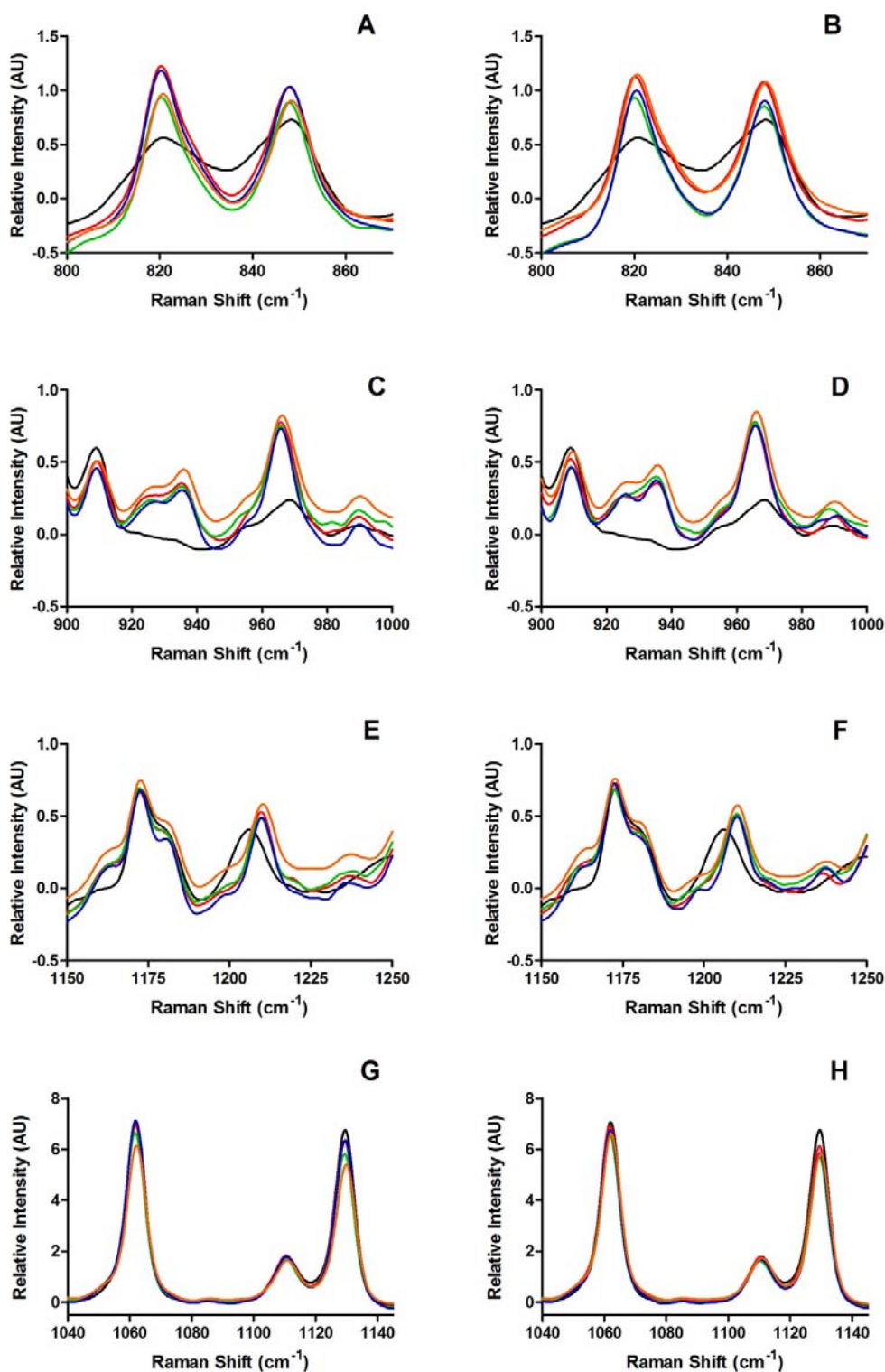
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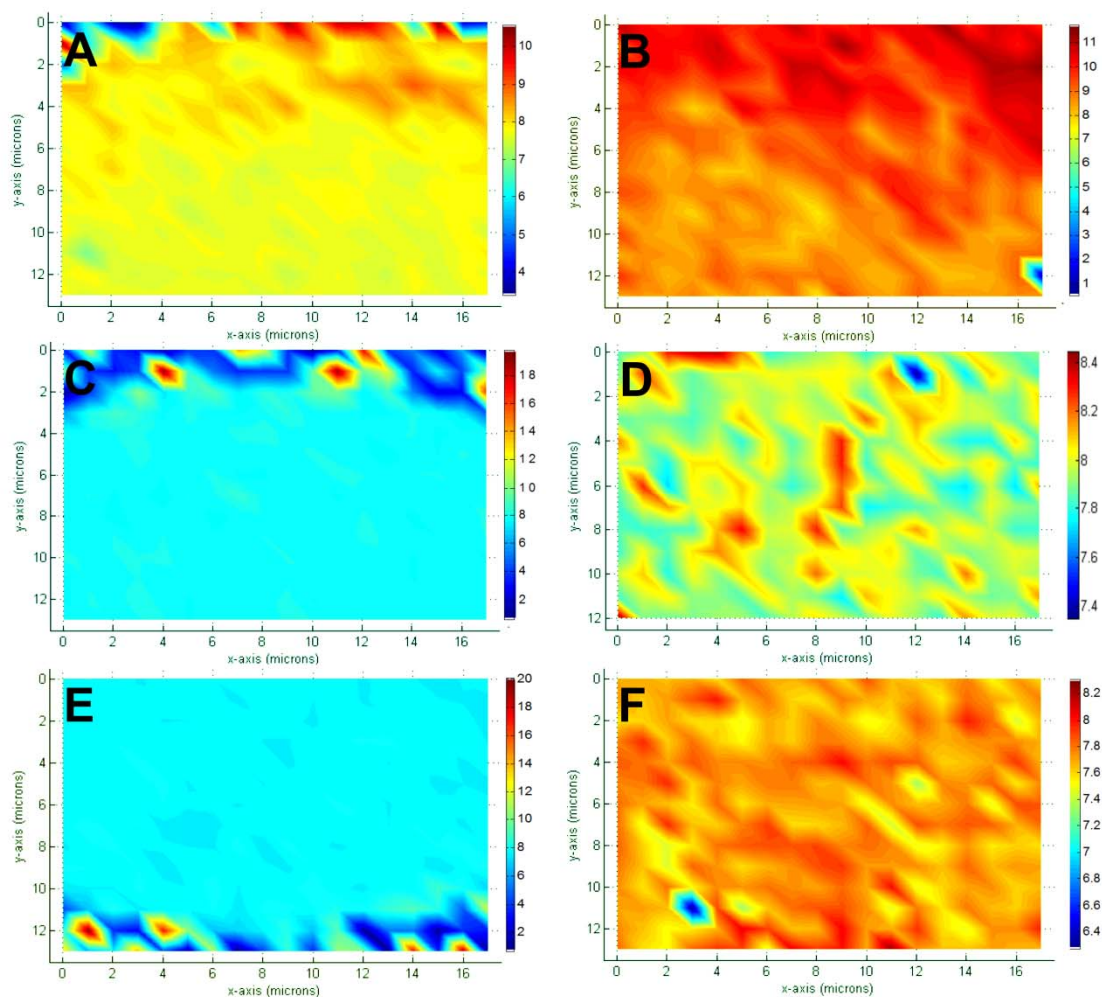
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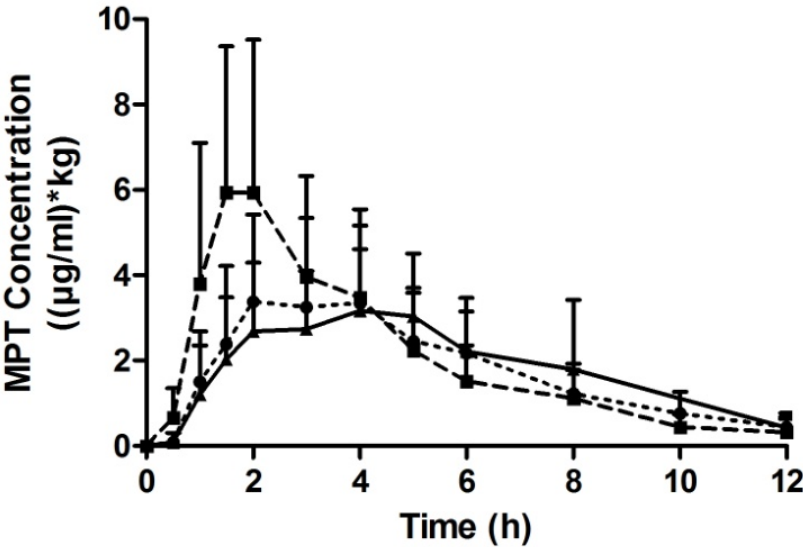
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% MPT	% Behenic Acid	% Stearic Acid	Mean FD (mm)	AR
10	90	-	2.35 ± 0.07	1.07 ± 0.03
20	80	-	2.36 ± 0.05	1.07 ± 0.04
30	70	-	2.37 ± 0.06	1.09 ± 0.05
40	60	-	1.83 ± 0.03	1.05 ± 0.02
30	-	70	2.35 ± 0.05	1.08 ± 0.04

Table 2. Thermal behavior of the prills during 6 months storage at 25 °C and 40 °C. The melt onset, peak melting temperature (T_m) and the melting enthalpy of the prills immediately after manufacturing were 69.8 ± 0.5 °C; 73.7 ± 0.1 °C and 144.8 ± 4.2 J/g, respectively.

	Melt Onset Temp. (°C)		T_m (°C)		Melting enthalpy (J/g)	
	25 °C	40 °C	25 °C	40 °C	25 °C	40 °C
1 Week	70.4 ± 0.3	70.5 ± 0.1	73.6 ± 0.2	73.8 ± 0.1	154.8 ± 5.8	161.7 ± 2.2
1 Month	70.4 ± 0.2	70.8 ± 0.2	73.7 ± 0.2	73.9 ± 0.1	145.7 ± 3.9	153.0 ± 3.4
3 Months	70.7 ± 0.2	70.9 ± 0.2	74.1 ± 0.1	74.1 ± 0.1	157.2 ± 1.1	157.4 ± 2.3
6 Months	70.5 ± 0.1	70.8 ± 0.1	73.7 ± 0.1	74.0 ± 0.1	166.3 ± 1.9	164.9 ± 3.4

Table 3. Mean pharmacokinetic parameters (± S.D.) after oral administration of 200 mg metoprolol tartrate to dogs (n = 6) as prills containing 30 % MPT and 70 % behenic acid (F1), as prills containing 40 % MPT and 60 % behenic acid (F2) and as Slow-Lopresor® 200 mg Divitabs® (F3).

	C_{max} ((µg/ml)*kg	t_{max} (h)	AUC ((µg/ml)*kg*h)	$HVD_{t50\%Cmax}$ (h)
F1	4,20 ± 1,82	2,33 ± 1,03	21,25 ± 10,96	4,29 ± 1,23
F2	6,75 ± 3,79	1,67 ± 0,52	25,01 ± 11,41	3,37 ± 0,94
F3	3,44 ± 1,52	3,83 ± 2,23	22,18 ± 12,16	5,98 ± 2,88