

## **2. MATERIALS AND METHODS**

### 2.1. MATERIALS

#### Model drugs

Theophylline (Synopharm GmbH, Barsbüttel, Germany; unretarded pellets (94 % drug content): Klinge Pharma GmbH, München, Germany), chlorpheniramine maleate (CPM) (STADA GmbH, Bad Vilbel, Germany), acetylic salicylic acid (ASS), paracetamol (Knoll AG, Ludwigshafen, Germany)

#### Polymers

Zein (grade F 4000 and F 6000) (Kaul GmbH, Elmshorn, Germany), shellac (SSB Renschel GmbH, Bremen, Germany), HPMC (Methocel E5, Colorcon Ltd., Kent, UK), low viscosity Na-alginate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), ethylcellulose (Ethocel Standard premium 10 or Standard premium 10 FP and 7 FP, DOW Chemical Company, Midland, USA), Eudragit<sup>®</sup> RS (Roehm GmbH, Darmstadt, Germany), Surelease<sup>®</sup> (aqueous ethylcellulose dispersion plasticised with MCT (~20 % w/w), Colorcon Ltd., Kent, UK), Aquacoat<sup>®</sup> ECD (unplasticised aqueous ethylcellulose dispersion, FMC BioPolymer, Newark, USA).

#### Plasticisers

glycerol, castor oil, sorbic acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), propylene glycol (BASF AG, Ludwigshafen, Germany), triethylcitrate (TEC), tributylcitrate (TBC), acetyltriethylcitrate (ATEC), acetyltributylcitrate (ATBC) (Morflex Inc., Greensboro, NC, USA), medium chain triglycerides (MCT 812) (Sasol Germany GmbH, Witten, Germany), glycerol, polyethylene glycol (PEG 400, PEG 1500) (BASF AG, Ludwigshafen, Germany), tartaric acid (Jungbunzlauer GmbH, Ladenburg, Germany), castor oil (Caelo), acetylated monoglycerides (AMG) (Myvacet 9-45, Quest International, Zwijndrecht, Netherlands), dibutyl sebacate (DBS), acetyldibutyl citrate (ATBC) and tributyl citrate (TBC) (Morflex Inc., Greensboro, USA).

### Other excipients

talc (Luzenac Val Chisone S.p.A., Pinerolo (TO), Italy), pepsin and pancreatin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), sorbic acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), aspartic acid (Merck KGaA, Darmstadt, Germany), polysorbate (Tween<sup>®</sup> 20, Tween<sup>®</sup> 80) (Carl Roth GmbH, Karlsruhe, Germany), sorbitan monooleate (Span 80) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), ethylene oxide - propylene oxide block copolymer (Pluronic<sup>®</sup> F68, Pluronic<sup>®</sup> F127) (BASF AG, Ludwigshafen, Germany), sodium dodecyl sulphate (SDS ultra pure) (Carl Roth GmbH, Karlsruhe, Germany), nonpareil beads (NP Pharma S.A.S., Bazainville, France).

## 2.2. METHODS

### 2.2.1. Plasticiser evaluation

10 ml of a 10 % (v/v) zein solution in ethanol (80 % v/v) or aqueous zein dispersion was mixed with 0-40% w/w plasticiser, based on the formulation, on a magnetic stirrer and cast into glass petri dishes ( $\varnothing$  5 cm) with Teflon<sup>®</sup> foil covered bottoms. The films were dried at room condition or the indicated temperature for 24 h and examined with regard to their appearance, flexibility, shrinkage and stickiness.

### 2.2.2. Differential scanning calorimetry (DSC)

DSC-measurements (Mettler Toledo DSC 821e, Schwerzenbach, Switzerland) were performed on samples of 5-10 mg in the range of  $-100$  to  $200$  °C at a heating rate of  $10^{\circ}\text{C}/\text{min}$  ( $\text{N}_2$ -atmosphere:  $80$  l/min). The glass transition temperature ( $T_g$ ) was evaluated with the Stare-software version 6.01 (Mettler Toledo, Schwerzenbach, Switzerland; calibration with indium and zink). Zein films were stored over silica gel or at different relative humidities (r.h.) for 5 d prior to measurement to achieve different water contents. The relative humidity (r.h.) were controlled by saturated salt solutions (KCH<sub>3</sub>COO: 22% r.h.; NaCl: 75% r.h.; ZnSO<sub>4</sub>: 85% r.h.; pure water: 100% r.h.)

The predicted  $T_g$ -values were calculated with the Gordon-Taylor-equation (Gordon and Taylor, 1952) according:

$$T_g(\text{predicted}) = \frac{w_1 \cdot T_{g1} + k \cdot w_2 \cdot T_{g2}}{w_1 + k \cdot w_2}$$

with  $w_1$  and  $T_{g1}$  as the weight fraction and glass transition temperature of zein and  $w_2$  and  $T_{g2}$  the respective values for water. The  $T_g$  of dry zein is  $451.9$  K and of water  $139.15$  K (Beck et al., 1996; de Graaf et al., 1993).

The constant  $k$  can be calculated from the densities of the two components by:

$$k = \frac{\rho_1 \cdot Tg_1}{\rho_2 \cdot Tg_2}$$

For the zein-water system  $k$  is 5.1 (de Graaf et al., 1993).

### 2.2.3. Zein-shellac compatibility

A 10% (w/v) zein solution in ethanol (80% v/v) was mixed with varying amounts of a 10% (w/v) shellac solution in ethanol (80% v/v) on a magnetic stirrer and cast into glass petri dishes where the bottom was covered by a Teflon<sup>®</sup> foil. The films were dried at room condition for 24 h and examined for their appearance.

### 2.2.4. Preparation of aqueous dispersions of zein

Four different methods were used for the preparation of aqueous zein dispersions:

Method 1 and 2 were prepared by use of an ethanolic (80% v/v) solution of zein (5% w/w). The polymer solution was introduced into an aqueous phase (volume ration 1:1) by a syringe either by the use of a needle ( $\varnothing$  0.45 mm) (method 1) or without a needle (method 2), whereby the aqueous phase was stirred at high speed by a magnetic stirrer (Heidolph Instruments GmbH & Co.KG, Schwabach, Germany, stirring speed: 750 rpm).

Method 3 started with an organic polymer solution of zein, to which water was added in 1 ml-steps. After a certain water volume added, phase inversion occurred and the appearance of the system changed from a transparent yellow solution to a slightly yellow milky dispersion. For method 1-3, the solvent and parts of the aqueous phase were subsequently evaporated by further stirring, thus the polymer concentration increased. The exact polymer concentration was determined gravimetrically after drying of 5 ml aqueous dispersion in an aluminium pan at 105 °C for 2 h.

Method 4 used zein as a powder. The zein powder (5 g) was dispersed directly into the aqueous phase (95 ml). Particle size reduction was performed by 5 min ultra turrax treatment at 12000 rpm (Ultra Turrax T25, Jahnke & Kunkel GmbH, IKA® Labortechnik, Staufen, Germany).

### **2.2.5. Particle size measurements**

The mean particle size of aqueous zein dispersions after preparation was evaluated in demineralised water by laser light scattering including polarized intensity differential scattering (PIDS) technology (Coulter LS 230, small volume module, Coulter Electronics, Krefeld, Germany). The relative frequency of the diameter of the particles was shown by the calculation based on volume distribution.

### **2.2.6. Surface tension measurements**

The plasticiser was added to the aqueous phase in the indicated amount, mixed by a magnetic stirrer and the surface tension was measured by a ring-tensiometer (Interfacial Tensiometer K 8600, KRÜSS GmbH, Hamburg, Germany). Then the aqueous dispersion was prepared according the standard method as described above.

### **2.2.7. Zeta-potential measurements**

The zeta-potential of the prepared aqueous zein dispersions was measured after dilution with demineralised water and adjustment of the pH and conductivity to 50  $\mu\text{S}/\text{cm}$  (with NaCl) (Malvern Zetasizer 4, 7032 Multi-8-Correlator, Malvern Instruments, Herrenberg, Germany). The measured electrophoretic mobility at electric field strengths of 20 mV/cm was converted into the zeta-potential by the Helmholtz-Smoluchowski equation.

### **2.2.8. Stabilisers for aqueous zein dispersions**

To evaluate the stabilizing ability against agglomeration of different additives, the substances were added to the aqueous dispersions after preparation. The dispersions were up-concentrated by evaporating the dispersing medium. The polymer concentration was determined gravimetrically whereas the corresponding particle size was measured by laser light scattering. The highest polymer concentration with unchanged particle size is given.

### **2.2.9. Centrifugation**

1 ml aqueous dispersion was centrifuged in an eppendorf vial (Heraeus Biofuge 22R, Sepatech GmbH, Germany; insert for eppendorf vials HFA 22.2 no.3042) at 3000 rpm for 15 min at 25 °C. The volume of the supernatant after centrifugation was determined.

### **2.2.10. Lyophilisation**

Aqueous zein dispersions were lyophilised after preparation in a lab-scale freeze-dryer (Christ Alpha I-5, Christ Medizinischer Apparatebau, Osterode/Harz, Germany). The dispersion was frozen in a deep freezer (Gallenkamp Super Cold 85, Haake Messtechnik GmbH, Karlsruhe Germany) at -80 °C and lyophilised under vacuum (0.02 mbar) for 48 h.

### **2.2.11. Spray-drying**

Aqueous zein dispersions after preparation or ethanolic zein solutions (5% w/v in 80 % ethanol) were spray-dried on a lab-scale spray-dryer (Büchi 190 Mini Spray Dryer, Laboratoriumstechnik AG, Flowil, Switzerland) at following conditions: inlet air: 70±1 °C, outlet air: 50±1 °C, flow indicator: 600 norm.l/h, aspirator position: 6, atomising air pressure: 45 mbar, spray rate: 3 g/min.

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Aquacoat<sup>®</sup> (containing 20 % MCT, based on the polymer, plasticisation: 24 h stirring at room condition on a magnetic stirrer (IKA Combimag RCT; Jahnke&Kunkel GmbH, Staufen, Germany)) or Surelease<sup>®</sup> were diluted 1:1 with deionised water and spray-dried at following conditions: inlet air: 105±3 °C, outlet air: 59±2 °C, atomising pressure: 19 mbar, flow indicator: 600 norm l/h, aspirator positioning: 6, spray rate: 4.5 g/min.

### 2.2.12. Redispersion

200 mg lyophilised or spray-dried zein powder were added to 2 ml dispersion medium (water or phosphate buffer pH 7.4 (Ph.Eur)) in a small glass vials and stirred on a magnetic stirrer (Variomag Electronicrührer Multipoint HP, H+P Labortechnik GmbH, Oberschleißheim, Germany) for 10 min at 750 rpm. The particle size distribution was measured by laser light scattering.

### 2.2.13. Micronised polymer powders

Ethylcellulose is commercially available as a fine powder of micron-sized range (DOW, 1996a; b), whereas Eudragit<sup>®</sup> RS was milled to the micron-size range size (Axiva GmbH, Frankfurt, Germany).

Plasticised ethylcellulose was obtained by mixing the standard ethylcellulose powder with the plasticiser (20 % w/w, based on the polymer amount) in a planetary mixer (Kitchen Aid Ultra Power, Michigan, USA; speed position: 8) for 30 min. The mixture was then hot melt extruded (HME) at 140 °C (Haake Minilab, Thermo Electron GmbH, Karlsruhe, Germany; co-rotating twin screws, torque: 20±5 Ncm, screw speed: 30 rpm, force feeder speed: 50 rpm, die: 1 mm diameter). The extrudate was cut into pieces of 2-3 cm and cryogenically milled on a high-speed rotor mill under cooling with liquid nitrogen in two steps with sieve inserts of 0.75 mm and 0.12 mm grain size at a rotor speed of 10000 rpm (Rotor Speed Mill Pulverisette 14, Fritsch GmbH, Idar-Oberstein, Germany)



### 2.2.14. Drug layering of pellets

A solution of 90 g CPM and PEG 400 (0.42 g) were dissolved in 168.75 g (96 % v/v) ethanol and 112.5 g water. Thereafter, 42.2 g of an aqueous 10 % w/w HPMC solution was added. Drug-loaded pellets (10 % w/w, drug loading) were prepared by layering the drug-binder solution onto 900 g nonpareil beads (710-850  $\mu\text{m}$ ) using a fluidized bed ball coater (Hüttlin<sup>®</sup> HKC-05, Steinen, Germany). The drug-layering conditions were: inlet air temperature, 50°C; product temperatures, 42°C-45°C; spray rate, 4-5 g/min; atomizing air pressure, 0.2/0.4 bar; spray nozzle diameter, 0.8 mm.

### 2.2.15. Coating of solid dosage forms

#### a. Pure zein and zein-shellac mixed coatings

Drug containing pellets were coated in a fluidized bed ball coater (Hüttlin<sup>®</sup> HKC-05, Steinen, Germany) up to 20 % coating level at following conditions: inlet air temperature: 31 $\pm$ 1 °C, product air temperature: 29 $\pm$ 2 °C, outlet air temperature: 30 $\pm$ 2 °C.

Tablets:

Drug containing tablets were coated in a drum coater (Glatt Lab-Coater GC 300, Pratteln, Switzerland) up to a coating level of 10 % at: inlet air temperature: 45 $\pm$ 1 °C, outlet air temperature: 35 $\pm$ 2 °C and pan speed of 21 rpm.

The coating solution contained zein (10 % w/w) in ethanol (80 % v/v) with 10 % (w/w) propylene glycol based on the polymer amount. As antitacking-agent 30 % talc based on the polymer amount was added.

For coating with aqueous zein dispersions the required amount of the dispersion was prepared the day prior the coating was performed. After evaporation of the solvent after the preparation of the aqueous dispersion the exact polymer content was determined and 30 % (w/w) tartaric acid as plasticiser based on the polymer amount added. 30 % (w/w) talc as antitacking-agent was added based on the polymer amount. Theophylline pellets were

coated in a fluidized bed ball coater (Hüttlin® HKC-05, Steinen, Germany) (inlet air temperature:  $47\pm 2$  °C, product air temperature:  $38\pm 2$  °C, outlet air temperature:  $42\pm 2$  °C).

### **b. Shellac topcoated pellets**

Pellets containing the model drug were coated in a fluidized bed ball coater (Hüttlin® HKC-05, Steinen, Germany) with the polymers dissolved in 70%(v/v) ethanol at following conditions: inlet air temperature:  $31\pm 1$  °C, product air temperature:  $28\pm 2$  °C, outlet air temperature:  $30\pm 2$  °C at a spray rate of 3.5-5 g/min. The coating solution contained 10% (w/w) propylene glycol as plasticiser and 30% (w/w) talc as antitacking agent based on the amount of polymer. After coating the pellets were cured at 40, 60 or 80 °C for the indicated time period.

### **c. Dry powder coating**

CPM-loaded pellets were dry powder coated in a fluidized bed ball coater (Hüttlin® HKC-05, Steinen, Germany). This coater has two nozzles from which one was used to introduce the micronised EC powder by a volumetric helix dosing feeder (Secudos, G+K Fuchs GmbH, Wiehl, Germany). The plasticiser was supplied by a peristaltic pump (Modell 323S/D/X, Watson+Marlow GmbH, Rommerskirchen, Germany) and sprayed simultaneously through the second (nozzle diameter: 0.6 mm). For the coating with Eudragit® RS the polymer was used in a 1:1 mixture with talc, to prevent agglomeration and sticking of the pellets during coating. The process conditions are summarised in Table 2. The pellets were cured after coating at the indicated conditions.

**Table 2** Process parameters for dry powder coating with micronised polymer powders

<b>Parameter</b> \ <b>System</b>	<b>EC 10 cp</b>	<b>EC 7 cp</b>	<b>Eudragit® RS</b>	<b>Pre-plasticised EC</b>
Inlet air temperature, °C	50 ± 1	50 ± 1	42 ± 2	42 ± 2
Outlet air temperature, °C	51 ± 1	51 ± 2	45 ± 2	47 ± 1
Product air temperature, °C	44 ± 1	44 ± 2	38 ± 4	36 ± 2
Powder feeding rate, g/min	10	10	10	12
Plasticiser feeding rate, g/min	5	5	5	3

### 2.2.16. Drug release from coated pellets

Drug release was determined according to Ph.Eur. in a paddle apparatus (VK700, Vankel Industries, Edison, NJ, USA) in simulated gastric medium of pH 1.2 (Ph.Eur., HCl-solution ± 3.2 g/l pepsin ) or in simulated intestinal medium of pH 6.8 (Ph.Eur. phosphate buffer ± 5 g/l pancreatin) in 900 ml medium at 37 °C and 100 rpm. Samples were taken at predetermined time points and the drug content quantified spectrophotometrically (UV-2101 PC, Shimadzu Scientific Instruments, Columbia, MD, USA) at following wavelengths: theophylline: 270 nm, CPM: 264 nm, ASS: 227 nm, salicylic acid: 302 nm, paracetamol: 241 nm. For long-term stability the coated pellets were stored at ambient or accelerated conditions (40 °C/75 % r.h.).

### 2.2.17. Swelling of isolated zein films and coated pellets

Thin isolated films were casting from 10 % (w/w) ethanolic zein solution with a casting knife (Multicator 411, Erichsen, Hemer, Germany) and dried on Teflon® plates at room conditions. Film-specimen of approximately 2x2 cm<sup>2</sup> were incubated at 37 °C in 200 ml medium in a horizontal incubation shaker (GFL3032, Gesellschaft für Labortechnik mbH, Burgwedel, Germany) at two osmolality levels: low (~100 mosmol/kg) and high (~400 mosmol/kg). The

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investigated media were pH 1.2 chloride buffer (HCl-solution; low osmolality; high osmolality adjusted with NaCl), pH 4.5 acetate buffer (prepared with NaCH<sub>3</sub>COO and acetic acid), pH 4.5 phosphate buffer (Ph.Eur., prepared with KH<sub>2</sub>PO<sub>4</sub>) pH 6.8 phosphate buffer (Ph.Eur., prepared with KH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub>); pH 6.8 chloride medium (low osmolality: purified water with NaCl).

The films were fixed within the incubation-vessels during the experiment to avoid folding or sticking. Specimen of the films were taken after predefined time points, blotted gently by a tissue paper to remove excess medium and examined gravimetrically. The weight increase was calculated as:

$$\text{weight increase} = \frac{m_{\text{wet}} - m_{\text{dry}}}{m_{\text{dry}}} * 100 [\%]$$

with  $m_{\text{wet}}$  = mass of the wet film and  $m_{\text{dry}}$  = initial mass of the dry film (n=3).

The films did not contain plasticiser or any other water soluble components, therefore leaching was negligible.

Coated pellets were incubated in pH 1.2 in a horizontal shaker at 37 °C. The pellets were observed by a microscope and their size determined after predefined time points. The increase in diameter was calculated as:

$$\text{diameter increase} = \frac{d_{\text{wet}} - d_{\text{dry}}}{d_{\text{dry}}} * 100 [\%]$$

with  $d_{\text{wet}}$  = diameter of the swollen pellet and  $d_{\text{dry}}$  = initial diameter of the dry pellet (n=5).

### 2.2.18. Viscosity measurements

Ethylcellulose, 1 or 2.5 % w/w, was dissolved in 10 ml plasticiser on a magnetic stirrer (Variomag Electronicrührer Multipoint HP, H+P Labortechnik GmbH, Oberschleissheim, Germany). The viscosity of the plasticisers and of the ethylcellulose- plasticiser solutions was measured with a rotational viscosimeter (cone-plate setup, cone: C60/1°, cone-plate gap: 0.052 mm, Haake Rheostress RS100, Thermo Electron GmbH, Karlsruhe, Germany) at a

shear rate of 60/s for 60s (data acquisition: 2 values/s). The relative viscosity  $\eta_{rel}$  was calculated as follows:

$$\eta_{rel} = \frac{\eta(\text{ethylcellulose} - \text{plasticizer solution})}{\eta(\text{plasticizer})}$$

### 2.2.19. Mechanical properties of films

Zein films were cast from 10% (w/w) ethanolic solution with a casting knife (Multicator 411, Erichsen, Hemer, Germany) and dried on Teflon<sup>®</sup> plates at room conditions. The thickness of the films was 100-160  $\mu\text{m}$  (Minitest 600, Erichsen, Hemer, Germany). Films with a higher water content were prepared by storage at 100% r.h. for 5 d or incubation directly in pH 1.2 medium for 2 h at 37 °C prior measurement.

Ethylcellulose films mixed with 20 or 40 % w/w plasticiser (based on the formulation) were cast from 10 % w/w chloroform solutions (10 ml) at 1 mm thickness with a casting knife (Multicator 411, Erichsen, Hemer, Germany) and dried on Teflon<sup>®</sup> plates at room conditions. The thickness of the films was 100-150  $\mu\text{m}$ .

Mechanical properties of films were evaluated by a puncture test with a texture analyzer (TAXT Plus, Winopal Forschungsbedarf GmbH, Ahnsbeck, Germany). The film specimen were mounted on a holder (n=6) and the puncture probe (spherical end:  $\varnothing$  5 mm) was driven down by the load cell (5 kg) with a cross-head speed of 0.1 mm/s. Load vs. displacement curves were recorded until the rupture of the film and used to calculate the following parameters:

$$\text{Puncture strength} = \frac{F}{A} \quad [MPa]$$

$$\text{Elongation at break} = \frac{\sqrt{R^2 + D^2} - R}{R} * 100 \quad [\%]$$

$$\text{Energy at puncture} = \frac{AUC}{V} \quad [MJ / m^3]$$

$$\text{Modulus at puncture} = \frac{\Delta\left(\frac{F}{A}\right)}{\Delta(\% \textit{ elongation})} \text{ [MPa]}$$

with: A = the cross-sectional area of the film

R = radius of the film

V = volume of the film, located in the cylindrical opening of the film holder

F = load required for puncture

D = displacement of the probe from the point of contact until puncture

AUC = area under the load vs. displacement curve

### 2.2.20. Dynamic mechanical analysis (DMA)

Plasticised ethylcellulose films with 20 or 40 % w/w plasticiser (based on the formulation) were prepared as described above and used for DMA-measurements, where the application of a sinusoidal force resulted in a small deformation of 10  $\mu\text{m}$  at a frequency of 3.3 Hz (DMA 242C, Netzsch Gerätebau GmbH, Selb, Germany). The amplitude of the deformation, the force and the phase shift were measured on ethylcellulose-films by the use of tension clamps in the range of 0-150 °C. The measurement parameters enabled the calculation of the storage and loss modulus as a function of temperature.

### 2.2.21. Dynamic vapour sorption (DVS) measurements

DVS-measurements (DVS1000, Surface Measurement Systems Ltd., Alperton, UK) were conducted after an initial drying of the sample (sample size  $\sim 10$  mg) at 0 % r.h. for 6 h. Thereafter the vapour sorption of the sample was determined by increasing the r.h. in a ramp mode from 0-98%r.h. at a rate change of 10 % r.h./h.

**2.2.22. Optical and environmental scanning electron microscopy (ESEM)**

The particle size of micronised ethylcellulose powders was measured by an optical microscope (magnification: 20x; Axioscop, Carl Zeiss Jena GmbH, Jena, Germany; n=200).

The mean particle size and standard deviation was calculated.

Coated pellets were examined without further conditioning in an Environmental Scanning Electron Microscope (ESEM, FEI XL30, Eindhoven, The Netherlands) without the necessity to coat a conductive metal film onto the surface. To prevent the samples from charging during electron exposure a partial pressure of water vapour between 0.2 and 0.8 mbar was applied. Imaging was performed using acceleration voltages between 4 and 16 kV.

