Controlling the Release of Indomethacin from Glass Solutions Layered
with a Rate Controlling Membrane Using Fluid-Bed Processing. Part 2:
The Influence of Formulation Parameters on Drug Release

Peer-reviewed author version

Dereyrnaker, Aswin; Pelgrims, Jirka; Engelen, Frederik; ADRIAENSENS, Peter &
Van den Mooter, Guy (2017) Controlling the Release of Indomethacin from Glass
Solutions Layered with a Rate Controlling Membrane Using Fluid-Bed Processing.
Part 2: The Influence of Formulation Parameters on Drug Release. In: MOLECULAR
PHARMACEUTICS, 14(4), p. 974-983.

DOI: 10.1021/acs.molpharmaceut.6b01024

Handle: <http://hdl.handle.net/1942/24183>

Controlling the release of indomethacin from glass solutions layered with a rate controlling membrane using fluid-bed processing. Part 2: The influence of formulation parameters on drug release

Aswin Dereymaker¹, Jirka Pelgrims¹, Frederik Engelen¹, Peter Adriaenssens², Guy Van den Mooter^{1}*

¹Drug Delivery and Disposition, KU Leuven, Campus Gasthuisberg O&N2, Herestraat 49, Box 921, 3000 Leuven, Belgium

²Applied and Analytical Chemistry, Institute for Materials Research (IMO), Hasselt University, Campus Diepenbeek, Agoralaan 1- Building D, 3590 Diepenbeek, Belgium

*Guy Van den Mooter – guy.vandenmooter@kuleuven.be

Address: Drug Delivery & Disposition, Campus Gasthuisberg O&N 2, Box 921, Herestraat 49, 3000 Leuven, Belgium. Tel.: +32 16 330304; fax: +32 16 330305

TABLE OF CONTENTS/GRAPHIC ABSTRACT

ABSTRACT This study aimed to investigate the pharmaceutical performance of an indomethacin-polyvinylpyrrolidone (PVP) glass solution applied using fluid bed processing as a layer on inert sucrose spheres, and subsequently top-coated with a release rate controlling membrane consisting of either ethyl cellulose or Eudragit RL. The implications of the addition of a pore former (PVP) and the coating medium (ethanol or water) on the diffusion and release behavior were also considered. In addition, the role of a charge interaction between drug and controlled release polymer on the release was investigated.

Diffusion experiments pointed to the influence of pore former concentration, rate controlling polymer type and coating solvent on the permeability of the controlled release membranes. This can be translated to drug release tests, which show the potential of diffusion tests as a preliminary screening test and that diffusion is the main factor influencing release. Drug release tests also showed the effect of coating layer thickness. A charge interaction between INDO and ERL was demonstrated, but this had no negative effect on drug release. The higher diffusion and release observed in ERL-based rate controlling membranes was explained by a higher hydrophilicity, compared to EC.

KEYWORDS Solid dispersions, Controlled release, Fluid bed coating, Drug diffusion, Drug release, Solid-state nuclear magnetic resonance

ABBREVIATIONS EC, ethyl cellulose; ERL, Eudragit RL[®]; ERS, Eudragit RS[®]; INDO, indomethacin; PVP, polyvinylpyrrolidone; TEC, triethyl citrate; Tg, glass transition temperature; NSAID, non-steroidal anti-inflammatory drug; ss-NMR, solid-state nuclear magnetic resonance; CP/MAS NMR, cross polarization/magic angle spinning nuclear magnetic resonance; EtOH, ethanol; ANOVA, analysis of variance

INTRODUCTION

Research on solid dispersions has been spanning for over half a century. Since being first described in 1961 by Sekiguchi and Obi ¹, over being defined and classified 10 years later by Chiou and Riegelman ², to ultimately being a widespread concept as a poorly soluble drug formulation strategy, solid dispersions have gathered some attention over time ³. Typically, solid dispersions are formulated in combination with hydrophilic polymers as immediate release formulations. A lot of hydrophilic polymers (semi-crystalline and amorphous) have been tested for their potential use in solid dispersions ^{4, 5}, but only few have been successfully utilized into marketed solid dispersions ⁶. When using these polymers, which show good water solubility, supersaturated gastrointestinal drug concentrations occur relatively fast after administration. However, supersaturation is not always maintained for a sufficient period of time leading to sub-optimal bioavailability enhancement. Even in vitro-in vivo comparisons cannot be readily made, as was reported in a study by Six *et al.* ⁷. Transforming rapidly dissolving but fast-precipitating

solid dispersions into slow-release formulations might therefore improve their absorption enhancing potential ⁸.

Different techniques have been proposed and used to prepare controlled release formulations for solid dispersions, like hot melt extrusion, powder compression, granulation and emulsion methods, or more recently electrospraying ⁹. Hot-melt extrusion, for example, has been one of the well explored systems to make slow-release drug-polymer systems. The drugs incorporated are mainly water soluble compounds that are combined with a controlled release polymer ^{10, 11} that can be part of the matrix or used as a rate controlling membrane ¹². In the current study, fluid bed coating is proposed as an alternative and relatively unexplored technique. Beten *et al.* showed the feasibility of loading controlled release drug-polymer coevaporates of dipyridamole and enteric Eudragit polymers using an industrial scale fluid bed coating system ¹³. One of the main advantages of this process is that additional or multiple steps in the preparation process like milling, sieving, compression or additional tablet coating can be omitted. The ability of fluid bed coating to produce multiple layered systems is ideal for the preparation of controlled release solid dispersions. The solubilization can be maximized by choosing an appropriate (polymeric) carrier for the solid dispersion (or, ideally, the glass solution) layer. Subsequently, an additional rate controlling membrane can be applied on top of the glass solution layer to optimize the release during a well-defined time frame. The feasibility of this approach is described in the companion paper ('Controlling the release of indomethacin from glass solutions layered with a rate controlling membrane using fluid-bed processing. Part 1: Surface and cross-sectional chemical analysis'). Two clearly defined coating layers were observed. The surface properties of different rate controlling membranes applied could be explained to a large extent by the polymer mixing behavior. The physical structure of the underlying glass solution layer was also shown not being

affected by the slow-release top coating, even when this last was sprayed from an aqueous dispersion. The focus of the current paper is the investigation of the pharmaceutical performance of these formulations, i.e. their release behavior and the effect of formulation changes on the drug release. The glass solution layer is always made up of indomethacin (INDO) in polyvinylpyrrolidone K25 (PVP) in a 30:70 % w/w ratio. Two different rate controlling polymers will be tested, ethyl cellulose (EC) and Eudragit RL (ERL). PVP will also be used as a pore former and triethyl citrate (TEC) will be used as a plasticizer. Special emphasis in this study will be put on the possible charge interaction between negatively charged INDO (above pH 4.5) and the positively charged ERL, which is a water insoluble polymer, but the presence of quaternary ammonium groups in ERL is responsible for pH-independent swelling ^{14, 15}. Drug release from ERL systems however, can be sensitive to the presence of other anionic species like buffer components or organic acids ¹⁶⁻¹⁸. This charge interaction has also been reported with other NSAID's in the past ¹⁹⁻²¹. This possible charge interaction will be studied using Solid State Nuclear Magnetic Resonance (ss-NMR) and the implications of this interaction will be studied in sorption, permeability and drug release tests.

EXPERIMENTAL SECTION

Materials

Indomethacin was purchased from FAGRON Ltd. (Waregem, Belgium). Polyvinylpyrrolidone K 25 was a generous gift from BASF (Ludwigshafen, Germany). Sucrose spheres (diameter 710 - 850 μm) were kindly donated by Hanns G. Werner GmbH (Tornesch, Germany). Ethyl cellulose (ethoxy content 48.0-49.5% w/w) powder and triethyl citrate (TEC) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Eudragit RS[®] PO (ERS), Eudragit RL[®] PO and Eudragit RL[®] 30D were purchased from Evonik Industries (Darmstadt, Germany).

Fluid bed coating

Coated beads were prepared using an Aeromatic MP 1 multiprocessor (GEA, Bubendorf, Switzerland) in a bottom spray setup, equipped with a Würster insert. A 30:70 (w:w) INDO-PVP (w/w) glass solution with a total solid content of 250,0g was coated onto 500,0g of sucrose beads from a 10% (w/v) ethanol solution. The sucrose spheres were loaded into the preheated coating chamber at 50°C and heated for 10 minutes. The drug-polymer solution was coated onto the sucrose pellets, using a feed rate of 13cm³/min. This feed was atomized at an air pressure of 1.5 bar. Meanwhile the heated air stream was passing through the fluid bed coater at a rate of 1.78 m³/min. When the spraying was finished, the pellets were dried until immobilization due to electrostatic charges was observed. The coated spheres were unloaded, weighed and dried for an additional 48 hours in an oven at 50°C. In the case when a top layer (rate controlling membrane) was applied, the feed solution was immediately changed after completion of the glass solution layer. The controlled release top layer consisted of a rate controlling polymer (ERL or EC) with an added pore former (PVP K25) in a 10% or 25% ratio to the total solid content and the plasticizer TEC, added in a concentration of 20% w/w relative to the amount of rate controlling polymer. The rate controlling membrane was applied from a 10% w/v ethanolic solution. Also, ERL-PVP 90-10% (w-w) was applied as an aqueous dispersion (10% w/v) instead of an ethanolic solution. The coating process parameters are the same for the top coating layer as for the glass solution layer, except for the feed rate with the ERL ethanolic solutions, where the feed rate was reduced to 6.5cm³/min) because of the electrostatic charges created inside of the fluid bed coater. After completion of the coating, the beads were dried in the coater for at least 10 minutes, followed by further drying in an oven for at least 48 hours. Ethanolic solutions are dried at 50°C, aqueous dispersions at 60°C to allow for curing of ERL.

During coating of the rate controlling membrane, samples of 10-15g were taken at different time points to measure the coating layer thicknesses, expressed as percentage weight gain, relative to the weight of the glass solution coated beads.

Drug diffusion

Drug diffusion through a rate controlling membrane is tested with a diffusion cell set up, represented in Figure 1.

Figure 1. Schematic representation of the diffusion cell set-up.

The polymer film was clamped between a donor and acceptor compartment. The diameter of polymer film in contact with donor and acceptor compartment is 18 or 25mm depending on the used cells. The diffusion medium used was a 100mM phosphate buffer solution of pH 6.8 with 15% propylene glycol to enhance INDO solubility in the donor compartment. For the donor solution 1.00mg/ml INDO was dissolved in the diffusion medium, the acceptor solution contained the blank medium. Volume of both donor and acceptor compartment was 100ml. During diffusion experiments, both compartments were also sealed from the air and constantly stirred with magnetic stirrers to ensure homogeneous distribution.

Controlling rate membranes, tested in the diffusion experiments, were prepared by film casting from an ethanol solution or aqueous dispersion onto a Teflon plate and drying it for 24 h at room

temperature under a funnel. After this, they were put into an oven and dried for an additional 24h at 50°C for the solutions and for 48h at 60°C for the dispersions. Eudragit RL and ethyl cellulose are used as controlled release polymers (ERL100% and EC 100%), PVP K25 is optionally added as a pore former in 10 or 25% (w/w) based on the total solid content (ERL-PVP 90-10%, ERL-PVP 75-25%, EC-PVP 90-10% and EC-PVP 75-25%). TEC was added as a plasticizer in all membranes in a 20% (w/w) concentration, based on the controlled release polymer mass. Ethanol solutions were prepared for ERL and EC based samples and aqueous dispersions for ERL based samples, both in a 10% w/v ratio.

Diffusion tests were performed with an automated sampling device, a Gilson Liquid Handler 215 (Gilson, Middleton, WI, USA). All rate controlling membranes were tested in triplicate, except for EC 100% and EC-PVP 90-10% which were tested in duplicate. At each sampling point, a 1ml sample was removed from the acceptor compartment and replaced with 1ml of blank medium. Samples are directly injected into the HPLC system for analysis.

Drug concentrations at different time points were used to calculate the permeability coefficient (P, cm/s) using following equation ²²:

$$\frac{2PS}{V}t = -\ln\left(\frac{C_0 - 2C_a}{C_0}\right)$$

In this equation; S is the diffusion surface area (cm²), V is the volume of donor and acceptor (ml), C₀ is the initial donor concentration and C_a the acceptor concentration (mg/ml) at time t (s).

High Performance Liquid Chromatography (HPLC)

Quantitative analysis of the samples was performed using a Merck-Hitachi Lachrom HPLC system consisting of a Merck Hitachi L-7100 pump, an L-7420 UV-VIS detector, an L-7200 autosampler and a D-7000 interface (Merck, Darmstadt, Germany). Injections were run through a Chromolith performance RP-18 column of 100mm x 4.6mm (Merck, Darmstadt, Germany). The

mobile phase was 30% orthophosphoric acid solution (0.5% v/v), 40% acetonitrile and 30% methanol. Flow rate was set at 1.0 ml/min and injection volume at 20 μ l. INDO absorption was measured at 320nm and the retention time was approximately 3.3min.

Drug sorption tests

Powders or films of ERL, ERS and EC were added in a test tube filled with 5.0ml of either a 200 μ g/ml or 100 μ g/ml INDO solution (medium: phosphate buffer pH 6.8) and rotated for 48 hours in a rotary mixer. After 48 hours, a sample was taken from the solution, filtered and analyzed with HPLC . Drug sorption to the polymer was calculated from the INDO concentration decline. All conditions were tested 5 times.

Solid-state NMR

The ^{13}C -CP/MAS NMR spectra were recorded at room temperature on an Agilent VNMRS DirectDrive 400MHz spectrometer (9.4 T wide bore magnet) equipped with a T3HX 3.2 mm probe dedicated for small sample volumes and high decoupling powers. Magic angle spinning (MAS) was performed at 15 kHz with ceramic rotors of 3.2 mm (22 μ l rotors). The aromatic signal of hexamethylbenzene was used to determine the Hartmann-Hahn condition ($\omega_{1\text{H}} = \gamma_{\text{H}} B_{1\text{H}} = \gamma_{\text{C}} B_{1\text{C}} = \omega_{1\text{C}}$) for cross-polarization and to calibrate the carbon chemical shift scale (132.1 ppm). Other acquisition parameters were: a spectral width of 50 kHz, a 90° pulse length of 2.5 μ s, a spin-lock field for CP of 100 kHz, a contact time for CP of 1.0 ms, an acquisition time of 20 ms, a recycle delay time of 7.5 s and 350-45000 accumulations (350 scans for INDO, 2700 scans for Eudragit RL[®] and 45000 scans for the INDO-ERL solid dispersion from an ethanol solution (INDO-ERL EtOH) as well as for the INDO-ERL powder isolated from a phosphate buffer pH6.8 (INDO-ERL pH 6.8)). High power proton dipolar decoupling during the acquisition time was set to 100 kHz.

Two different INDO-ERL samples were prepared. The first one was prepared by dispersing ERL particles in a 200µg/ml INDO solution in phosphate buffer pH 6.8. After 48h of constant stirring, ERL particles were filtered and dried in an oven at 50°C. The second sample was prepared by spray drying an equivalent INDO-ERL ratio from an ethanol solution (10% w/v) using a Büchi Mini Spray Dryer B-191 (Flawil, Switzerland) and applying an inlet air flow rate of 0.56 m³/min, an inlet air temperature of 50°C, an atomizing air flow rate of 0.02 m³/min and a feed rate of 4.8 cm³/min. After collecting of the powder, it was additionally dried in an oven at 50°C.

Water permeation through isolated polymer films

Water permeation through isolated polymer films was tested using aluminium cups containing 5.0ml of water. A schematic representation is provided in Figure 2.

Figure 2. Schematic representation of the diffusion cup set-up.

Films were prepared using the solvent casting method described in the drug diffusion section. Water evaporation was tested for the ERL 100%, ERL-PVP 90-10%, ERL-PVP 75-25%, EC-PVP 90-10% and EC-PVP 75-25% polymer films. Cups were stored at ambient conditions and weighed at fixed time intervals for at least 126 hours to constant mass. This allowed calculating the percentage loss of water at each weighing interval. Tests were performed in duplicate.

Drug release

Drug release tests of the different controlled release formulations were performed using a Hanson SR8PLUS dissolution station (SpectraLab Scientific Inc., Markham, Canada). The release medium was 500ml of a 100mM phosphate buffer pH6.8. Paddle speed was set at 120rpm and the temperature at 37.0 °C (± 1.0). Samples were accurately weighed and were equivalent to an INDO dose of 75mg. At sampling time intervals, a 1ml sample was taken, filtered (pore size: 0.45 μ m) and put into an HPLC vial. The sample taken was replaced by 1ml of blank dissolution medium. Sampling times were 30, 60, 90, 120, 180, 240, 300 and 360 min. The quantitative analysis of the samples was performed with HPLC using the above described method. All formulations were tested in triplicate.

Content analysis

Accurately weighed formulations (with a theoretical INDO content of 3mg) were transferred into a test tube with 5.0ml of ethanol and rotated for 24h. Thereafter 1ml of ethanol solution was withdrawn, filtered (pore size 0.45 μ m) and analyzed with HPLC.

Statistical analysis

Differences between the permeability coefficients of the different membranes were evaluated using ANOVA and unpaired t-testing. Results were considered statistically significantly different if $p < 0.05$.

The similarity factor f_2 was used to compare drug release profiles of different formulations²³. Two profiles are considered not significantly different when the f_2 value is between 50 and 100.

RESULTS

Indomethacin diffusion through rate controlling membranes

Permeability coefficients are calculated from the concentration change in the acceptor compartment. Values are shown in Figure 3. In general, if the amount of PVP increases, the

permeability of INDO increased as well. This is noticed for all three formulation groups, EC based films casted from an ethanol solution, ERL based films casted from an ethanol solution and ERL based samples casted from an aqueous dispersion (so-called 'latex'). The differences in the permeability of the films when changing the PVP content are significant, except for ERL-PVP 90-10% and ERL-PVP 75-25% ($p = 0,2371$), ERL 100% and ERL-PVP 75-25% ($p = 0,1104$), and also, ERL-PVP 90-10% latex and ERL-PVP 75-25% latex ($p = 0,1493$). Apart from differences related to the amount of PVP incorporated, also differences related to the rate controlling polymer were observed. The permeability of INDO is always higher for ERL based formulations (solution or dispersion) as compared to EC based formulations (given the same amount of PVP). All these permeability differences are statistically significant, except for ERL-PVP 75-25% and EC-PVP 75-25% ($p = 0,2035$). Both of these films show a very large standard deviation in the value of P. Also ERL-PVP 75-25% latex and EC-PVP 75-25% do not show a significant difference since they have the same P-value. Films casted from a solution show higher P-values compared to films prepared from an aqueous dispersion, but the difference was only statistically significant for films of ERL 100% and ERL latex 100%; for films containing 10% and 25% PVP the difference was not significant ($p = 0,1052$ and $p = 0,2027$).

Figure 3. Permeability coefficients of INDO through different rate controlling membranes

Indomethacin sorption to rate controlling polymers

Because the rate controlling polymers EC, ERL and ERS are not soluble in aqueous media, INDO can sorb onto or into the swollen polymer particles. Figure 4 shows the amount (μg) of INDO that was sorbed into/onto the polymer particles (A) or the films (B) per milligram of CR polymer. These graphs clearly show that ERL powder and films incorporated the largest amount of INDO, irrespective of the INDO concentration in the medium. Moreover, INDO sorption in ERL doubled when the INDO concentration doubled, from $22.4\mu\text{g}/\text{mg}$ to $44.9\mu\text{g}/\text{mg}$ for the powder and from $20.4\mu\text{g}/\text{mg}$ to $44.0\mu\text{g}/\text{mg}$ for the film. EC showed the second largest INDO sorption in powders but lowest in the films, where it almost didn't sorb any INDO ($0.0394\mu\text{g}/\text{mg}$ and $0.186\mu\text{g}/\text{mg}$ for 100 and $200\mu\text{g}/\text{ml}$ INDO concentration, respectively). INDO sorption in EC powder also almost doubled when the concentration doubled ($8.76\mu\text{g}/\text{mg}$ and $16.4\mu\text{g}/\text{mg}$). INDO sorption on ERS only slightly increased with increasing INDO concentration in films (from $4.74\mu\text{g}/\text{mg}$ to $6.71\mu\text{g}/\text{mg}$) and powders (from $4.63\mu\text{g}/\text{mg}$ to $5.51\mu\text{g}/\text{mg}$). Sorption in films was also slightly higher than sorption in powders.

Figure 4. Sorption of INDO into/onto different controlling rate polymers in the form of powder (A) or a film (B) in phosphate buffer medium pH 6.8

¹³C-CP/MAS solid-state NMR investigation into drug-polymer interactions

INDO and ERL reference spectra, together with the spectra of INDO-ERL solid dispersion from an ethanol solution (INDO-ERL EtOH) and INDO-ERL powder isolated from phosphate buffer pH6.8 (INDO-ERL pH 6.8) are shown in Figure 5. Each INDO carbon nucleus shows a single

signal without splitting, indicative for the stable γ -form²⁴. Specially the signal at 156.7 ppm, attributed to the aromatic carbon bearing the methoxy group, is highly specific and confirms that the INDO starting material was γ -indomethacin^{24,25} (other peak assignments can be found in these references as well). The spectrum of INDO-ERL EtOH still shows the characteristic INDO peak at 156.7 ppm next to other INDO signals around 114.5 ppm, 131.5 ppm and 167.7 ppm. These peaks are situated at exactly the same position as for the INDO reference. The spectrum of the material prepared by soaking ERL in an INDO solution above its pKa (INDO-ERL pH 6.8) however shows clearly two significant chemical shift changes, i.e. an upfield shift of the signal at 156.7 ppm to 153.9 ppm, and an upfield shift of the signal at 114.5 ppm to 111.5 ppm. Moreover, the 167.7 ppm signal is shifted downfield and so coincides with the intense signal of the ERL carbonyl carbon. These changes point to an electrostatic interaction between INDO and ERL.

Figure 5. ^{13}C -CP/MAS NMR spectra of INDO (red), ERL (black), INDO-ERL solid dispersion from ethanol solution (green) and INDO-ERL powder isolated from phosphate buffer pH6.8 (blue). In the detailed view on the right, INDO intensity is reduced by factor 3.

Water vapour diffusion through rate controlling membrane

The permeation of water through the polymer films (expressed as percentage water loss) was measured over time and plotted in Figure 6. The evaporation rate increased with increasing pore former concentration for both ERL based and EC base rate controlling films. The time to lose 50% of water was calculated according to the equation of the trendline and was found to be 68h for ERL-PVP 75-25%, 80h for ERL-PVP 90-10% and 97h for ERL 100%. The time to reach 50% water content was 83h for EC-PVP 75-25% and 103h for EC-PVP 90-10%. In case the same amount of pore former is present in the films, ERL based films have faster mass loss compared to EC based samples.

Figure 6. Water evaporation (in content %) through rate controlling membranes as a function of time.

Drug release

Crystalline indomethacin and INDO-PVP glass solutions

The dissolution of crystalline indomethacin and the release from INDO-PVP 30-70% (w/w) glass solutions coated onto sucrose beads is shown in Figure 7. Already at the first time point (60 min), INDO-PVP glass solutions showed full release. Glass solutions were considered to have an

immediate release profile. Crystalline INDO on the other hand, only gradually reached a plateau of 95% release after 5 hours.

Figure 7. INDO release in function of time for crystalline INDO (blue dots) and INDO-PVP 30-70% (w/w) glass solution coated around inert carriers (red squares).

EC rate controlling membranes

INDO release was tested for EC-PVP 90-10% and EC-PVP 75-25% coatings on top of INDO-PVP glass solutions. Figure 8 shows the INDO release (cumulative %) in function of time for beads coated with an EC-PVP 90-10% rate controlling outer membrane. The coating thickness was varied from 9.7% to 36.6%. Increasing coating layer thicknesses showed decreased and slower INDO release. Maximum release after 6h was 96% for 9.7% coating, 38% INDO release for 19.1% coating, 3% for 28.0% coating and 9% for 36.6% coating. The profiles were different. Initial

release is slow and shows a lag time, which increases with increasing coating layer thickness. This is followed by a period of high release rate for the 9.7% (between 60 and 180 min) and 19.1% (between 180 and 360 min) coating level. The lowest coating level shows a decreased INDO release between 180 and 360 min. The release profiles of the formulations with the highest coating levels (28.0 and 36.6%) were still in a lag phase which lasted for the entire 6 hours. All release profiles are significantly different, except for 28.0 and 36.6% ($f_2=81.2$), which explains why 36.6% coating level has a higher mean release compared to 28.0% coating level.

Figure 8. INDO release in function of time for INDO-PVP 30-70% (w/w) glass solution and an EC-PVP 90-10% (w/w) rate controlling membrane. Different coating levels were tested: 9.7% (blue), 19.1% (red), 28.0% (green) and 36.6% (purple).

INDO release from EC-PVP 75-25% beads was quasi immediate. All beads reach their maximum release after 60-100min and this irrespective of the coating layer thickness, which varied from 9.5% to 35.8% (a release profile has been provided in the Supporting Information, Figure A)

ERL rate controlling membranes

The INDO release from INDO-PVP glass solutions was tested with ERL-PVP 90-10% (coated from either an ethanol solution or an aqueous dispersion) and 75-25% rate controlling membranes on top of the glass solutions. The release profiles of ERL-PVP 90-10% rate controlling membranes, coated from a solution are shown in Figure 9. Also in this case, increasing the coating layer thickness resulted in slower and decreased INDO release. While a 11.2% coating layer still shows 84% release after 6h, the release drops to 75% for 21.7% coating level, to 55% for 31.7% coating level and, ultimately, to a mere 50% for the highest coating level (36.3%). ERL-PVP 90-10% beads showed initial high drug release rate (burst release) followed by a slower INDO release for the lowest coating level. Formulations having higher coating levels showed an initial slow release followed by an increased release rate up until 90 minutes followed by a decreased release rate. All release profiles are significantly different from each other except for 31.7% and 36.6% ($f_2=66.2$).

Figure 9. INDO release in function of time for INDO-PVP 30-70% (w/w) glass solution and an ERL-PVP 90-10% (w/w) rate controlling membrane. Different coating levels were tested: 11.2% (blue), 21.7% (red), 31.7% (green) and 36.3% (purple).

ERL-PVP 75-25% coated beads also showed an INDO release which was highest (93%) and fastest from beads with the smallest coating amount (11.3%). Similar to the other formulations, this decreased to 68% and 57% with an increasing coating level of 21.9% and 32.0% respectively. The release profiles are shown in Figure 10. Formulations having the lowest coating level showed a high release rate during 180min, followed by a quasi-plateau level for the remaining 3 hours. The higher coating levels both showed a lag phase for 10min followed by an enhanced release for 50min and a decreased release rate for the remaining 5 hours. All curves with different coating layer thicknesses are significantly different.

Figure 10. INDO release in function of time for INDO-PVP 30-70% (w/w) glass solution and an ERL-PVP 75-25% (w/w) rate controlling membrane. Different coating levels were tested: 11.3% (blue), 21.9% (red) and 32.0% (green).

Finally in Figure 11, the ERL-PVP 90-10% rate controlling membrane, coated from an aqueous dispersion, also showed a decreased and slower release with increasing coating level. After 6 hours the release was 87% for 8.7% coating level and 69% for 17.1% coating level. Beads with 8.7% coating level showed high initial release (68% after 120min) followed by a slower release for the remaining 4 hours. After an initial (90min) high release for the beads having 17.1% coating level, INDO release approached zero-order release kinetics for the remainder of the release experiment. Both profiles showed to be significantly different from one another.

Figure 11. INDO release in function of time for INDO-PVP 30-70% (w/w) glass solution and an ERL-PVP 90-10% (w/w) rate controlling membrane, coated from an aqueous dispersion (latex). Different coating levels were tested: 8.7% (blue) and 17.1% (red).

DISCUSSION

The difference of INDO diffusion through the different rate controlling membranes can be calculated based on the permeability coefficient. The applied equation has the benefit of being independent of membrane thickness. It is, however, important when calculating from a slope, that only the linear part of the plot is used. In other words, steady-state diffusion has to be reached to be able to calculate the permeability coefficient. For EC-based as well as ERL-based samples, it is clear that an increased amount of PVP, which is added as a pore former, led to an increased permeability coefficient. This is to be expected because PVP is a hydrophilic polymer which is

expected to leach out of the rate controlling membrane. This allows for a higher permeation of the drug because the EC or ERL polymer chains are not as closely packed as they would be in absence of a pore former. In the ERL based samples, the permeability coefficient increases gradually from 0% over 10% to 25% PVP; in the EC based samples P is very small when 0% and 10% of PVP is added but increases significantly when 25% of PVP is added. This can be explained by the miscibility of both polymers, which has been reported in the companion paper. Because PVP and EC are completely immiscible, PVP is not able to interpenetrate the EC polymer chains as much as in ERL. So while the amount of PVP in EC-PVP 90-10% is not sufficient for an adequate pore former functionality (similar P-value with EC 100%), EC-PVP 75-25% led to the formation of weak spots in the rate controlling membrane with a higher permeability as a result. The partial miscibility of ERL and PVP allowed for a more gradual increase of the rate controlling membrane permeability with increasing pore former concentration. Increasing pore former concentration also led to a higher variability of the permeability coefficient in case films were casted from an ethanol solution. This can also be explained by the (partial) immiscibility of the rate controlling polymer and the pore former. Increasing pore former concentration increased the heterogeneity of the films, resulting in a larger variation in permeability coefficients. When EC-based films are compared to ERL-based samples, a lower permeability coefficient was noticed for the same pore former concentration. This was most noticeable for the 0% and 10% pore former concentrations. Although there is a difference in the 25% PVP samples, this difference is not statistically significant due to the large standard deviation. When ERL films from an ethanolic solution are compared to films from an aqueous dispersion with equal pore former concentration, the second ones tended to have lower permeability coefficients. The main difference between film casting from a solution versus casting from a dispersion is that the latter requires a curing step. The polymer disposes itself as a

small polymer particle (instead of spread out polymer chains in the solution) and needs to be stored at a temperature above the minimal film forming temperature for a certain amount of time to allow optimal coalescence of the dispersed polymer particles and polymer chain inter-diffusion for efficient film formation.

INDO sorption studies showed clear differences in the amount of INDO incorporated into the insoluble controlled release polymers ERL, ERS and EC. ERL showed the highest INDO sorption in all conditions, being different concentrations and form (powder or film). INDO sorption is the lowest in the case of ERS powder and second lowest in case of the films. As with ERL, the sorption into powders or films doesn't differ much, indicating that sorption in this case is independent of the available surface area. There is no relationship between ERS sorption and the INDO concentration in the medium. This observation also justifies the inclusion of ERS into this test as to show that the sorption of INDO in these polymers is independent upon the amount of quaternary ammonium groups present in these polymers (the part in the ter-co-polymer mainly responsible for the swelling). This is evidenced by the fact that ERS has half of the quaternary ammonium groups compared to ERL and shows less than half of the amount of INDO sorbed. The link between the quaternary ammonium groups and INDO sorption also shows that an interplay of different forces is responsible for the sorption; not only a charge interaction, but also a decreased swelling of these particles because of the decreased amount of quaternary ammonium groups. Since these particles or films swell upon contact with the medium, INDO has a certain affinity for the hydrophobic environment of the polymers and will diffuse in them. This diffusion does not seem to be dependent on a charge interaction because it is also observed in the neutral EC polymer. Here, INDO sorption into/onto EC showed the second highest amount sorbed for the powders and lowest for the films. In the films almost no INDO is sorbed, which could be an indication for the

dependence on the available surface area for INDO sorption. It can be presumed that the films have less surface area in contact with the medium compared to the powder. In the test with EC powder, INDO sorption is doubled when the INDO concentration in the medium is doubled. This can be in a direct way, pointing to sorption into or onto the available surface area or in an indirect way, meaning that the available surface area relates to the swelling of the polymer and that swelling has an impact on the diffusion into the polymer. The sorption of the active pharmaceutical ingredient into or onto the controlled release polymer can have certain implications towards incomplete drug release. These implications will only be enhanced when the coating layer thickness increases or if ERL should be used as part of the matrix of a different formulation.

To confirm the electrostatic charge interaction between INDO (above its pKa at pH 6.8) and ERL during drug diffusion through the controlled release membrane, solid-state NMR experiments were performed. The significant changes in chemical shift (3ppm) observed for INDO-ERL pH 6.8 are undeniably the result of an interaction between INDO and the cationic quaternary ammonium groups of the polymer. Moreover, this was not observed for the INDO-ERL solid dispersion prepared from EtOH in which INDO was not present in its ionic form. Thus, the observed interaction is only present when INDO and ERL are both in their ionic form. Due to this interaction, the electron density (chemical environment) of some of the INDO carbons is affected, leading to the observed changes in chemical shift ^{24, 25}.

Diffusion through the rate controlling membranes was not only measured for INDO molecules in solution, also water vapor diffusion was monitored. Because of the uncharged nature of water molecules, the diffusion through the rate controlling membranes is not affected by possible charge interactions. The results of the water vapor diffusion tests were along the same lines as INDO diffusion tests, i.e. diffusion of ERL-based membranes is always higher compared to EC-based

samples with the same amount of PVP pore former. ERL-PVP 90-10% and EC-PVP 75-25% have comparable water mass losses in function of time, which is in accordance with INDO diffusion results. Water vapor diffusion tests confirm that diffusion is generally higher for ERL-samples compared to EC-samples (given an equal amount of pore former), and this is irrespective of a possible charge interaction between the diffusing molecules (INDO) and (part of) the rate controlling membrane (ERL). This leads to conclude that ERL is more hydrophilic, compared to EC.

When comparing the release from INDO-PVP coated beads and crystalline INDO in Figure 7, it can be seen that release from the glass solutions is faster and more uniform, opposed to crystalline INDO (larger standard deviation). This shows the poor dissolution characteristics of the pure crystalline drug, and the need to formulate INDO into a glass solution prior to sustaining the release.

INDO release from coated beads is dependent on the coating layer thickness in all formulations, except for those with a top-coating made up of EC-PVP 75-25% where all INDO is released immediately irrespective of the coating layer thickness. Increasing the coating layer thickness slows down and decreases the release of INDO from the coated beads, which can be attributed to the longer diffusion pathways for the drug²⁶ and diffusion through a somewhat denser polymer network. Hydrophobic drugs have also been reported to have slower release profiles compared to hydrophilic drugs²⁷. The decrease in release can also be attributed to the increasing presence of rate controlling polymer which will not dissolve into the medium and sorb INDO as observed in the this study. A combination of these effects will lead to a decreased drug release rate and amount with increasing rate controlling membrane thickness. There is also an increase in lag-time with

increasing coating thickness, which is also explained by the increased diffusion path for the drug

26.

Large differences are observed concerning the influence of the pore former depending on the rate controlling polymer used. When 10% and 25% PVP are compared in ERL-based samples with similar coating levels, the former led to a slightly faster and higher release compared to the latter, but the observed differences are never significant. In EC-PVP 90-10% and EC-PVP 75-25% on the other hand, differences could not be more remarkable. While 10% pore former is able to slow down the INDO release (given an appropriate coating level), EC-PVP 75-25% does not slow down the release for any coating level. This inability to control the release points to a defect in the coating layer which can originate from a too high pore former concentration. The reason behind the coating defect could be explained by results obtained in the first part of this study (details are provided in the companion paper). Here, differences in polymer miscibility and, subsequently, pore former distribution along the surface were observed. The immiscibility between EC and PVP resulted in more isolated PVP presence along the surface which could lead to local coating defects when leached out in early stages of release experiments. When using 10% pore former concentrations, the isolated PVP regions are smaller and the dissolving pore former can still be replaced by the swelling EC polymer, successfully slowing down the release. The partial miscibility between ERL and PVP explains why the PVP is more homogeneously spread along the rate controlling membrane surface and why no coating defects are observed when using a 25% pore former concentration. Miscibility studies between rate controlling polymer and pore former can indicate formation of isolated pore former regions in the rate controlling membrane and may thus give an idea about the success rate in formulating rate controlling membranes.

Different release profiles are observed when different rate controlling polymers with equal coating levels and an equal pore former concentration (10% w/w) are compared. Only the lowest coating level shows a higher INDO release for EC after 6 hours (ERL: 84%, EC 90%). With higher coating levels, INDO release is always higher after 6 hours from ERL based samples. This difference becomes larger with increasing coating levels up to a point where, at ca. 36% coating level, INDO release for EC-PVP 90-10% is 3% and for ERL-PVP 90-10% it is 49%. All release profiles with comparable coating level have significantly different release profiles. Not only the release after 6 hours is smaller for EC, these formulations also show an extended lag time. For example, after 120 minutes, beads with coating levels of ca. 20% show an INDO release of 6% in EC-PVP 90-10% and 63% in ERL-PVP 90-10%. The burst release in the lower coating levels of ERL-PVP 90-10% could point to INDO release before complete swelling of the ERL coating. When 25% pore former concentration is applied, the situation is quite different. Because of the quasi immediate release (60-90 min) in case of EC-PVP 75-25%, these controlling rate membranes are not considered suitable for controlled release purposes. ERL-PVP 75-25% is, on the other hand, able to slow down the release in a way similar to ERL-PVP 90-10%. The reason for this difference is polymer miscibility as discussed in the previous paragraph.

Lastly, a comparison is made between identical rate controlling membranes coated from either an ethanol solution or an aqueous dispersion. While release profiles at 10% coating levels are not significantly different (f_2 : 63,0), at 20% coating level, the rate controlling membrane coated from a dispersion shows a slower and lower (after 6h: 69% for latex and 76% for solution) INDO release compared to the membrane coated from a solution. The release curves are significantly different from each other. Already 63% of INDO has been released after 120 min from the formulation coated from a solution. In the following 4 hours, an additional 13% INDO was released. In the

formulation with the latex membrane the release after 120 minutes is 42% INDO and in the following 4 hours an additional 27%. This shows that the beads with a latex top layer show less burst release which allows for higher release after the initial phase. Indeed, after 90 minutes quasi-zero order release kinetics are observed, and from linear regression it can be calculated that all INDO will be released (given continuation of zero-order) at 10 hours and 42 minutes.

CONCLUSIONS

The pharmaceutical performance of a glass solution coated onto inert carriers with an additional rate controlling membrane was assessed. Firstly, diffusion through rate controlling membranes showed influence of pore former concentration, rate controlling polymer used and coating process, i.e. from a solution or a dispersion. Drug release experiments generally showed similar influences of formulation parameters and an additional influence of coating layer thickness. A link can be made between permeability coefficients and release behavior which shows, on one hand, that diffusion through the rate controlling membrane is the rate limiting step during drug release, and on the other hand, that drug diffusion results can be used as indicative values when screening for an appropriate controlled release dosage form.

Although INDO and ERL are an interacting system, this has no negative effect on drug release. On the contrary, ERL generally shows higher diffusion rates and faster release, but this can be mainly attributed to its more hydrophilic nature, compared to EC.

REFERENCES

1. Sekiguchi, K.; Obi, N. Studies on absorption of eutectic mixture. I. A comparison of the behavior of eutectic mixture of sulfathiazole and that of ordinary sulfathiazole in man. *Chem. Pharm. Bull.* **1961**, 9, 866-872.

2. Chiou, W. L.; Riegelman, S. Pharmaceutical applications of solid dispersion systems. *J. Pharm. Sci.* **1971**, 60, 1281-1302.
3. Baghel, S.; Cathcart, H.; O'Reilly, N. J. Polymeric Amorphous Solid Dispersions: A Review of Amorphization, Crystallization, Stabilization, Solid-State Characterization, and Aqueous Solubilization of Biopharmaceutical Classification System Class II Drugs. *J. Pharm. Sci.* **2016**, 105, 2527-2544.
4. Van Duong, T.; Van den Mooter, G. The role of the carrier in the formulation of pharmaceutical solid dispersions. Part I: crystalline and semi-crystalline carriers. *Expert Opin. Drug Deliv.* **2016**, 13, 1583-1594.
5. Van Duong, T.; Van den Mooter, G. 2016. The role of the carrier in the formulation of pharmaceutical solid dispersions. Part II: amorphous carriers. *Expert Opin. Drug Deliv.* **2016**, <http://dx.doi.org/10.1080/17425247.2016.1198769>
6. Van den Mooter, G. The use of amorphous solid dispersions: A formulation strategy to overcome poor solubility and dissolution rate. *Drug Discov. Today Technol.* **2012**, 9, e79-e85.
7. Six, K.; Daems, T.; de Hoon, J.; Van Hecken, A.; Depre, M.; Bouche, M. P.; Prinsen, P.; Verreck, G.; Peeters, J.; Brewster, M. E.; Van den Mooter, G. Clinical study of itraconazole prepared by hot-stage extrusion. *Eur. J. Pharm. Sci.* **2005**, 24, 179–186.
8. Augustijns, P.; Brewster, M. E. Supersaturating drug delivery systems: fast is not necessarily good enough. *J. Pharm. Sci.* **2012**, 101,7-9.

9. Nguyen, D. N.; Palangetic, L.; Clasen, C.; Van den Mooter, G. One-step production of darunavir solid dispersion nanoparticles coated with enteric polymers using electrospraying. *J. Pharm. Pharmacol.* **2016**, 68, 625-633.
10. Lang, B.; McGinity, J. W.; Williams, O. W. Hot-melt extrusion – basic principles and pharmaceutical applications. *Drug Dev. Ind. Pharm.* **2014**, 40, 1133-1155.
11. Crowley, M. M.; Zhang, F.; Repka, M. A.; Thumma, S.; Upadhye, S. B.; Battu, S. K.; McGinity, J. W.; Martin, C. Pharmaceutical Applications of Hot-Melt Extrusion: Part I. *Drug Dev. Ind. Pharm.* **2007**, 33, 909-926.
12. Tran, P. H. L.; Tran, T. T. D.; Park, J. B.; Lee, B. J. Controlled release systems containing solid dispersions: strategies and mechanisms. *Pharm. Res.* **2011**, 28, 2353-2378.
13. Beten, D. B.; Amighi, K.; Moës A. J. Preparation of controlled-release coevaporates of dipyridamole by loading neutral pellets in a fluidized-bed coating system. *Pharm. Res.* **1995**, 12, 1269-1272.
14. Thakral, S.; Thakral, N. K.; Majumdar, D. K. Eudragit: a technology evaluation, *Expert Opin. Drug Deliv.* **2013**, 10, 131-149.
15. Yoshida, T.; Lai, T. C.; Kwon, G. S.; Sako, K. pH- and ion-sensitive polymers for drug delivery, *Expert Opin. Drug Deliv.* **2013**, 10, 1497-1513.
16. Bodmeier, R.; Guo, X.; Sarabia, R. E.; Skultety, P. F. The influence of buffer species and strength on diltiazem HCl release from beads coated with the aqueous cationic polymer dispersions, Eudragit RS, RL 30D. *Pharm. Res.* **1996**, 13, 52-56.

17. Narisawa S., Nagata M., Hirakawa Y., Kobayashi M., Yoshino H. An organic acid-induced sigmoidal release system for oral controlled-release preparations. 2. Permeability enhancement of Eudragit RS coating led by the physicochemical interactions with organic acid. *J. Pharm. Sci.* **1996**, 85, 184-8.
18. Narisawa, S.; Nagata, M.; Danyoshi, C.; Yoshino, H.; Murata, K.; Hirakawa, Y.; Noda, K. An organic acid-induced sigmoidal release system for oral controlled-release preparations. *Pharm. Res.* **1994**, 11, 111-116.
19. Pignatello, R.; Spadaro, D.; Vandelli, M. A.; Forni, F.; Puglisi, G. Characterization of the mechanism of interaction in ibuprofen-Eudragit RL100 coevaporates. *Drug Dev. Ind. Pharm.* **2004**, 30, 277-288.
20. Mollica, G.; Geppi, M.; Pignatello, R.; Veracini, C. A. Molecular properties of flurbiprofen and its solid dispersions with Eudragit RL100 studied by high- and low-resolution solid-state nuclear magnetic resonance. *Pharm. Res.* **2006**, 23, 2129-2140.
21. Geppi, M.; Guccione, S.; Mollica, G.; Pignatello, R.; Veracini, C. A. Molecular properties of ibuprofen and its solid dispersions with Eudragit RL100 studied by solid-state nuclear magnetic resonance. *Pharm. Res.* **2005**, 22, 1544-1555.
22. Van den Mooter, G.; Samyn, C.; Kinget, R. Characterization of colon-specific azo polymers: A study of the swelling properties and the permeability of isolated polymer films. *Int. J. Pharm.* **1994**, 111, 127-136.

23. FDA, S. Guidance for Industry: SUPAC-MR: Modified Release Solid Oral Dosage Forms; Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls. *In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* **1997**.
24. Masuda, K.; Tabata, S.; Kono, H.; Sakata, Y.; Hayase, T.; Yonemochi, E.; Terada, K. Solid-state ^{13}C NMR study of indomethacin polymorphism. *Int. J. Pharm.* **2006**, 318, 146-153.
25. Ukmar, T.; Kaucic, V.; Mali, G. Solid-state NMR spectroscopy and first-principles calculations: a powerful combination of tools for the investigation of polymorphism of indomethacin. *Acta Chim. Slov.* **2011**, 58(3), 425-433.
26. Kranz, H.; Gutsche, S. Evaluation of the drug release patterns and long term stability of aqueous and organic coated pellets by using blends of enteric and gastrointestinal insoluble polymers. *Int. J. Pharm.* **2009**, 380, 112-119.
27. Mehta, R.; Teckoe, J.; Schoener, C.; Workentine, S.; Ferrizzi, D.; Rajabi-Siahboomi, A. Investigation into the Effect of Ethylcellulose Viscosity Variation on the Drug Release of Metoprolol Tartrate and Acetaminophen Extended Release Multiparticulates-Part I. *AAPS PharmSciTech.* **2016**, DOI: 10.1208/s12249-015-0465-z