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Research paper

In-vitro and in-vivo evaluation of enteric-coated starch-based pellets prepared via extrusion/spheronisation

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Abstract

Pellet cores containing modified starch (high-amylose, crystalline and resistant starch) as the main excipient were enteric-coated with an Eudragit® L30 D-55 based dispersion. The polymer weight gain was from 15% to 30% (w/w). Pellet cores were prepared using piroxicam (2.5% w/w, poor water solubility) and anhydrous theophylline (2.5% and 25% w/w, coarse and micronised powder, medium water solubility) as model drugs. Next to the water solubility, particle size and concentration of the model drugs, the influence of sorbitol (0% and 10%, w/w) and drying method (oven and fluid-bed) on pellet yield, size (Feret mean diameter), sphericity (aspect ratio, AR and two-dimensional shape factor, e_R), friability, surface morphology and drug release were evaluated. Binder (HPMC) and granulation liquid (water) concentration were optimised to obtain maximum yield (size fraction between 900 and 1400 μm) and acceptable sphericity (AR < 1.2). Pellet friability was <0.01% for all formulations, while the mean pellet diameter was lower for pellets with sorbitol and the ones dried in an oven. Mercury intrusion porosimetry combined with scanning electron microscopy revealed an influence of drying method and sorbitol level on the surface structure: the surface of fluid-bed dried pellets without sorbitol and with 2.5% of model drug was cracked, which correlated with a Hg-intrusion peak at the 6–80 μm pore size range. Due to improved mechanical properties of the wet mass, sorbitol addition smoothened the pellets as the main peak of Hg-intrusion shifted to a smaller pore size range. Using a higher drug concentration and micronised theophylline shifted the main peak of Hg-intrusion further towards the smaller pore size range. Oven-dried pellets showed no Hg-intrusion and no cracks were observed. When applying the highest coating thickness (30% weight gain), all theophylline pellet formulations were successfully coated (<10% drug release after 2 h in acid dissolution medium), while pellets with the lowest coating thickness (15% weight gain) released from 5% to about 30% theophylline. The extent of drug release depended on the pellet composition and drying method as these factors determined the surface properties. Piroxicam release in acid medium was less than 1% irrespective of the surface characteristics, due to its poor water solubility. In basic medium (phosphate buffer, pH 6.8) all pellets released the drug in less than 45 min. The bioavailability of coated and uncoated piroxicam pellets was determined after oral administration to six dogs. Values of $AUC_{0\rightarrow 72h}$, C_{max} and t_{max} after oral administration of piroxicam pellets to dogs were not significantly different from the values obtained for immediate release capsules ($P > 0.05$).

Keywords: Extrusion/spheronisation; Pellets; Starch; Enteric-coating; Bioavailability

1. Introduction

Due to their multiparticulate nature, pellets are mainly coated in order to either sustain drug release or to deliver a drug to the specific absorption site in the gastro-intestinal tract (e.g. enteric-coated or colon-targeted drug delivery).

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Enteric-coated pellets as dosage forms are especially suited for administration of drugs which are not stable in gastric fluids or can cause irritation of gastric mucosa and which are absorbed in the duodenum or upper intestine [1]. After the acid-resistant coating has dissolved in the intestine, immediate drug release is essential, but this can be difficult to obtain if a poorly water-soluble drug is formulated in microcrystalline cellulose pellets due to the lack of disintegration of these pellets [2].

It was recently demonstrated that using a specific type of modified starch as the main excipient in pellet formulations promoted pellet disintegration and consequently fast dissolution of poorly soluble drugs [3]. This modified starch grade (UNI-PURE®EX starch, National Starch and Chemical Co., Bridgewater, NJ, USA) was introduced by the same authors as an alternative to microcrystalline cellulose (MCC) for production of pellets by means of extrusion and spheronisation [3,4]. UNI-PURE®EX starch is obtained by enzymatic debranching of amylose-rich starch, followed by retrogradation [5], which provides crystallinity (double-helical chain structure) and resistance to α-amylase in the small intestine (resistant starch).

The aim of this study was to produce enteric-coated pellet formulations with modified starch as the main excipient, using piroxicam (poor water solubility) or theophylline anhydrous (medium water solubility) as model drugs. Next to the drug water solubility, the influence of pellet core composition (drug load, sorbitol level) and drying method on the quality of the coating has been evaluated. Finally, an in-vivo study in dogs was performed to compare piroxicam plasma levels after oral administration of enteric-coated and uncoated starch-based piroxicam pellets to the plasma levels after administration of a commercially available piroxicam capsule formulation.

2. Materials and methods

2.1. Materials

Piroxicam ($D[v, 0.5] = 9.9 \pm 0.6$ μm, $n = 3$; Sagran, Milan, Italy) and anhydrous theophylline (coarse ($D[v, 0.5] = 157.9 \pm 3.7$ μm, $n = 3$ (Roig Farma, Terrassa, Spain) and micronised grade ($D[v, 0.5] = 19.2 \pm 0.5$ μm, $n = 3$ (Bufa, Uitgeest, The Netherlands)), were used as model drugs with poor and medium aqueous solubility, respectively. The main excipient was UNI-PURE®EX starch (high-amylose, crystalline and resistant starch), donated by National Starch and Chemical Co. (Bridgewater, New Jersey, USA). Hydroxypropylmethylcellulose (HPMC) (Methocel® E15 LV EP Pharm), donated by Colorcon (Dartford, UK), was used as binder and sorbitol (Sorbidex® P 16616, Cerestar, Vilvoorde, Belgium) was added to modify the consistency of the wet mass. Demineralised water was used as granulation liquid. Microcrystalline cellulose (MCC, Avicel® PH 101, FMC, Cork, Ireland) was used to prepare reference pellets.

Pellet cores were coated with a 30% aqueous dispersion of methacrylic acid and ethylacrylate copolymer (1:1) (Eudragit® L30 D-55, Röhm, Darmstadt, Germany) used as acid-resistant film-forming polymer in combination with triethylcitrate (Sigma–Aldrich Chemie, Steinheim, Germany) as plasticizer, polysorbate 80 (Tween® 80, Alpha Pharma, Nazareth, Belgium) as wetting agent and glycerol monostearate (Federa, Braine-l'Alleud, Belgium) as glidant.

For determination of piroxicam in dog plasma, meloxicam (Boehringer Ingelheim, Ingelheim, Germany) was used as internal standard, acetonitrile and methanol were obtained from Biosolve (Valkenswaard, The Netherlands), triethylamine and acetic acid were purchased from Sigma–Aldrich Chemie (Steinheim, Germany) and finally, diethylether and hydrochloric acid 37% from WVR International (Leuven, Belgium). All solvents and reagents were of HPLC-grade.

2.2. Methods

2.2.1. Experimental set-up

Table 1 lists the formulation variables of the performed experiments. Pellets were prepared using two model drugs: piroxicam (2.5% w/w) and anhydrous theophylline (2.5% and 25% w/w). To evaluate the influence of drug particle size, additional pellets were prepared using micronised anhydrous theophylline in two concentrations (2.5% and 25% w/w). Based on preliminary experiments, a binder was added in a concentration depending on the drug level: 7% and 5% w/w HPMC (Methocel® E15 LV) at a drug load of 2.5% or 25% w/w, respectively. Each drug formulation was prepared without or including sorbitol (10% w/w, dry mass). In order to obtain maximum process yield and acceptable pellet sphericity, optimal water content (% w/w wet mass, determined by preliminary experiments) was used in the wet massing step, followed by extrusion/spheronisation. Pellets were dried either in an oven or fluid-bed drier. Other process parameters were the same for all batches and were selected based on previous studies with UNI-PURE®EX starch [3,4]. The drug release from enteric-coated starch-based pellets was compared to the release from coated pellets containing microcrystalline cellulose as the main excipient.

2.2.2. Pellet production

A uniform dry powder mixture (batch size: 250 g) containing model drug and excipients was obtained by mixing in a Turbula® mixer (model T2A, W.A. Bachofen, Basel, Switzerland) for 15 min. Water was added during first 30 s of the granulation phase, performed by means of a planetary mixer (Kenwood Chief, Hampshire, UK) during 10 min and with a mixing speed of 60 rpm. To ensure uniform water distribution during granulation, the material was repeatedly scraped from the mixing bowl walls. The wet mass was extruded at a speed of 50 rpm using a single screw extruder (Dome extruder lab model DG-L1, Fuji Paudal, Tokyo, Japan) equipped with a dome-shaped

Table 1
Overview of the formulation variables used in the experimental set-up

Formulation	Model drug	Drug conc.[a]	Sorbitol conc.[a]	HPMC conc.[a]	Water content[b,c]
Px-0	Piroxicam	2.5	0	7	50.0
Px-10			10		45.2
TC-2.5-0	Theophylline anhydrous (coarse)	2.5	0	7	49.5
TC-2.5-10			10		44.5
TC-25-0		25	0	5	44.0
TC-25-10			10		38.0
TM-2.5-0	Theophylline anhydrous (micronised)[d]	2.5	0	7	49.5
TM-2.5-10			10		44.5
TM-25-0		25	0	5	44.7
TM-25-10			10		38.5

[a] % (w/w, dry mass).
[b] % (w/w, wet mass).
[c] Water content has been optimised in order to obtain maximal pellet yield and sphericity.
[d] Pellets with micronised theophylline were only dried in fluid-bed.

extrusion screen (thickness: 1.2 mm, perforation diameter: 1 mm). The extrudates were spheronised at 850 rpm during 3 min in a spheroniser with a cross-hatched friction plate (Caleva Model 15, Caleva, Sturminster Newton, Dorset, UK) and a diameter of 38.1 cm. Wet pellets were dried for 24 h at 40 °C in an oven or for 20 min at 50 °C (piroxicam) and 60 °C (theophylline) in a fluid-bed (GPCG1, Glatt, Binzen, Germany).

2.2.3. Coating of pellets

Three subbatches of pellets were combined to obtain 400 g pellets of the 900–1400 μm size fraction for coating trials using a bottom-spray fluid-bed coating technique with Wurster insert (GPCG1, Glatt, Binzen, Germany). A coating dispersion containing 15.3% dry polymer was prepared. Triethylcitrate (final concentration in coating suspension: 3.1% w/w; 20% w/w on polymer weight), 33% aqueous solution of polysorbate 80 (1.6% w/w) and water were mixed and heated to 70–80 °C (i.e. above the melting point of glycerol monostearate). Glycerol monostearate (1.3% w/w, suspension weight) was added to this solution and homogenised for 10 min by means of a rotor–stator mixer (Silverson, Bucks, UK). The dispersion was left to cool down to room temperature while mixing with a magnetic stirrer. After cooling, the glycerol monostearate dispersion was added to an aqueous pseudolatex dispersion of Eudragit® 30L D-55 and gently stirred with a magnetic stirrer for at least 30 min to stabilize the dispersion before starting the coating process. The dispersion was further gently mixed throughout the entire coating process. Prior to suspension spraying, pellets were pre-heated to 23–26 °C. The coating dispersion was sprayed at a rate of 4.0–4.5 g/min, through a 0.8 mm nozzle using an atomizing air pressure of 1.5 bar. The inlet air temperature was set between 30 and 33 °C in order to maintain product temperature between 25 and 26 °C. After coating, pellets were dried at the same product temperature for 15 min. Pellets were coated until 10%, 15%, 25% and 30% of dry polymer weight gain were obtained.

2.2.4. Pellet characterisation

Pellet cores were characterised by process yield (900–1400 μm fraction), pellet size (Feret diameter), sphericity (aspect ratio, AR and two-dimensional shape factor, e_R) and friability as previously described by Dukić-Ott et al. [3].

2.2.4.1. Raman spectroscopy. Pellets containing 25% (w/w) theophylline (with and without sorbitol, dried in an oven and fluid-bed) were evaluated by Raman microscopy in order to evaluate the hydration state of theophylline. A RamanRxn 1 Microprobe (Kaiser Optical Systems, Ann Arbor, USA) equipped with an air-cooled CCD detector (back-illuminated deep depletion design) was used to inspect the pellet surface and core. Per pellet, five spectra, each representing a different place, were collected on the surface and inside of the pellet using a 10× long working distance objective lens (spot size laser = 50 μm). The laser wavelength during the experiments was the 785 nm line from a 785 nm Invictus NIR diode laser. All spectra were recorded at a resolution of 4 cm^{-1} using a laser power of 400 mW and a laser light exposure time of 5 s per spectrum. Before data analysis, spectra were baseline-corrected and normalized. Data collection and analysis were done using the HoloGRAMS™ data collection software package, the HoloMAP™ data analysis software package and the Matlab® software package (version 6.5).

2.2.4.2. Mercury intrusion porosimetry. Mercury intrusion porosimetry was used for determination of pore size distribution of uncoated pellets. Prior to measurement, pellet cores were dried in an oven at 40 °C for a minimum of 72 h, in order to minimise residual water in the pellets and facilitate the evacuation phase. Mercury porosimetry was performed using an AutoPore III (Micromeritics Instrument, Norcross, Georgia, US). Sample size (from 0.7 to 1.7 g) was adjusted in order to use 20–80% of the stem volume. The sample was evacuated to 6.67 kPa, followed by low-pressure mercury intrusion in a pressure

range from 3.4 to 193 kPa, with a mercury filling pressure of 3.4 kPa, maximal intrusion volume of 0.001 mL/g and equilibration time of 10 s. High-pressure mercury intrusion was performed in a pressure range from 0.193 to 71 MPa (due to pellet compression when applying higher pressures than 71 MPa [6]), using the same maximal intrusion volume and equilibration time. Measurements were performed in duplicate for each sample.

2.2.4.3. Dissolution profiles. The dissolution tests were performed using the USP apparatus (VK 8000, VanKel, New Jersey, USA) with paddles (piroxicam pellets) or baskets (theophylline pellets, since they floated) at a rotational speed of 100 rpm, in 900 mL dissolution medium at 37 °C. For enteric-coated pellets, acidic dissolution medium (0.1 N HCl) was used during the first 2 h, followed by 1 h in pH 6.8 phosphate buffer (PB, USP 27). The pellet amount used for analysis was adjusted to obtain sink conditions: for piroxicam pellets, the sample size was set to 72 mg, equivalent to 1.8 mg of piroxicam (maximum piroxicam solubility values are 43.8 and 112.5 μg/mL in 0.1 N HCl and PB pH 6.8, respectively); the sample size of theophylline pellets was set to 300 mg, equivalent to 7.5 and 75 mg of drug for pellet cores with 2.5% and 25% theophylline (maximum theophylline solubility in water is 8.3 mg/mL, at 25 °C). Samples of 5 mL were withdrawn from the dissolution vessel at 5, 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min during dissolution in 0.1 N HCl and at 5, 10, 15, 20, 30, 45 and 60 min in pH 6.8 PB. Drug concentration was determined spectrophotometrically at 272 nm for theophylline pellets and at 334 and 354 nm for piroxicam pellets in 0.1 N HCl and pH 6.8 PB, respectively, using a double-beam spectrophotometer (UV-1650PC, Shimadzu, Kyoto, Japan). From each batch three samples were taken for analysis. According to the requirements from USP 27, an enteric coat was successfully applied if less than 10% of drug had been released after 2 h of dissolution in acid dissolution medium (0.1 N HCl).

2.2.4.4. Scanning electron microscopy (SEM). A scanning electron microscope (Jeol JSM 5600 LV, Jeol, Tokyo, Japan) was used for visualisation of the pellet and powder surface morphology. Prior to visualisation, the samples were coated with platinum using a sputter coater (Auto Fine Coater, JFC-1300, Jeol, Tokyo, Japan).

2.2.5. Bioavailability testing

2.2.5.1. Determination of piroxicam by HPLC. Two enteric-coated (until 15% polymer weight gain, with and without sorbitol) and one uncoated (without sorbitol) piroxicam pellet formulation (filled into hard gelatine capsules), as well as immediate release Feldene® capsules (Pfizer, NY, USA) containing piroxicam were orally administered to six male mixed-breed dogs (aged 1–4 years, weighing 21–42 kg) in a randomised cross-over study. Each dog was weighed one day before each drug administration in order to receive 0.3 mg piroxicam/kg body weight. Food was restricted to dogs for 12 h before dosage administration, until the 12 h sample was taken. Water was always available. A minimum wash-out period of one-week was respected between experiment. A blood sample was taken from the sphenoid vein at 0, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24, 48, 60 and 72 h after oral administration and collected into heparinised borosilicate test tubes, centrifuged at 1400*g* for 10 min and stored at −20 °C until analysed.

A validated high-performance liquid chromatography (HPLC) method was used to determine piroxicam plasma concentrations (adapted from Debunne et al. [7]). The HPLC-system consisted of an isocratic pump (L-7110, Merck Hitachi, Tokyo, Japan), an automatic injection system (234 Autoinjector, Gilson, Middleton, WI, USA) with a 50 μL loop, a precolumn (LiChrospher® 100 RP-18, 4 × 4 mm, 5 μm, Merck, Darmstadt, Germany) followed by a reversed-phase C-18 column (LiChrospher® 100 RP-18 e, 125 × 4 mm, 5 μm, Merck, Darmstadt, Germany) and a variable wavelength UV/vis detector (L-7400, Merck Hitachi, Tokyo, Japan). The software package D-7000 HSM Chromatography Data Station (version 4.1, Hitachi Instruments, San Jose, CA, USA) was used for integration of the chromatographic peaks. The mobile phase consisted of acetonitrile, 0.1% (v/v) aqueous solution of triethylamine and 30% (v/v) aqueous solution of acetic acid (40/55/5; v/v/v). The pump flow was set to 1.0 mL/min and the wavelength of a detector was 357 nm.

Five-hundred microlitres of plasma sample was added to the residue after drying 20 μL internal standard solution (20 μg/mL meloxicam dissolved in methanol) under N_2-stream at 40 °C. The mixture was sonicated for 10 s and vortexed for 10 s. After adding 250 μL of 1 M HCl, the sample was vortexed for 10 s. The extraction was performed by adding 5 mL of diethyl ether. The mixture was further shaken for 5 min and finally centrifuged for 5 min at 1420*g*. The organic layer was transferred into a new test tube and evaporated under N_2-stream at 40 °C. The residue was dissolved in 200 μL of mobile phase, vortexed for 10 s and 50 μL of this solution was injected into HPLC-system.

To prepare a calibration curve, 20 μL of piroxicam solution (diluted from a 50 μg/mL stock solution in methanol) and 20 μL of internal standard solution were dried. After adding 500 μL blank plasma to obtain piroxicam plasma concentrations of 0.2, 0.4, 0.6, 1.0, 1.6, 2.0 and 2.4 μg/mL, the samples were analysed as previously described.

Interference of piroxicam and meloxicam with endogenous components was not detected. The calibration curves were linear ($r^2 = 0.99655$; SD = 0.00247; $n = 10$) in the 0.2–2.4 μg/mL concentration range. The recovery of piroxicam after extraction varied between 77.3% and 82.4% depending on the concentration, while 81.6% of IS was recovered. The method was precise: the repeatability and coefficients of variation for intermediate precision ranged from 7.2% to 9.9% and from 1.8% to 11.9%, respectively. The limits of detection and quantification were 0.05 and 0.16 μg/mL, respectively.

2.2.5.2. Pharmacokinetic and statistical analysis. Piroxicam plasma concentrations were plotted against the time to obtain the concentration–time profiles and to determine C_{max} and t_{max}. The pharmacokinetic program MW/Pharm (version 3.0, Mediware, Utrecht, The Netherlands) was used to calculate $AUC_{0\rightarrow 72h}$. Data were statistically analysed using SPSS 14 software (SPSS, Chicago, USA). Multiple comparisons of $AUC_{0\rightarrow 72h}$ and C_{max} were performed by means of repeated measures multivariate ANOVA analysis within-subjects with the formulation as factor (P-value < 0.05).

3. Results and discussion

Previous work from the same authors [3,4] showed that a binder is needed to obtain starch-based pellets with acceptable yield. Furthermore, it was shown that including sorbitol into a starch-based formulation had a twofold effect on pellet properties: firstly, a higher mechanical strength of the extrudate (higher wet mass consistency) increased pellet yield and secondly, pellet surface properties improved as less cracks appeared on the surface. In-vitro release of model drugs like anhydrous theophylline [4], hydrochlorothiazide and piroxicam [3] was immediate, irrespective of drug solubility, pellet formulation and process parameters due to quick disintegration of starch-based pellets.

In this study, sorbitol was included as a formulation variable due to its influence on pellet surface structure. Binder (HPMC) and water concentration were previously optimised to obtain pellets with maximal yield and acceptable sphericity. Extrusion and spheronisation parameters were also selected based on previous work [3,4]. Prior to coating, wet pellets were dried in an oven or in fluidised-bed in order to investigate the influence of drying method on pellet core properties as well as on drug release from coated pellets.

Table 2 lists the formulations and the corresponding values of pellet yield, sphericity (aspect ratio, AR and two-dimensional shape factor, e_R) and size (mean Feret diameter, FD) of pellet cores dried by means of oven or fluid-bed drying. The yield has been defined as the pellet fraction between 900 and 1400 μm, since a broader pellet size distribution could influence coating thickness uniformity due to differences in available pellet surface area [8,9]. As discussed previously [4], the optimal water level for successful extrusion/spheronisation (Table 1) was lower when introducing sorbitol as water-soluble excipient and when increasing the drug concentration in the pellet formulation. Aspect ratio (AR) and two-dimensional shape factor (e_R) ranged from 1.11 to 1.16 and from 0.51 to 0.57, respectively. Based on the values suggested by Chopra et al. [10] sphericity can be described as acceptable. In addition, the data presented at Table 2 showed that pellets tended to be more spherical (lower AR and higher e_R) when sorbitol was added and when dried in a fluid-bed. Mean pellet core

Table 2
Pellet yield (%), sphericity (aspect ratio, AR; two-dimensional shape factor, e_R) and size (mean Feret diameter, FD) for pellets dried in an oven (O) and fluid-bed (FB)

Formulation	Yield (%)		AR		e_R		Mean FD (μm)	
Drying method:	O	FB	O	FB	O	FB	O	FB
Px-0	77.1	75.3	1.14	1.13	0.53	0.54	1101	1129
Px-10	78.5	78.3	1.12	1.12	0.56	0.57	1032	1109
TC-2.5-0	77.2	76.5	1.16	1.13	0.51	0.54	1110	1152
TC-2.5-10	79.9	79.3	1.15	1.11	0.53	0.57	1072	1119
TC-25-0	76.9	72.3	1.14	1.13	0.52	0.55	1144	1179
TC-25-10	82.0	81.4	1.14	1.13	0.53	0.53	1102	1145
TM-2.5-0	–	72.6	–	1.13	–	0.54	–	1108
TM-2.5-10	–	78.3	–	1.13	–	0.55	–	1102
TM-25-0	–	71.3	–	1.11	–	0.55	–	1176
TM-25-10	–	86.3	–	1.11	–	0.57	–	1080

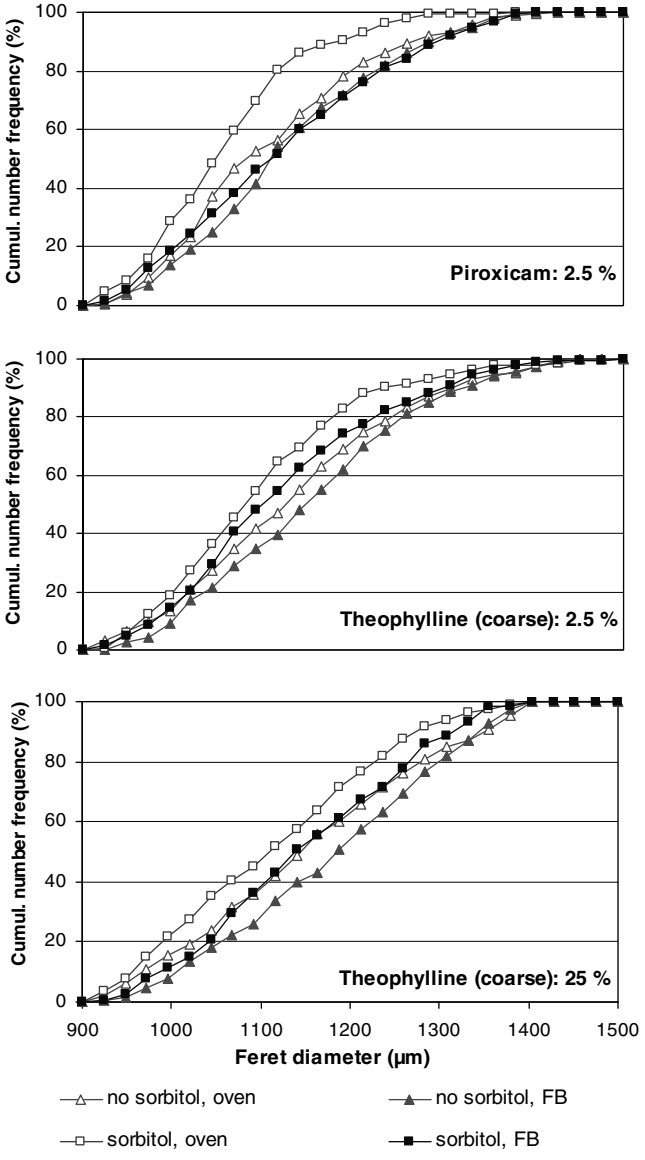

Fig. 1. Cumulative number frequency distributions of pellets containing piroxicam (2.5%) and coarse theophylline (2.5% and 25%) (FB-pellets dried in fluid-bed drier).

size was around 1100 μm for all formulations. Nevertheless, pellets containing sorbitol as well as oven-dried pellets were always slightly smaller compared to pellets without sorbitol and dried in fluidised-bed (Fig. 1). As reported by Kleinebudde [11], pellets made from water-absorbing excipients tend to shrink during drying and the extent of

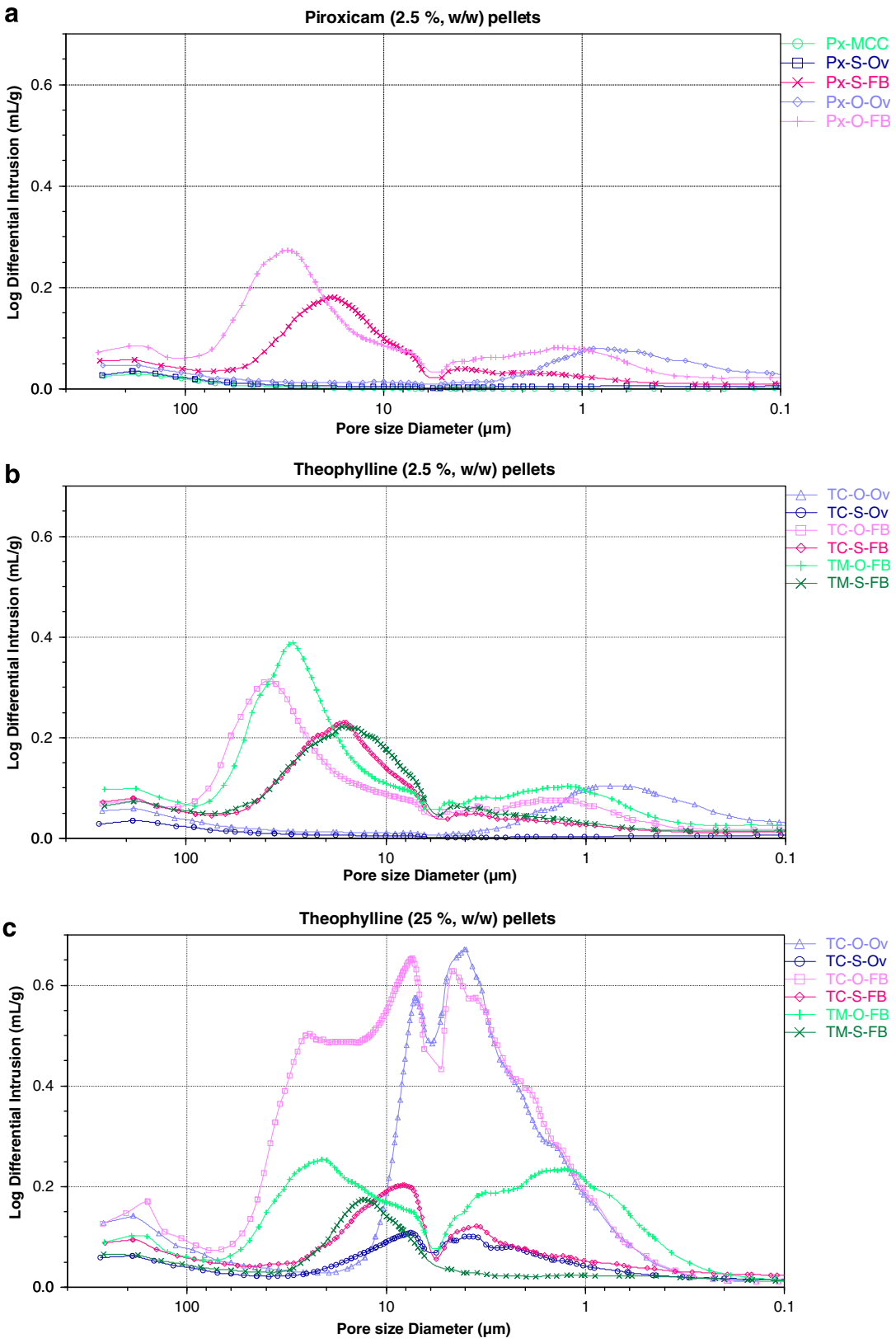

Fig. 2. Mercury intrusion volumes vs. pore size distribution (mean curve, $n = 2$) of pellets (O-without and S-with sorbitol), dried in oven (Ov) and fluid-bed (FB), containing (a) piroxicam (Px), and theophylline (coarse, TC and micronised, TM) at (b) 2.5% and (c) 25% load.

shrinkage depended on the drying method. Bashaiwoldu et al. [12] studied the influence of several drying methods on pellet properties: compared to fluid-bed dried pellets, oven drying enabled a higher extent of shrinkage and pellet size was smaller. This was related to static nature of oven drying, where water slowly evaporated over a longer period and the contraction of the solid material is enabled via generation of capillary pressure due to the surface tension of water. Pellet friability was less than 0.01% for all formulations, which is an important feature of pellets intended for coating.

Mercury intrusion porosimetry was used to determine the pore size distribution of pellet cores. Since a smooth pellet surface structure is important for a successful coating process, the evaluation of cracks and large pores on the pellet surface was of major interest. Mercury intrusion volumes in the low-pressure range (corresponding to pore sizes between 6 and 100 μm) were used to evaluate pellet surface properties. Mercury intrusion in the pore range above 100 μm was observed for all pellets, but related to mercury intrusion into the voids between pellets. Logarithmic differential intrusion volumes (mL/g) plotted against pore diameter (R, μm) in logarithmic scale are presented in Fig. 2. Due to the logarithmic transformation ($\Delta V/\Delta \log R$), the large pore fraction is overemphasised, which is in this case useful for comparison of the 6–100 μm pore range of the different pellet cores [13,14]. Fig. 2a displays the pore volume vs. size distribution of starch-based pellets containing piroxicam (2.5% w/w) and for comparison the pore volume vs. size distribution of MCC-based pellets containing piroxicam (fluid-bed dried) is also presented. For MCC pellets as well as starch-based pellets dried in an oven (irrespective of sorbitol level) no intrusion of mercury was observed in the pore range of interest, indicating that no cracks were present on the surface of the pellets. In contrast to oven drying, starch-based pellets dried in a fluid-bed yielded a high mercury intrusion in the large pore range, which indicated an irregular pellet surface. Moreover, addition of sorbitol reduced the total intrusion volume in the large pore range: the mercury intrusion peak shifted from 6 to 80 μm pore size range for pellets without sorbitol to 6–60 μm for pellets with sorbitol, indicating a lower extent of surface roughness and less cracks on the surface.

The above mentioned observations were supported by SEM photos of the pellets: cracks on the surface of fluid-bed dried starch-based pellets and a smooth surface in the case of oven-dried starch-based pellets and MCC-based pellets (Fig. 3).

A similar influence of drying method and sorbitol addition on pellet surface morphology was observed for pellets containing anhydrous theophylline as model drug (Fig. 2b and c). In general, the surface cracking during drying is a consequence of differential shrinkage of the solid material and the likelihood of fracturing depends on the evaporation rate and the strength of the network [15]. A major difference between oven and fluid-bed drying is the drying rate. As mentioned previously, in static-bed systems like ovens, drying is driven by capillary forces which allow water to slowly migrate to the surface and evaporate. Consequently, pellets shrink and the surface is smoother [16]. In contrast, fluid-bed drying is a dynamic process which involves turbulent movement of particles in an air stream and due to the intensive contact of each particle with the heated air fast evaporation of water occurs [17]. This faster drying rate and therefore higher pressure gradient of evaporating liquid might be a driving force for uneven shrinkage of pellets and crack formation [15,18,19]. The probability of crack formation can be reduced by increasing the network strength [15]. It was shown in the previous study [3] that the wet mass consistency (mean torque values) of starch-based granules was lower compared to MCC-based granules. This is not surprising, since MCC particles have fibrous structure (Fig. 4a) which – in contrast to globular starch particles (Fig. 4b) – provides a higher mechanical strength. Although during extrusion and spheronisation additional material densification occurs, it can be assumed that resulting starch-based wet pellets have a lower mechanical strength and compared to MCC-based pellets, these formulations are more sensitive to the faster evaporation rate during fluid-bed drying. Similarly, the lower extent of crack formation during fluid-bed drying in the case of sorbitol addition may be a consequence of

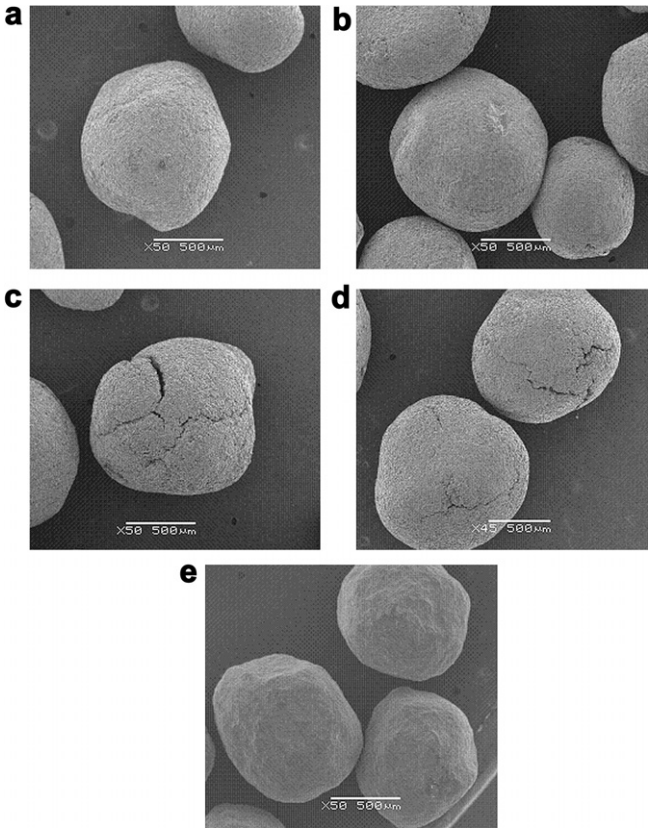

Fig. 3. Scanning electron micrographs of starch-based piroxicam pellets dried in oven without (a) and with (b) sorbitol, of starch-based piroxicam pellets dried in fluidised-bed without (c) or with sorbitol (d) and of microcrystalline cellulose-based piroxicam pellets dried in fluidised-bed (e).

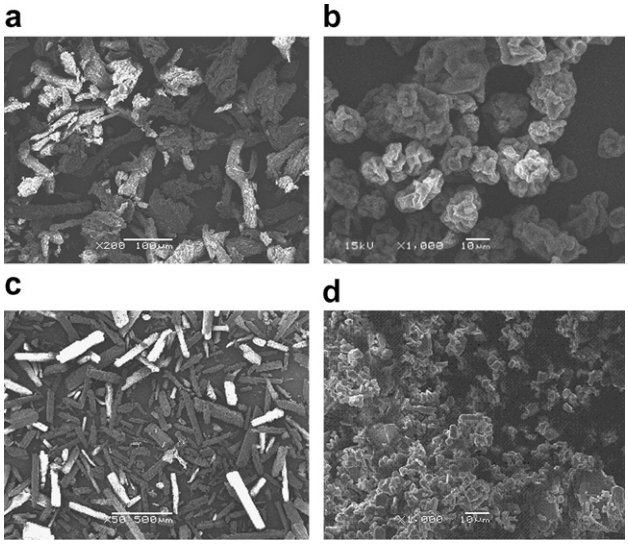

Fig. 4. Scanning electron micrographs of (a) Avicel® PH 101, (b) UNI-PURE®EX starch, (c) coarse and (d) micronised anhydrous theophylline powder.

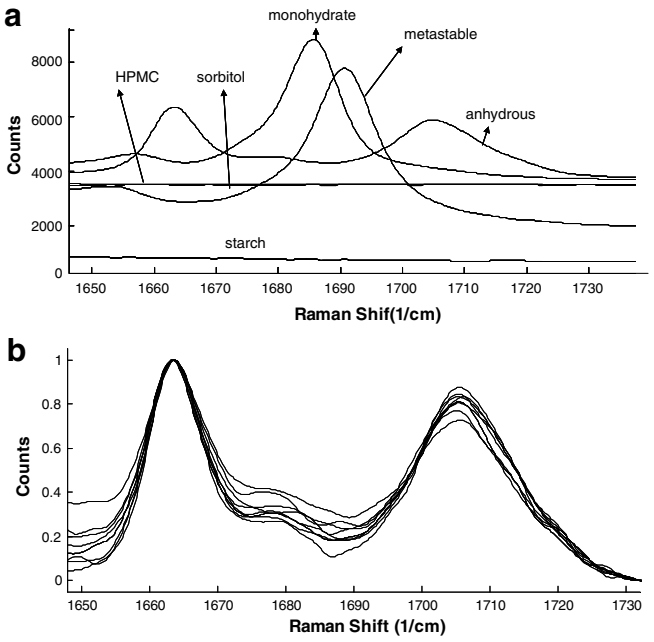

Fig. 5. Raman spectra: (a) raw materials including theophylline monohydrate and metastable form; (b) pellets dried in oven and fluidised-bed, with and without sorbitol (25% theophylline) and sampled at the surface and inside of the pellets.

improved mechanical strength of starch-based wet extrudates, as shown in the previous study [3].

Intrusion volume vs. pore size distribution graphs also show a difference in mercury intrusion volumes and peak position for fluid-bed dried pellets containing different theophylline concentrations (Fig. 2b and c): pellets with 2.5% drug have an intrusion peak in the same range as starch-based piroxicam pellets, while for pellets at higher theophylline concentration (25%) the intrusion peak is shifted to the smaller pore size range. This shift may be linked to a higher wet mass consistency (i.e. higher network strength) and therefore lower extent of crack formation: addition of a higher concentration of needle-like (coarse) theophylline powder (Fig. 4c) and a lower level of globular starch particles (Fig. 4b) increased the wet mass consistency due to mechanical interlocking of particles. Moreover, increasing the concentration of micronised theophylline powder (Fig. 4d) increased the wet mass consistency due to a larger particle surface area [20]. Compared to pellets with coarse theophylline, introducing micronised theophylline at the same concentration promoted a shift of peak intrusion range towards smaller pores and a reduction of the total intrusion volume. Those results comply with the results of Niskanen [21] who reported that reducing the particle size of theophylline powder in pellet formulations decreased the fraction of large pores.

The Raman spectra at the 1650–1730 cm^{-1} range of different theophylline polymorphs (anhydrous, monohydrate and metastable) as well as the spectra of excipients used in pellet formulations are presented in Fig. 5a. It can be observed that all theophylline forms are easily distinguished and there is no spectral overlap originating from other excipients. Fig. 5b shows the Raman spectra of pellet formulations containing different sorbitol levels and dried in an oven and fluidised-bed. Anhydrous theophylline peaks were detected in the samples irrespective of sampling place (at the surface or inside the pellets) and drying method.

The theophylline polymorphs have a significant influence on drug release. Herman et al. [22] reported that the transition of anhydrous theophylline into a monohydrate polymorph occurred during wet granulation. If theophylline dehydration during drying is not complete, the dissolution might be prolonged due to theophylline monohydrate having a lower aqueous solubility [23]. Furthermore, depending on the drying conditions, theophylline monohydrate dehydration into a stable anhydrous form can occur via a metastable form with lower dissolution rate compared to the stable anhydrous polymorph [24,25].

Figs. 6 and 7 show theophylline release profiles from enteric-coated pellets (with 15% and 30% polymer weight gain, respectively) during 2 h in acidic dissolution medium (0.1 N HCl). For pellets coated with 15% of weight gain theophylline release ranged from 5% to about 30%, while after applying a higher coating thickness all theophylline pellet formulations were successfully coated (<10% drug release after 2 h in acidic dissolution medium, USP 27). Furthermore, since the reference pellets (MCC-based) containing 25% theophylline (coarse), fluid-bed dried and coated until 15% of polymer weight gain released only 3% of the drug after 2 h in acidic medium (Fig. 6), the differences in theophylline release from enteric-coated starch-based pellets were related to the pellet-surface properties. Therefore, the drug release depended on pellet composition and drying method as these factors determined the surface properties. It was already reported that surface roughness

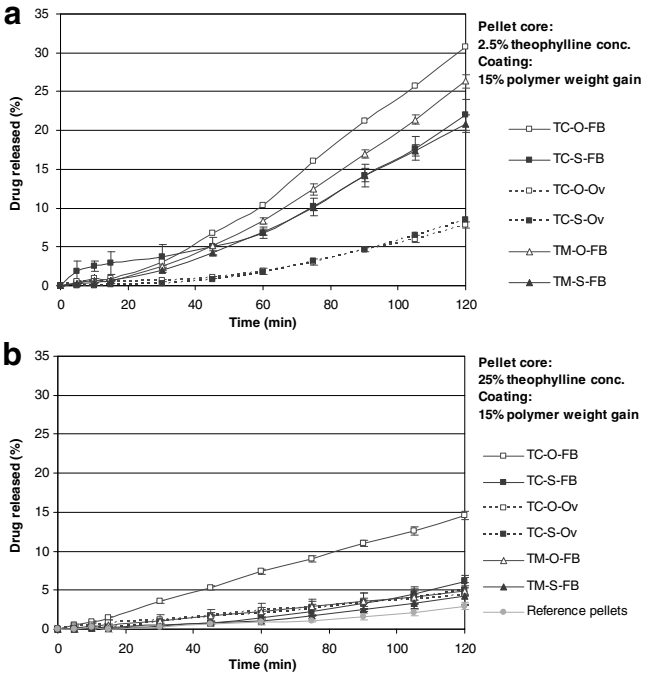

Fig. 6. In-vitro drug release in 0.1 N HCl during 2 h from enteric-coated pellets (15% of polymer weight gain) containing (a) 2.5% (w/w, dry mass) and (b) 25% (w/w, dry mass) of theophylline anhydrous. Legend: TC and TM – coarse and micronised theophylline; S and O – formulation with and without sorbitol; FB and Ov – pellet cores dried in fluidised-bed and oven.

promoted the formation of an uneven coating thickness (especially in the case of larger pores or cracks) and resulted in a faster drug release [26]. For pellets coated with 15% of polymer weight gain and at the lower theophylline concentration, the highest release was observed for pellets without sorbitol and dried in fluidised-bed (25% and 30% release from pellets with micronised and coarse theophylline, respectively) (Fig. 6a). This correlated with the worst surface defects as identified via mercury intrusion. The slightly lower drug release for micronised theophylline is also linked to a shift of mercury intrusion peak towards smaller pore size. In case of oven-dried pellets dried, drug release was less than 10% as a smooth pellet surface was identified. Enteric-coated pellets containing a higher drug level (Fig. 6b) released about 15% from fluid-bed dried pellets containing coarse theophylline and no sorbitol, whereas all other formulations released about 5% of theophylline. Furthermore, when comparing pellets of the same formulation but with different coating thickness (15%, 20%, 25% and 30% of polymer weight gain), theophylline release progressively reduced with an increase of polymer coat thickness.

The influence of pellet surface properties was negligible for pellets containing piroxicam: drug release from enteric-coated pellets during 2 h in acid medium was less than 1%, irrespective of the coating level. In this case the poor water solubility of piroxicam reduced the drug diffusion rate through the water-filled pores. Drug release from uncoated piroxicam pellets was fast (more than 80% in less than

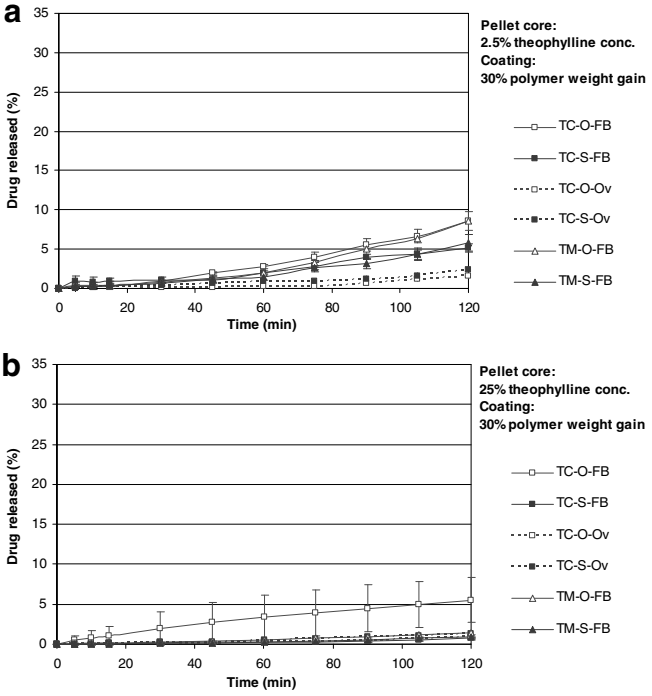

Fig. 7. In-vitro drug release in 0.1 N HCl during 2 h from enteric-coated pellets (30% of polymer weight gain) containing (a) 2.5% (w/w, dry mass) and (b) 25% (w/w, dry mass) of theophylline anhydrous. Legend: TC and TM – coarse and micronised theophylline; S and O – formulation with and without sorbitol; FB and Ov – pellet cores dried in fluidised-bed and oven.

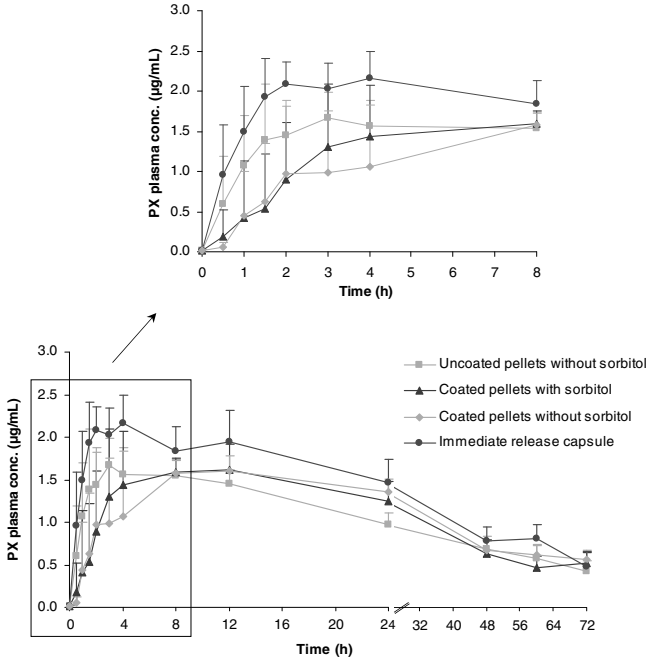

Fig. 8. Mean piroxicam plasma concentration–time profiles (±SD; $n = 6$) obtained after administration of an oral dose of 0.3 mg piroxicam/kg body weight, formulated as uncoated and coated pellets as well as immediate release capsules.

Table 3
Mean $AUC_{0\rightarrow 72h}$, C_{max} and t_{max} values (±SD) after oral administration of piroxicam (0.3 mg/kg body weight) to dogs ($n = 6$)

Formulation	$AUC_{0\rightarrow 72h}$ (μg h/mL)	C_{max} (μg/mL)	t_{max} (h)
Uncoated pellets without sorbitol	66.4 (±7.7)[a]	1.7 (±0.3)[a]	6.1 (±3.9)
Coated pellets without sorbitol	71.2 (±6.8)[a]	1.8 (±0.1)[a]	7.7 (±4.1)
Coated pellets with sorbitol	67.1 (±14.3)[a]	1.8 (±0.3)[a]	5.7 (±2.6)
Immediate release piroxicam capsule	87.5 (±13.5)[a]	2.2 (±0.3)[a]	2.8 (±1.1)

[a] Treatments are not significantly different ($P > 0.05$, multivariate repeated measures test).

45 min) since these pellets disintegrated rapidly in the dissolution medium.

Due to pellet disintegration, the release of theophylline and piroxicam in phosphate buffer was complete for all formulations in less than 30 and 45 min, respectively (data not shown).

Three piroxicam pellet formulations (one uncoated without sorbitol, and two coated with and without sorbitol) were used in an in-vivo study to compare their bioavailability with a commercial formulation (Feldene® capsules used as a reference). Piroxicam is a highly potent non-steroidal anti-inflammatory drug and exhibits a gastric irritation as the major side effect associated with the use of non-steroidal anti-inflammatory drugs. Formulating an enteric-coated multiparticulate solid dosage would be an advantage due to the protection provided to the gastric mucosa by such a dosage form. Fig. 8 presents the mean ($n = 6$) piroxicam plasma concentration vs. time profiles of the pellet formulations and the immediate release capsule, while Table 3 summarises the pharmacokinetic parameters.

Table 3 shows that there are no statistically significant differences of $AUC_{0\rightarrow 72h}$ and C_{max} between the pellet and reference formulations ($P > 0.05$, multivariate repeated measures test), indicating a similar drug availability at the absorption site. It can also be observed that application of an enteric coat did not influence the bioavailability of piroxicam. However, in Fig. 8 a lag time of 30 min was observed for the drug release from coated pellet formulations. This can be attributed to acidoresistivity of the coating polymer (Eudragit® L30 D-55), which dissolves only at pH > 6 (i.e. when the coated pellets are emptied from the stomach). In addition, 1 and 4 h after administration, a small increase of mean piroxicam plasma concentration was observed. As reported by several authors, this could indicate an enterohepatic recirculation of piroxicam [27,28,7].

4. Conclusion

Pellets of acceptable sphericity, process yield and containing modified starch as the main excipient were successfully enteric-coated (<10% drug release after 2 h in acidic dissolution medium). However, the extent of drug release during 2 h in acidic medium ranged from <1% to about 30%, depending on model drug solubility, particle size and concentration, pellet formulation and drying method as these factors determined the pellet core surface properties. The influence of pellet core surface roughness was reduced by increasing the coating thickness up to 30% of polymer weight gain. Due to pellet disintegration, the drug release in phosphate buffer was immediate for all formulations. Values of $AUC_{0\rightarrow 72h}$ and C_{max} after oral administration of piroxicam pellets to dogs were comparable to the values obtained from immediate release capsules.

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