HOT-MELT CO-EXTRUSION FOR THE PRODUCTION OF FIXED-DOSE COMBINATION PRODUCTS WITH A CONTROLLED RELEASE ETHYLCELLULOSE MATRIX CORE

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Abstract

In this study, hot-melt co-extrusion was evaluated as a technique for the production of fixed-dose combination products, using ethylcellulose as a core matrix former to control the release of metoprolol tartrate and a polyethylene oxide-based coat formulation to obtain immediate release of hydrochlorothiazide. By lowering the concentration of the hydrophilic additive polyethylene oxide in the plasticized ethylcellulose matrix or by lowering the drug load, the in vitro metoprolol tartrate release from the core was substantially sustained. The in vitro release of hydrochlorothiazide from the polyethylene oxide/polyethylene glycol coat was completed within 45 min for all formulations. Tensile testing of the core/coat mini-matrices revealed an adequate adhesion between the two layers. Raman mapping showed no migration of active substances. Solid state characterization indicated that the crystalline state of metoprolol tartrate was not affected by thermal processing via hot-melt extrusion, while hydrochlorothiazide was amorphous in the coat. These solid state characteristics were confirmed during the stability study. Considering the bioavailability of metoprolol tartrate after oral administration to dogs, the different co-extruded formulations offered a range of sustained release characteristics. Moreover, high metoprolol tartrate plasma concentrations were reached in dogs allowing the administered dose to be halved.

Keywords: co-extrusion, hot-melt extrusion, fixed-dose combination product, sustained release, immediate release, ethylcellulose, matrix

Chemical compounds studied in this article

Hydrochlorothiazide (PubChem CID: 3639); Metoprolol tartrate (PubChem CID: 441308).

1. INTRODUCTION

The need for novel combination therapies, primarily focusing on fixed-dose combinations (FDC), has been reported by various authors and is seen as a driver for innovative drug development (Woodcock et al., 2011 and Zhang et al., 2011). Besides their benefits in life cycle management, FDC products have shown to improve patient adherence by decreasing the number of required pills and thus reducing the complexity of the medication regimen (Pan et al., 2008). Fixed-dose combinations offer benefits to a lot of drugs due to the additive nature of therapeutic effect and the reduced level of side-effects associated with the use of complementary drugs (Hiremath et al., 2011). 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. Sustained release formulations have been shown to offer many other advantages over conventional drug products, such as the controlled administration of a therapeutic dose at a desired delivery rate in order to gain more constant plasma concentrations of the drug. Moreover, the production of sustained release multiparticulate dosage forms is advantageous since in vivo the subunits spread into the gastro-intestinal tract as soon as the dosage unit, e.g. capsule or tablet, disintegrates. Since high local drug concentrations are avoided, less inter- and intra-subject variability and a decreased risk of dose dumping can be expected (De Brabander et al., 2003).

While hot-melt extrusion (HME) has proven to be a successful processing technique used in pharmaceutical industry to produce drug products in a continuous way, co-extrusion is quite new in pharmaceutical applications (Dierickx et al., 2012 and Quintavalle et al., 2008). Nevertheless co-extrusion of polymers is widely applied in the plastics and packaging industry. The pharmaceutical co-extrusion process consists of the simultaneous hot-melt extrusion of two or more drug loaded formulations creating a multi-layered extrudate. HME as a continuous manufacturing technology has shown some other major advantages over conventional techniques, like improving the bioavailability of poorly water soluble drugs via molecular dispersions (Breitenbach and Magerlein, 2003), without the requirement for

processes based on organic solvent or agueous spray drying. Moreover via HME matrix formulations can be manufactured using polymers that act as drug depots (Crowley et al., 2007). The added value of co-extrusion is that it allows to modulate the release of each drug independently, to enable simultaneous administration of non-compatible drugs and to produce fixed-dose combinations in a continuous single-step process. By processing the co-extrudate into mini-matrices that can be easily filled into gelatin capsules a multi-particulate formulation is created. A specific challenge during co-extrusion is to establish a core/coat polymer combination fit for purpose considering required release characteristics of the incorporated drugs, similarity in extrusion temperature and appropriate adhesion between the layers. So far, no co-extruded dosage forms for oral use are on the market. In this study a contribution is made to enable the use of co-extrusion in pharmaceutical industry for the production of oral FDC drug products that offer multiple release profiles.

The aim of this study was to evaluate the use of co-extrusion for the manufacturing of a fixed-dose combination drug product for oral application, using a core matrix former that offers a range of controlled release profiles for highly water soluble drugs. For this purpose ethylcellulose, a thermoplastic polymer that has been intensively used as a matrix former in hot-melt extrusion (Follonier et al., 1994, Verhoeven et al., 2009), was combined with polyethylene oxide as a hydrophilic additive and metoprolol tartrate as model drug. The combination of this beta-blocker with the diuretic hydrochlorothiazide is well known (Lewanczuk and Tobe, 2007). It offers the opportunity for a co-extrudate with hydrochlorothiazide incorporated in the coat as immediate release model drug and metoprolol tartrate incorporated in the core as model for a highly water soluble drug. The in vitro performance of the different formulations was assessed. The solid state of the model drugs in the formulations was characterized using modulated differential scanning calorimetry (MDSC), X-ray diffraction (XRD) and Raman spectroscopy. Furthermore, the physical stability of the co-extruded mini-matrices was monitored during 6 months storage at 25°C/60%RH and 40°C/75%RH. Finally, the bioavailability of the different formulations was

evaluated after oral administration to dogs and compared to a commercially available fixed-dose combination product.

2. MATERIALS AND METHODS

2.1 Materials

Metoprolol tartrate (Esteve Quimica, Barcelona, Spain) and hydrochlorothiazide (Utag, Amsterdam, the Netherlands) were selected as sustained release and immediate release model drugs, respectively. Ethylcellulose (Ethocel® std 10, Colorcon, Dartford Kent, United Kingdom), dibutyl sebacate (Sigma-Aldrich, Bornem, Belgium), polyethylene oxide 1M (MW: 1000000 g/mol, Sentry™ Polyox® WSR N12K, Colorcon, Dartford Kent, United Kingdom), polyethylene oxide 100K (MW: 100000 g/mol, Sentry™ Polyox® WSR N10, Colorcon, Dartford Kent, United Kingdom), polyethylene glycol 4K (MW: 4000 g/mol, Fagron, Waregem, Belgium), polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus®, BASF, Ludwigshafen, Germany), poloxamer 188 (Lutrol F68®, BASF, Ludwigshafen, Germany) and an 8:2 blend of polyvinyl acetate and polyvinylpyrrolidone (Kollidon SR®, BASF, Ludwigshafen, Germany) were used as excipients. All other chemicals were of analytical grade.

2.2 Methods

2.2.1 Co-extrusion

Co-extrusion was carried out with two co-rotating, fully intermeshing, Prism Eurolab 16mm twin screw extruders (ThermoFisher Scientific, Karlsruhe, Germany), both connected to a co-extrusion die (Guill, West Warwick, USA). The co-extrusion die combined both layers into a rod-like co-extrudate consisting of a core and a concentric coat. The five heating segments of both extruders were heated to 80/90/100/100/100 °C from feed opening to die-end. The co-extrusion die was heated to 100 °C. Both formulated premixes were fed separately into the corresponding extruder by a Brabender Flexwall® loss-in-weight powder feeder (Duisburg, Germany) at a feed rate of 200 g/h for the coat and 300 g/h for the core material. A screw speed of 40 rpm for the extruder producing the outer layer and 150 rpm for the extruder producing the inner layer was used. The core of the co-extrudate, with a diameter of 3 mm, was surrounded by a coat with a thickness of 0.5 mm, which led to a total

co-extrudate diameter of 4 mm. Four different co-extrudates were manufactured consisting of a specific core and coat formulation, by combining the following components in different concentrations: ethylcellulose (EC), dibutyl sebacate (DBS), polyethylene oxide (PEO), polyethylene glycol (PEG), metoprolol tartrate (MPT) and hydrochlorothiazide (HCT) (Table 1). After cooling down the co-extruded rod to room temperature, the cylindrical co-extrudate was manually cut into mini-matrices of 2 mm length. Those mini-matrices had an average weight of 27.2 mg (SD = 1.8 mg, n = 20).

2.2.2 In vitro drug release

In vitro dissolution was performed using USP dissolution apparatus 1 (baskets). The equipment consisted of an Evolution 6300 dissolution system (Distek, New Brunswick, New Jersey, USA) coupled with an Evolution 4300 automatic dissolution sampler (Distek, New Brunswick, New Jersey, USA). The temperature of the dissolution medium (900ml) was maintained at 37 ± 0.5 °C while the rotational speed of the baskets was set at 100 rpm. For the first hour a 0.1 N solution of hydrochloric acid (pH 1) was used as dissolution medium in order to mimic the pH of the stomach. Afterwards the baskets containing the mini-matrices were transferred to vessels filled with phosphate buffer pH 6.8 (USP) as a dissolution medium, to which they were exposed for the next 23 hours. Samples (filtered using Distek 45 µm filters) of 5 ml were withdrawn at 5, 10, 15, 20, 30, 45 and 60 minutes for the determination of hydrochlorothiazide in the first dissolution medium and at 1, 2, 4, 6, 8, 12, 16, 20 and 24 h for the determination of metoprolol tartrate in the second dissolution medium. The inner layer extrudate was analyzed separately to cover for the metoprolol tartrate release during the first hour. Samples were analyzed spectrophotometrically at 316.6 nm and 222 nm respectively, by a UV-spectrophotometer, type UV-1800 (Shimadzu, Deurne, Belgium), using an appropriate calibration curve for quantification of hydrochlorothiazide and metoprolol tartrate, respectively. Each experiment was performed in triplicate.

2.2.3 Modulated differential scanning calorimetry

The crystallinity of the drug in the matrices and the thermal behavior of pure compounds, physical mixtures and corresponding extrudates were studied using a differential scanning calorimeter Q2000 V24.8 equipped with a refrigerated cooling system (TA Instruments, Leatherhead, UK). Nitrogen was used as a purge gas through the DSC cell (50 ml/min) and the RCS unit (300 ml/min). Samples (± 5 mg) were run in hermetically closed Tzero pans with perforated lid, supplied by TA Instruments, 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). Mass of sample pan and empty reference pan were taken into account. Temperature and enthalpy calibration was performed with an indium standard, whereas calibration of the heat capacity was performed using a sapphire standard. MDSC data were analyzed using the TA instruments Universal Analysis 2000 V4.7A software. Melting enthalpies were determined in the total heat flow signal. Melting temperatures were reported as peak temperatures. The glass transition temperature corresponds to the temperature at the midpoint of the heat capacity change (or Cp jump).

2.2.4 X-ray diffraction

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

2.2.5 Adhesion

The adhesion between core and coat was analyzed using a tensile tester with a load cell capacity of 100 N (LF Plus, Lloyd Instruments, West Sussex, UK). The co-extruded mini-matrices were placed on a metal disk with a central opening of 3.3 mm, above which the core of the co-extruded mini-matrices was positioned to make sure only the coat was supported by the device. A probe with a diameter of 2 mm was used to apply a downward force on the

core (preload 1 N; extension rate 100 mm/min) and the maximum force needed to separate coat from core was measured. The test was repeated 10 times for each tested formulation.

2.2.6 Raman spectroscopy

The distribution of the different components in the coat and core of the co-extrudates was evaluated with Raman microscopic mapping using a Raman Rxn1 Microprobe (Kaiser Optical Systems Inc, Ann Arbor, MI, USA), equipped with an air-cooled CCD detector. The laser wavelength employed was 785 nm from a Invictus NIR diode laser (Kaiser Optical Systems Inc, Ann Arbor, MI, USA). All spectra were recorded with a resolution of 4 cm^{-1} and an exposure time of 2 s, using a laser power of 400 mW. Cross sections of co-extrudates were scanned by a 10 x long working distance objective lens in point-by-point mapping mode using a step size of 50 µm in both the x and y directions (18 x 13 = 234 spectra per mapping). The resulting images provide information about the distribution of different components in the co-extrudates. Data collection and data transfer were automated using the HoloGRAMS™ data collection software (version 2.3.5, Kaiser Optical Systems Inc, Ann Arbor, MI, USA), the HoloMAP™ data analysis software (version 2.3.5, Kaiser Optical Systems Inc, Ann Arbor, MI, USA) and Matlab® software (version 7.1, The MathWorks Inc., Natick, MA, USA). In order to correct for the uneven surface of the co-extrudates, manually cut in half using a surgical blade, all spectra were preprocessed using Pearsons method to perform a baseline correction.

In order to attribute specific Raman peaks in the spectra to the different components in the formulations, Raman spectra were collected from the pure components and the physical mixtures. All spectra were recorded with a resolution of 4 cm^{-1} and an exposure time of 5 s. Standard normal variate (SNV) pre-processing was applied on the collected spectra prior to analysis, to correct for the variation in path length/sampling distance between probe and sample.

2.2.7 Stability study

A sufficient number of mini-matrices from the 3 different formulations was manufactured to perform a stability study. Immediately after co-extrusion, the formulations were filled in an amber glass container and stored in closed condition at 25°C/60%RH and in open condition at 40°C/75%RH. To investigate the influence of storage MDSC, XRD, and in vitro drug release were performed on the co-extrudates immediately after manufacturing, after 1 month, 3 months and 6 months storage, respectively.

2.2.8 In vivo evaluation

2.2.8.1 Study design

All procedures were performed after approval by the Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO) (Merelbeke, Belgium). To study the drug plasma profiles for hydrochlorothiazide and metoprolol tartrate, the formulations listed in Table 1 were administered to 6 male mixed-breed dogs (23 - 41.5 kg). For the test formulations A, B, C and D the co-extruded mini-matrices were filled in hard-gelatin capsules, whereas the reference formulation was given as 2 tablets Zok-Zid® (Pfizer, Brussels, Belgium). During the cross-over study the formulations were administered in randomized order, taking into account a wash-out period of at least 8 days. The dogs were fasted for 12 h prior to and after the administration of the formulations, but water was available ad libitum. At the start of the study an intravenous cannula was placed in the lateral saphenous and a blank blood sample was collected. The formulations were orally administered with 20 ml of water and blood samples were subsequently collected in dry heparinized tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h after administration. The blood samples obtained were centrifuged for 5 min at 1500 *g* within 1 h after collection. The plasma was separated and frozen at -20 °C before analysis.

2.2.8.2 Metoprolol tartrate and hydrochlorothiazide assay

Two different validated HPLC methods were used to determine metoprolol tartrate and hydrochlorothiazide plasma concentrations in the dog plasma. After extraction of metoprolol tartrate and the internal standard bisoprolol hemifumarate from the plasma using a solid

phase extraction (SPE) procedure, with Oasis® MCX 1 cc (30 mg) cartridges (Waters, Brussels, Belgium), a HPLC method with fluorescence detection was used for the determination of metoprolol tartrate (Fang et al., 2004). Hydrochlorothiazide was extracted from the plasma samples by means of a liquid-liquid extraction with the internal standard hydroflumethiazide and afterwards determined using a HPLC-UV method (Vervaet and Remon, 1997). The HPLC system consisted of an isocratic solvent pump (L-7100, Merck, Hitachi LaChrom, Tokyo, Japan), an automatic autosampler (L-2200, Merck, Hitachi Elite LaChrom, Tokyo, Japan). For the MPT analysis 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) were used. The pump flow was set at 1.1 ml/min and the excitation and emission wavelength were 275 nm and 300 nm, respectively. For the HCT analysis a guard column (LiChroCart® 4-4, LiChrospher® 100 RP-18e (5 µm), Merck, Darmstadt, Germany) followed by a reversed phase column (LiChroCart® 250-4, LiChrospher® 100 RP-18 (5 µm), Merck, Darmstadt, Germany) and a UV-detector (L-7400, Merck, Hitachi LaChrom, Tokyo, Japan) were used. The pump flow was set at 0.8 ml/min and the detection wavelength was 272 nm. Since metoprolol tartrate did not cause an interfering peak during the determination of hydrochlorothiazide, and vice versa, the specificity of the methods was secured. Peak integration was performed using the software package D-7000 HSM Chromatography Data Station (Hitachi Instruments, San Jose, CA, USA).

2.2.8.3 Data analysis

The metoprolol tartrate and hydrochlorothiazide plasma concentrations were normalized for dose and body weight of the dogs, by dividing the respective plasma concentrations by dose per kg body weight for each dog. The peak plasma concentration (C_{max}), the extent of absorption ($AUC_{0\text{-}12h}$) and the time to reach the peak plasma concentration (t_{max}) were calculated. The controlled release characteristics of the core formulation were evaluated by

means of the $HVD_{t50\%Cmax}$, defined as the width of the plasma concentration profile at 50% of the C_{max} (Meier et al., 1974). The bioavailability data were statistically evaluated using SPSS 17 (SPSS, Chicago, USA). To compare the effects of the different treatments on the pharmacokinetic 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 Formulation and production of co-extrudates

In order to produce a combination product with an immediate release coat and a controlled release core via co-extrusion some combinations of polymer-plasticizer mixtures were hot-melt extruded and evaluated for their processability. A successful co-extrusion process requires that both polymer melts can be processed at similar temperatures, match in melt viscosity and show adequate adhesion. Therefore combinations of polymer-plasticizer mixtures for core and coat were tested. As a first combination Kollidon SR® was assessed for the controlled release core and Soluplus® for the immediate release coat. At a metoprolol tartrate load of 30%, Kollidon SR® only slightly sustained the release (80 and 100% released at 2 and 8h, respectively). The addition of the hydrophobic plasticizer dibutyl sebacate (15%) only minimally improved the sustained release profile (with 80 and 100% released at 4 and 8h, respectively). Soluplus® was initially assessed in combination with a wide range of plasticizers. The lowest possible extrusion temperature (130°C) was reached for the formulation containing 10% Lutrol F68®. This coat formulation only offered an immediate release profile for hydrochlorothiazide concentrations of 5% or less. A second core/coat combination tested consisted of an ethylcellulose matrix for the controlled release core and a polyethylene oxide 100K/polyethylene glycol 4K immediate release coat. Dibutyl sebacate has previously been used to plasticize an ethylcellulose matrix and polyethylene glycol/polyethylene oxide was added in different concentrations to modify the metoprolol tartrate release (Quinten et al., 2011 and Verhoeven et al., 2009). As a core matrix ethylcellulose, plasticized with dibutyl sebacate, sustained metoprolol tartrate release in an efficient way, but by adding a hydrophilic additive to this matrix it became possible to offer a range of release profiles for metoprolol tartrate release. Polyethylene oxide 1M was selected as a hydrophilic additive and a design of experiments (DOE) methodology was employed to set up 11 experiments (8 experiments + 3 center points) in a mixture design in order to extrude metoprolol tartrate at a drug load of 30% in different combinations of ethylcellulose, dibutyl sebacate and polyethylene oxide 1M as a matrix. Two formulations with a similar

ethylcellulose/dibutyl sebacate ratio but a major difference in metoprolol tartrate release were finally selected. Both had a drug load of 30% metoprolol tartrate but a different matrix composition: (A) 53% ethylcellulose + 27% dibutyl sebacate + 20% polyethylene oxide 1M and (B) 62% ethylcellulose + 33% dibutyl sebacate + 5% polyethylene oxide 1M. In order to steer the metoprolol tartrate release not only by the amount of hydrophilic polymer (polyethylene oxide 1M) but also by drug load, this parameter was evaluated as a release-controlling factor as well. Therefore, a third formulation (C) was manufactured, containing 53% ethylcellulose + 27% dibutyl sebacate + 20% polyethylene oxide 1M with a drug load of 15% metoprolol tartrate in the core. For the corresponding coat formulation polyethylene oxide 100K, which has previously been used as a carrier in hot-melt extrusion, was chosen as main component (Crowley et al., 2002 and Li et al., 2006). To optimize this coat formulation different concentrations of polyethylene glycol 4K were added to polyethylene oxide 100K and the hydrochlorothiazide release was evaluated (Dierickx et al., 2012). A concentration of 15% polyethylene glycol 4K proved to be sufficient for immediate hydrochlorothiazide release and consistently yielded co-extrudates of good quality with a smooth surface and good adhesion between core and coat (Fig. 1). Therefore 85% polyethylene oxide 100K + 15% polyethylene glycol 4K was selected as matrix for the coat with the drug load adjusted in order to obtain a final formulation with the same metoprolol tartrate:hydrochlorothiazide ratio as the reference formulation (8:1). The metoprolol tartrate loaded plasticized ethylcellulose matrix, with the addition of polyethylene oxide 1M as a hydrophilic additive, was finally co-extruded with the hydrochlorothiazide loaded polyethylene oxide 100K/polyethylene glycol 4K coat at a temperature of 100°C. The obtained co-extrudates had a regular shape, a smooth surface, a white opaque inner layer and a transparent outer layer.

3.2 In vitro drug release

The influence of both the hydrophilic additive and the drug load on metoprolol tartrate release from the core is illustrated in Fig. 2. As previously reported the metoprolol tartrate

release from the ethylcellulose matrix containing 20% of polyethylene oxide 1M is diffusion controlled with a constant diffusion coefficient (Verhoeven, 2008). By reducing the amount of polyethylene oxide 1M in the matrix from 20 (formulation A) to 5% (formulation B) the metoprolol tartrate release was found to be delayed from 80 and 100% after 2 and 8h, respectively, in formulation A to 36, 60, 70 and 90% after 2, 8, 12 and 24h, respectively, in formulation B. At this low polyethylene oxide 1M concentration it has been described that the mobility of the high molecular weight polyethylene oxide is increased with increasing matrix porosity over time, leading to an metoprolol tartrate release approximating a zero-order release (Verhoeven, 2008). Reducing the polyethylene oxide concentration lowered the burst release to around 20% for formulation B. The same delaying effect on the in vitro metoprolol tartrate release profile was seen by lowering the drug load from 30% (formulation A) to 15% (formulation C). The reference formulation, where metoprolol tartrate is formulated in coated pellets which are compressed into tablets, has a slightly different release profile for metoprolol tartrate compared to the matrix formulations B and C, showing a slower release during the first 8 hours and a lower burst release (8% compared to 23 and 21% for the matrix formulations B and C, respectively). For hydrochlorothiazide the immediate release criteria are met, with complete release obtained after 30 min for all formulations (data not shown).

3.3 Physical state characterization

Fig. 3A shows the MDSC thermograms of the core components, ethylcellulose:dibutyl sebacate physical mixture in a 2:1 ratio, polyethylene oxide 1M, metoprolol tartrate, the physical mixtures of a core formulation and the corresponding extruded formulation. Metoprolol tartrate showed a peak melting endotherm at 123.9 °C, while polyethylene oxide 1M showed a peak melting temperature at 70.2 °C, indicating the crystalline state of these compounds. The reversing heat flow for the 2:1 ethylcellulose:dibutyl sebacate mixture showed a clear change in heat capacity in the temperature range from 34 to 48 °C (data not shown) with a glass transition (Tg) at 39.5 °C. The indication that the 2:1 ethylcellulose:dibutyl sebacate mixture is in an amorphous state was confirmed by XRD

355 analysis. Thermal analysis of the physical mixture and the extruded coat formulation revealed that metoprolol tartrate remained crystalline after hot-melt extrusion. Fig. 3B shows the MDSC thermograms for the components of the coat, hydrochlorothiazide, the 85:15 polyethylene oxide 100K:polyethylene glycol 4K matrix, the physical mixture and the corresponding extrudate. Hydrochlorothiazide showed a peak melting temperature at 267.2
360 °C, while the polyethylene oxide 100K:polyethylene glycol 4K matrix showed a peak endotherm at 66.2 °C, indicating the crystalline state of these compounds. Thermal analysis of the physical mixture and the extruded coat formulation revealed only a melting endotherm of the polyethylene oxide 100K:polyethylene glycol 4K 85:15 matrix, indicating that hydrochlorothiazide is amorphous in the coat matrix. For the physical mixture this result is
365 attributable to the heating of the sample during the MDSC experiment, where the small amount of hydrochlorothiazide in the physical mixture gradually dissolved in the molten polymer mixture during heating.

The solid state was also characterized using XRD and Raman spectroscopy. The X-ray diffraction patterns of the pure drug substances, the core and coat physical mixtures and the
370 corresponding formulations are shown in Fig. 4. The X-ray diffractogram of metoprolol tartrate showed distinct diffraction peaks at 2θ of 10.7°, 16.0°, 19.6°, 20.5° and 23.3°. Since these peaks were also detected in the diffractogram of the physical mixture and the extruded core formulation, it can be concluded that the crystalline state of metoprolol tartrate was maintained in the hot-melt extrudates. The diffraction pattern of pure hydrochlorothiazide
375 revealed several representative peaks, which also showed up in the diffractogram of the physical mixture of the coat. The absence of these peaks in the diffraction pattern of the extruded coat revealed that there were no hydrochlorothiazide crystals left in the coat of the co-extrudate, confirming that hydrochlorothiazide was amorphous in the polyethylene oxide 100K:polyethylene glycol 4K 85:15 matrix after hot-melt extrusion. These results were
380 confirmed with Raman spectroscopy. Raman peaks attributed to metoprolol tartrate, detected in the core of the co-extrudate, remained as sharp as in the physical mixture, indicating that

metoprolol tartrate stayed in its crystalline state and the extrusion process did not affect the solid state of the metoprolol tartrate (Fig 5). From the selected region of the Raman spectra in Fig. 6 it can be concluded that the Raman peaks attributed to hydrochlorothiazide in the coat of the co-extrudate showed broadening and a lower intensity when compared to the physical mixture, indicating the loss of crystallinity for hydrochlorothiazide.

3.4 Co-extrudate characterization

The microscopic image of the co-extrudate clearly showed the two distinct layers, properly attached to each other. An adhesion test was performed in order to measure the adhesion force between core and coat. For the formulation with ethylcellulose 62% + dibutyl sebacate 33% + polyethylene oxide 5% as a core matrix (formulation B), loaded with 30% metoprolol tartrate the average force needed to detach the core from the coat was 8.3 ± 2.6 N. The mini-matrices also passed a friability test (<0.1% weight loss), without any signs of detachment between core and coat layers.

To evaluate the distribution of the drug products in core and coat, Raman microscopic mapping was performed. The peak intensity of the Raman band of metoprolol tartrate in the 810 - 830 cm^{-1} region was monitored to map the distribution of metoprolol tartrate in the core and to check if there was diffusion of metoprolol tartrate in the coat. Fig. 7 shows the distribution of metoprolol tartrate, throughout different sections of the co-extruded core/coat formulation. A red color corresponds to a high peak intensity, indicating a high metoprolol tartrate concentration, while a blue color corresponds to a low metoprolol tartrate concentration. The metoprolol tartrate band in the 810 - 830 cm^{-1} region showed an intense peak in the core but not in the coat. The peak intensity of the Raman band of hydrochlorothiazide in the 700 - 720 cm^{-1} region was monitored to map the distribution of hydrochlorothiazide in the coat and to check if diffusion of hydrochlorothiazide in the core of the co-extrudate had occurred during hot-melt extrusion. The hydrochlorothiazide band in the 700 - 720 cm^{-1} region showed an intense peak in the coat but not in the core (data not

shown), indicating no intermigration of core and coat drug components during processing, given the spatial resolution of 50 µm.

3.5 Physical stability

Stability issues when using polyethylene oxide as a main matrix former in hot-melt extrusion have been reported previously. Polymer degradation of polyethylene oxide when stored below its melting point was occurring more rapidly for the lower molecular weight polymer in comparison to the higher molecular weight polyethylene oxide and is due to oxygen permeation in the amorphous region of the semi-crystalline polymer (Crowley et al., 2002). Therefore, influences of storage conditions on drug release were investigated for the co-extruded formulations.

The co-extrudates of the three different formulations (A, B and C) kept their original shape and integrity during the entire stability study. The influence of storage on metoprolol tartrate release for formulation B is shown in Fig. 8. The same observations were made for hydrochlorothiazide release during the stability study. For both drugs the overall release profile remains similar over time and in both storage conditions.

MDSC-profiles for the core extrudates after 1 and 3 months at different storage conditions indicated the stability of the crystalline state of the metoprolol tartrate fraction incorporated in the core. To evaluate the physical stability of the coat the X-ray patterns of the 5.6% hydrochlorothiazide formulation stored during 3 months at different storage conditions were compared with the X-ray pattern of the physical mixture and of the pure drug (Fig. 9). The diffraction patterns of the 3 months stability samples were similar with the formulation immediately after processing and indicated that hydrochlorothiazide stayed amorphous in the coat after 3 months at both conditions.

3.6 In vivo evaluation

To study the hydrochlorothiazide and metoprolol tartrate bioavailability, co-extrudates (formulation A, B, C and D) were administered to 6 dogs. The bioavailability was compared

435 with a commercially available reference formulation, Zok-Zid®, a tablet containing hydrochlorothiazide and coated metoprolol tartrate pellets. The mean normalized plasma concentration-time profiles (n = 6) for hydrochlorothiazide and metoprolol tartrate after oral administration of formulation A, B, C (25 mg hydrochlorothiazide and 200 mg metoprolol tartrate), formulation D (25 mg hydrochlorothiazide and 100 mg metoprolol tartrate) and the
440 reference (2 tablets) are illustrated in Fig. 10 and Fig. 11, respectively, while the mean pharmacokinetic parameters (AUC, $C_{max,}$ t_{max} and $HVD_{t50\%Cmax}$) are reported in Table 2 and Table 3, respectively. The drug plasma concentration profiles confirmed the in vitro dissolution results. The immediate in vitro hydrochlorothiazide release from the coat was confirmed in vivo with a Tmax value of about 2 hours for all co-extrudates. The rather fast in
445 vitro metoprolol tartrate release from formulation A was reflected in the in vivo study since this formulation showed a burst release with a mean C_{max} of 26.6 ng/ml obtained after 2.8 h, compared with a C_{max} of 12.8 ng/ml and 12.3 ng/ml obtained after 3.1 h and 3.5 h after administration of formulation B and C, respectively. The high metoprolol tartrate plasma concentrations reached after administration of the co-extruded matrix formulations in
450 comparison to the reference formulation, were indicating that the administered MPT dose could be reduced. Therefore, Formulation D was designed with the same core composition as formulation B in combination with a coat having a higher HCT load to be able to half the MPT dose while maintaining the HCT dose for administration to dogs. The pharmacokinetic parameters (AUC and C_{max}) of this formulation were not different at the 0.05 level of
455 significance from the reference formulation. Moreover, similar to formulation B and C, formulation D was characterized by a $HVD_{t50\%Cmax}$ of more than 7 h, illustrating better controlled release characteristics than the reference formulation with a $HVD_{t50\%Cmax}$ of 4.8 h. The difference in pharmacokinetic parameters for metoprolol tartrate between the co-extruded matrix formulations and the coated pellet reference formulation, as already noticed
460 in the in vitro results, can be observed clearly in vivo, where a lag phase of 2 hours is seen for the reference formulation. This might be attributed to the hydrophobic ethylcellulose coating of the pellets in comparison to the mini-matrices, causing metoprolol tartrate only to

be released further down the gastrointestinal (GI) tract and thus missing the absorption sites in the dogs small intestine. It has been documented that dogs have an unsacculated colon and therefore handle the transit of fluid and small particles in a fast way (Sutton, 2004). In contrast to the small pellets in the reference formulation it can be assumed that the larger mini-matrices are retained at the ileocecal junction, increasing the residence time in the ileum which offers a better absorption of the metoprolol tartrate, since it has been demonstrated previously that metoprolol is absorbed to the same degree from the duodenum and colon (Fara et al., 1985). It has been demonstrated that for metoprolol tartrate the co-extruded matrix formulations offer a higher bioavailability in dogs (AUC 3 to 7-fold higher and C_{max} 2 to 7-fold higher) than the reference pellet formulation. From the dog plasma concentrations it can be concluded that the metoprolol tartrate dose administered with formulation D could even be halved.

4. CONCLUSION

In this study we have demonstrated that fixed-dose combination mini-matrices with a matrix core offering a range of controlled release profiles and an immediate release coat were successfully developed by co-extrusion. The mini-matrices showed good adhesion and no migration of active drug substances between core and coat. Metoprolol tartrate release from the ethylcellulose matrix core could be sustained in function of the drug load and the content of the hydrophilic additive. High metoprolol tartrate plasma concentrations were obtained after oral administration to dogs, which indicated that MPT dose could be lowered to achieve the same bioavailability compared to a commercial reference formulation. However this advantage in dose reduction should be confirmed in humans. A stability study, monitoring the solid state of the drug compounds, indicated that the co-extrudates were stable for at least 6 months at different storage conditions. From this study it can be concluded that co-extrusion proved to be a valuable technique for the production of oral FDC drug products with multiple release profiles. Moreover the featured tests provided a thorough physical characterization of the co-extrudates manufactured.

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Figures

Fig. 1. Image of co-extrudate, before and after cutting into mini-matrices of 2 mm length. Core diameter 3 mm and coat thickness 0,5 mm.

Fig. 2. *In vitro* metoprolol tartrate release (in phosphate buffer pH 6.8) for formulation A ( ), formulation B ( ), formulation C ( ), Reference ( ) and mean hydrochlorothiazide release (in 0.1 N HCl) for Co-extrudate A to C ( ). Mean (n = 3) dissolution profiles (± SD) of co-extrudates and reference. Dissolution at 37 °C and 100 rpm.

Fig. 3A. MDSC thermograms of metoprolol tartrate (A), ethylcellulose:dibutyl sebacate 2:1 (B), polyethylene oxide 1M (C), physical mixture of 62% ethylcellulose + 33% dibutyl sebacate + 5% polyethylene oxide 1M with 30% metoprolol tartrate (D), extruded core formulation B: 62% ethylcellulose + 33% dibutyl sebacate + 5% polyethylene oxide 1M with 30% metoprolol tartrate (E).

Fig. 3B. MDSC thermograms of hydrochlorothiazide (A), physical mixture of 85% polyethylene oxide 100K + 15% polyethylene glycol 4K (B), physical mixture of 85% polyethylene oxide 100K + 15% polyethylene glycol 4K with 5.6% hydrochlorothiazide (C), extruded coat formulation B: 85% polyethylene oxide 100K + 15% polyethylene glycol 4K with 5.6% hydrochlorothiazide (D).

Fig. 4. X-ray diffraction patterns of metoprolol tartrate (A), physical mixture core (B), co-extrudate core (C), hydrochlorothiazide (D), physical mixture coat (E), co-extrudate coat (F) for formulation A.

Fig. 5. Selected regions of the Raman spectra of metoprolol tartrate, physical mixture of core and core of the co-extrudate for formulation B.

Fig. 6. Selected region of the Raman spectra of hydrochlorothiazide, physical mixture of the coat and coat of the co-extrudate for formulation B. (Left scale for the selected region of the

pure HCT spectrum. Right scale for the selected region of the spectra for physical mixture of the coat and co-extrudate of the coat.)

Fig. 7. Raman mapping of metoprolol tartrate in two sections of the co-extrudate containing 30% metoprolol tartrate in the core. A red color corresponds to a high peak intensity in the 810 - 830 cm^{-1} region, indicating the presence of metoprolol tartrate. A blue color corresponds to a very low peak intensity, indicating absence of metoprolol tartrate.

Fig. 8. *In vitro* metoprolol tartrate (MPT) release (in phosphate buffer pH 6.8) for co-extrudate formulation B (Core: 62% ethylcellulose + 33% dibutyl sebacate + 5% polyethylene oxide 1M with 30% metoprolol tartrate), initially () and after storage for 1 month at 25°C/60%RH () and 40°C/75%RH (), 3 months at 25°C/60%RH () and 40°C/75%RH () and 6 months at 25°C/60%RH () and 40°C/75%RH (). Mean (n = 3) dissolution profiles (± SD) of co-extrudates. Dissolution at 37 °C and 100 rpm.

Fig. 9. X-ray diffraction patterns of hydrochlorothiazide (A), physical mixture core (B), coat co-extrudate after 3 months storage at 25°C/60%RH (C) and coat co-extrudate after 3 months storage at 40°C/75%RH (D) for formulation A.

Fig. 10. Mean (n = 6) hydrochlorothiazide (HCT) plasma concentration-time profiles after oral administration of the co-extruded formulations A (), B (), C (), D () (25 mg hydrochlorothiazide) and Zok-Zid® tablets () (2 tablets) to dogs. The SD for formulation A and B are shown, for the other formulations SD is of the same magnitude.

Fig. 11. Mean (n = 6) metoprolol tartrate (MPT) plasma concentration-time profiles after oral administration of the co-extruded formulations A (), B (), C () (200 mg metoprolol tartrate) and D () (100 mg metoprolol tartrate) and Zok-Zid® tablets () (2 tablets) to dogs. The SD for formulation A and B are shown, for the other formulations SD is of the same magnitude.

Tables

Table 1. Composition of test and reference formulations and drug dose administered during the in vivo study. Formulation components are ethylcellulose (EC), dibutyl sebacate (DBS), polyethylene oxide (PEO), polyethylene glycol (PEG), metoprolol tartrate (MPT) and hydrochlorothiazide (HCT).

Table 2. Mean (n = 6) pharmacokinetic parameters (± SD) after oral administration of the co-extruded formulations A, B, C, D (25 mg hydrochlorothiazide) and Zok-Zid® tablets (2 tablets) to dogs.

Table 3. Mean (n = 6) pharmacokinetic parameters (± SD) after oral administration of the co-extruded formulations A, B, C (200 mg metoprolol tartrate) and D (100 mg metoprolol tartrate) and Zok-Zid® tablets (2 tablets) to dogs.