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Research paper

Immediate release of poorly soluble drugs from starch-based pellets prepared via extrusion/spheronisation

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Abstract

The aim of this study was to evaluate modified starch (high-amylose, crystalline and resistant starch) as the main excipient for immediate-release pellets containing poorly soluble drugs (hydrochlorothiazide and piroxicam) and prepared via extrusion/spheronisation. The bioavailability of pellets (containing 50 mg hydrochlorothiazide) was determined after oral administration to 6 dogs. A 2^4-factorial design with central point was used to evaluate the influence of hydrochlorothiazide (10% and 50%, w/w), HPMC (binder, 4% and 7%, w/w), sorbitol (0% and 10%, w/w) and water (granulation liquid, low and high level) on pellet yield, size (Feret mean diameter) and sphericity (aspect ratio and two-dimensional shape factor, e_R). Optimal granulation liquid content depended on drug and sorbitol level in the formulation. All factors except sorbitol content, as well as the interactions between drug concentration and binder level and between drug and water level, were significant ($P < 0.05$) for pellet yield, while a significant curvature ($P < 0.05$) suggested non-linearity of the response plots. The model was not significant for pellet shape, while hydrochlorothiazide and water level as well as their interaction were significant ($P < 0.05$) for pellet size. Pellet friability, disintegration, residual water content and in-vitro drug release were determined. Pellets containing 2.5% (w/w) piroxicam were also evaluated. For both model drugs, pellets with a high yield (>90%), acceptable sphericity (AR < 1.2) and low friability (<0.01%) were obtained. Due to pellet disintegration, fast dissolution of both hydrochlorothiazide and piroxicam was achieved: >80% drug released in 30 min. The bioavailability ($AUC_{0\rightarrow 24\ h}$, C_{max} and t_{max}) of hydrochlorothiazide pellets in dogs was not significantly different from fast-disintegrating immediate-release hydrochlorothiazide tablets ($P > 0.05$).

Keywords: Extrusion/spheronisation; Pellets; Starch; Hydrochlorothiazide; Piroxicam; *In-vivo* evaluation

1. Introduction

Being a multiparticulate solid dosage form, pellets offer several advantages when compared to tablets as classical "single-unit" dosage forms [1]. Physiological advantages (reduced gastric irritation effect, longer gastrointestinal transit times, minimised gastric emptying effect, etc.) are attributed to more even distribution of pellets in gastrointestinal tract, while technological advantages (good sphericity, smooth surface properties, narrow size distribution and low friability) ensure a drug content uniformity and successful coating with minimised risk of dose "dumping". Additionally, pellets offer greater flexibility in dosing and drug release design.

Extrusion/spheronisation is a well-established technique for the production of pellets. Microcrystalline cellulose (MCC) is up to now the most widely used excipient for pellets prepared via extrusion/spheronisation because it has good binding properties and provides the necessary plasticity to the wet mass, which both ensure successful extrusion and spheronisation [2]. Nevertheless, MCC-based pellets do not disintegrate and drug release occurs via diffusion through an insoluble inert matrix. Although pellet disintegration is not required if pellets are used for sustained drug delivery, disintegration is an important issue for

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enteric-coated pellets or colon targeted drug delivery, where immediate drug release is required after a functional coating has dissolved in gastrointestinal fluids. Lack of disintegration of MCC-based pellets becomes very critical if an active component has poor solubility in water, since the drug release is prolonged [3]. Due to this limitation, MCC formulations have been modified in order to increase a drug release [4–8] and alternatively, excipients as substitutes for MCC have been investigated and reported in the literature [9–17].

Modified starch (UNI-PURE® EX starch, National Starch and Chemical Co., Bridgewater, New Jersey, USA) has been recently identified as a material suitable for preparation of pellets via extrusion/spheronisation [18]. The most important feature of UNI-PURE® EX starch-based pellets is their disintegration, which might provide a solution for the slower release of poorly soluble drugs observed in MCC-based pellets.

UNI-PURE® EX starch is a crystalline material obtained by enzymatic debranching of amylose-rich starch, followed by retrogradation [19]. It is referred to as resistant starch because α-1,4-D-glycosidic bonds between D-anhydroglucose monomers are inaccessible to α-amylase in the small intestine due to formation of a double-helical crystalline chain structure.

In a previous paper [18] it was demonstrated that UNI-PURE® EX starch in combination with a suitable binder resulted in spherical pellets with a narrow particle size distribution and high process yield. Furthermore, due to pellet disintegration, the release of anhydrous theophylline as a model drug with medium water solubility was complete in less than 20 min. This study evaluates UNI-PURE® EX starch as the main excipient for pellets containing poorly soluble drugs. Two model drugs (hydrochlorothiazide at low and high drug content, and piroxicam at low drug content) were used to evaluate the pellet quality and drug release. An *in-vivo* study was conducted in order to determine the bioavailability of hydrochlorothiazide pellets compared to immediate-release tablets.

2. Materials and methods

2.1. Materials

Hydrochlorothiazide (HCT) (Bufa, Uitgeest, The Netherlands) and piroxicam (PX) (Sagran, Milan, Italy) were used as poorly soluble model drugs. UNI-PURE® EX starch (high-amylose, crystalline and resistant starch, used as the main excipient in the pellets) was donated by National Starch and Chemical Co. (Bridgewater, New Jersey, USA). Particle size D(v,0.5) of hydrochlorothiazide, piroxicam and UNI-PURE® EX starch, determined by laser diffraction, was 102.4 ± 9.6, 9.9 ± 0.6 and 43.1 ± 4.7 μm, respectively. Hydroxypropylmethylcellulose (HPMC) (Methocel® E15 LV EP Pharm), used as a binder, was a gift from Colorcon (Dartford, UK). Sorbitol (Sorbidex® P 16616, Cerestar, Vilvoorde, Belgium) was added to modify the consistency of the wet mass. Demineralised water was used as granulation liquid. Reference pellets were made with MCC (Avicel® PH 101, FMC, Cork, Ireland) as the main excipient and were used for comparing the drug release profiles.

The following materials of HPLC-grade were used for determination of hydrochlorothiazide in dog plasma: hydrochlorothiazide, hydroflumethiazide and methyl *tert*-butylether, purchased from Sigma Chemical Co. (St. Louis, MO, USA); acetonitrile, toluene and tetrahydrofurane, obtained from Biosolve (Valkenswaard, The Netherlands); NaOH and KH_2PO_4 from VWR International (Fontenay sous Bois, France).

2.2. Methods

2.2.1. Experimental design and data analysis

To identify significant formulation variables a 2^4-factorial design with a central point for curvature estimation was used. Table 1 lists the four factors at both levels used in the design. HCT (10% and 50% w/w, dry mass) and HPMC concentration (4% and 7% w/w, dry mass) were nominal variables in the design. The optimal range of the water content was determined based on preliminary experiments and two water levels providing the highest pellet yield were selected for the experimental design. Since the water concentration depended on the amount of water-soluble components in the formulation (sorbitol in this case), both sorbitol and water concentration were introduced in the design as categorical variables. The codes used for the categorical variables were O (formulation without sorbitol), S (formulation containing a sorbitol concentration which was 10% of the UNI-PURE® EX starch content in the formulation), L (low water level) and H (high water level). Table 2 lists all experiments with the coded and actual values of the variables. The total number of experiments was 20 (including four experiments using the central point of each nominal variable combined with each level of both categorical variables). Experiments were performed in a randomised order using the same process parameters. Pellet yield, sphericity (aspect ratio and shape factor) and size (Feret diameter) were determined for each batch of the experimental design and those values were used as responses for modelling. Results were analysed using

Table 1
Definition of the factors used in the experimental design

Factor	Low level (−1)	High level (+1)
A: HCT conc. (% w/w, dry mass)	10	50
B: HPMC conc. (% w/w, dry mass)	4	7
C: sorbitol level[a,b]	O	S
D: water level[b]	Low	High

[a] Sorbitol concentration is expressed as the percentage of UNI-PURE® EX starch amount in the formulation: O is the formulation without sorbitol, S is the formulation containing sorbitol in a concentration which is 10% of the UNI-PURE® EX starch content of the formulation.

[b] Sorbitol and water level are categorical variables in the design.

Table 2
2^4-Factorial design (randomised order) presented in coded and actual terms and the corresponding results of pellet yield, aspect ratio (AR), two-dimensional shape factor (e_R) and Feret diameter (FD). *A*: HCT conc. (% w/w, dry mass), *B*: HPMC conc. (% w/w, dry mass), *C*: sorbitol conc. (% w/w, dry mass), *D*: water conc. (% w/w, wet mass)

Run	Coded values				Actual values				Results			
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	Yield (%)	AR	e_R	FD (μm)
1	0	0	O	L	30	5.5	0	42.86	82.7	1.12	0.57	1100
2	1	−1	O	L	50	4	0	37.50	65.3	1.12	0.55	1133
3	−1	−1	O	H	10	4	0	50.00	46.1	1.12	0.58	1162
4	−1	1	S	L	10	7	8.3	42.86	90.9	1.12	0.56	1038
5	1	1	O	H	50	7	0	37.50	51.5	1.16	0.53	1246
6	0	0	S	L	30	5.5	6.5	40.30	86.9	1.26	0.45	1300
7	−1	−1	O	L	10	4	0	49.37	50.8	1.15	0.53	1096
8	−1	−1	S	H	10	4	8.6	45.95	53.9	1.15	0.55	1117
9	0	0	S	H	30	5.5	6.5	41.18	76.7	1.15	0.54	1210
10	1	−1	O	H	50	4	0	39.39	50.0	1.11	0.59	1147
11	1	1	O	L	50	7	0	35.48	85.1	1.12	0.56	1095
12	1	1	S	L	50	7	4.3	33.33	81.8	1.16	0.54	1155
13	1	−1	S	H	50	4	4.6	36.51	52.4	1.19	0.47	1246
14	−1	1	S	H	10	7	8.3	44.44	92.3	1.16	0.52	1038
15	1	1	S	H	50	7	4.3	35.48	37.1	1.16	0.51	1170
16	−1	−1	S	L	10	4	8.6	44.44	53.6	1.13	0.57	1071
17	1	−1	S	L	50	4	4.6	35.48	77.7	1.12	0.55	1093
18	0	0	O	H	30	5.5	0	44.44	80.7	1.14	0.55	1151
19	−1	1	O	L	10	7	0	48.05	89.0	1.14	0.55	1142
20	−1	1	O	H	10	7	0	48.72	83.5	1.15	0.52	1155

Design-Expert®, v.6.0.6. software (Stat-Ease, Minneapolis, USA). Analysis of variance (ANOVA) with $P < 0.05$ was performed for each response.

Piroxicam was used as a second model drug and was incorporated at a low concentration (2.5%, w/w, dry mass) in the pellets. Pellets were prepared with 7% HPMC (w/w, dry mass) and 10% sorbitol (w/w, dry mass). Piroxicam pellets without sorbitol were also prepared. The optimal range of the water content was determined based on preliminary experiments and pellets were prepared at two water levels which provided the highest yield.

In order to compare the drug release profiles, reference pellets were made with MCC as the main excipient, containing the same concentrations of model drug and using the same process parameters, as well as an optimised amount of water as granulation liquid.

2.2.2. Pellet preparation

The model drug, HPMC, sorbitol and modified starch were mixed (batch size: 250 g) for 15 min in a Turbula® mixer (model T2A, W.A. Bachofen, Basel, Switzerland), followed by granulation with demineralised water by means of a planetary mixer (Kenwood Chief, Hampshire, UK) (granulation time: 10 min; mixing speed: 60 rpm). Water was added during the first 30 s of the wet massing phase. During granulation, the material was repeatedly scraped from the mixing bowl walls, to ensure uniform water distribution. The wet mass was extruded at an extrusion speed of 50 rpm using a single screw extruder (Dome extruder lab model DG-L1, Fuji Paudal, Tokyo, Japan) equipped with a dome-shaped extrusion screen (thickness: 1.2 mm, perforation diameter: 1 mm). The extrudates were spheronised for 3 min at 850 rpm in a spheroniser with a "cross-hatched" friction plate (Caleva Model 15, Caleva, Sturminster Newton, Dorset, UK) and finally dried for 20 min at 50 °C in a fluid-bed drier (Uniglatt, Glatt, Binzen, Germany).

2.2.3. Pellet characterisation

2.2.3.1. Sieving. Pellets (100 g) were sieved for 10 min at an amplitude of 3 mm on a shaker (Type VE 1000, Retsch, Haan, Germany) using 1400, 1000, 710, 500 and 250 μm sieves (Retsch, Haan, Germany). The pellet yield was calculated based on the pellet fraction between 710 and 1400 μm and presented as the percent of the total pellet weight.

2.2.3.2. Pellet size and shape. An image analysis system was used to determine pellet size and shape. Photomicrographs of pellets were taken with a digital camera (Camedia® C-3030 Zoom, Olympus, Tokyo, Japan), linked with a stereomicroscope system (SZX9 DF PL 1.5x, Olympus, Tokyo, Japan). A cold light source (Highlight 2100, Olympus, Germany) and a ring light guide (LG-R66, Olympus, Germany) provided top light illumination of the pellets against a dark surface. The images were analysed by image analysis software (AnalySIS®, Soft Imaging System, Münster, Germany). The magnification was set in a way that one pixel corresponded to 5.7 μm and around 300 pellets were analysed for every batch. Each individual pellet was characterised by mean Feret diameter (FD) (average of 180 calliper measurements with an angle of rotation of 1°), aspect ratio (AR) (ratio of longest Feret diameter and its longest perpendicular diameter) and two-

dimensional shape factor (e_R) (as described by Podczeck and Newton [20])

$$e_r = \frac{2 \cdot \pi \cdot r}{P_m} - \sqrt{1 - \left(\frac{b}{l}\right)^2} \quad (1)$$

where r is the radius, P_m is the perimeter, l is the length (longest Feret diameter) and b is the breadth (longest diameter perpendicular to the longest Feret diameter) of a pellet.

2.2.3.3. Friability. A sample of pellets (F_s, 10 g) was placed in an abrasion wheel together with 200 glass beads (diameter: 4 mm) and fitted to a friabilator (Type PTF, Pharma Test, Hainburg, Germany). The sample was subjected to falling shocks for 10 min at a rotational speed of 25 rpm. Afterwards the fines were removed by sieving through a 250 μm mesh for 5 min (2 mm amplitude). The fraction above 250 μm (F_a) was used to calculate the friability of pellets according to the following equation

$$\text{Friability } (\%) = [(F_s - F_a)/F_s] * 100 \quad (2)$$

2.2.3.4. Water content. The water content of powders and pellets was determined by means of a Karl Fischer titrator (Mettler DL 35, Beersel, Belgium) coupled with infrared (IR) oven (Mettler DO 337, Beersel, Belgium). Hydroquant-Uniquant 2 (Biosolve, Valkenswaard, The Netherlands) and extra dry methanol (Biosolve, Valkenswaard, The Netherlands) were the titration reagent and solvent, respectively. The sample was placed in an IR oven for 10 min at 150 °C and a stream of dry nitrogen (150 mL/min) transported evaporated water into the titration vessel. Each batch was analysed in triplicate.

2.2.3.5. Disintegration. The pellet disintegration time (sample amount: 100 mg) was measured in a disintegrator (Type PTZ, Pharma Test, Hainburg, Germany) using a method modified from the Eur. Ph. 4th ed. monograph for tablet disintegration: a 500 μm mesh cloth was placed at the bottom of the tubes. The mechanical stress on the pellets was increased by means of discs and 0.1 N HCl was the disintegration medium. Results represent the average of six determinations.

2.2.3.6. Dissolution profiles. The dissolution tests were performed according to the USP paddle apparatus (VK 8000, VanKel, New Jersey, USA) at a rotational speed of 100 rpm and temperature of 37 °C. Sample amount used for analysis was adjusted to obtain sink conditions. The dissolution medium (900 mL) depended on the model drug: 0.1 N HCl for pellets containing HCT and phosphate buffer (pH 6.8) for pellets with PX. Samples of 5 mL were withdrawn from the dissolution vessel at 5, 10, 15, 20, 30, 45, 60 and 75 min and spectrophotometrically analysed at 272 and 354 nm for HCT and PX pellets, respectively, by means of a double-beam spectrophotometer (Shimadzu UV-1650PC, Shimadzu Co., Kyoto, Japan). Each batch was analysed in triplicate.

2.2.3.7. Scanning electron microscopy (SEM). Photomicrographs were taken with a scanning electron microscope (Jeol JSM 5600 LV, Jeol, Tokyo, Japan) for visualisation of the powder surface morphology. Powders were coated with platinum by means of a sputter coater (Auto Fine Coater, JFC-1300, Jeol, Tokyo, Japan) to assure conductivity.

2.2.4. Bioavailability testing

2.2.4.1. Determination of hydrochlorothiazide by HPLC. HCT plasma concentrations were determined using a validated high performance liquid chromatography (HPLC) method [21]. The HPLC-system consisted of an isocratic pump (L-7110, Merck Hitachi, Tokyo, Japan), automatic injection system (234 Autoinjector, Gilson, Middletown, WI, USA) with a 100 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, 250 × 4 mm, 5 μm, Merck, Darmstadt, Germany) and a variable wavelength UV/vis detector (L-7400, Merck Hitachi, Tokyo, Japan). A 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 a phosphate buffer pH 7.0 (USP XXVII), tetrahydrofurane and acetonitrile (85/10/5; v/v/v). The precolumn and column were conditioned at 40 °C, the pump flow was 0.8 mL/min and the wavelength of a detector was set to 272 nm.

A stock solution of hydrochlorothiazide (50 μg/mL) was used to prepare the standard solutions for method validation. Hydroflumethiazide (1.25 μg/mL) was internal standard. For the determination of calibration samples, 100 μL of IS solution was added to 400 μL of blank plasma together with 100 μL of standard HCT solutions to obtain eight plasma concentrations in the range from 10 to 2000 ng/mL.

No interference with endogenous components was detected. Calibration curves were linear in the whole concentration range ($r^2 = 0.99986 \pm 0.00026$; $n = 10$). The recovery of HCT (10–2000 ng/mL range) after extraction varied between 77.1% and 96.3%, while 84.8% of IS was recovered. The method was precise for the same concentration range, since the repeatability and intermediate precision coefficients of variation ranged between 1.01% and 8.63% and between 2.05% and 8.97%, respectively. The limits of detection and quantification were 4.45 and 13.49 ng/mL, respectively.

2.2.4.2. Oral administration. Six male mixed-breed dogs (aged 1–4 years) weighing from 21 to 42 kg were used for the study. Prior to oral dose administration food was restricted for 12 h as well as during the experiment (24 h), while access to water was unlimited. In a randomised

cross-over design, each dog received 50 mg of HCT on three occasions: twice as pellet formulation (filled into hard gelatine capsules) and once as an immediate-release tablet (Esidrex® 50 mg, Novartis Pharma, Bern, Switzerland) (reference formulation). One pellet formulation contained 10% of HCT, while the other was formulated with 50% HCT (both formulations had the same binder level, 7% w/w dry mass). There was a 1-week wash-out period in between each experiment. Each dosage form was administered to a dog with a small amount of water to prevent sticking to the buccal mucosa. A blood sample was taken from the sphenoid vein at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h, collected into heparinised borosilicate test tubes and after centrifugation at 1400*g* for 10 min, plasma was stored at −20 °C until analysed.

2.2.4.3. Analysis of plasma samples. One hundred microliters of IS solution was added to 500 μL of plasma sample, vortexed for 15 s and after adding 5 mL of methyl *tert*-butylether vortexed for another 2 min. After 5 min of centrifugation at 2500*g*, 4.5 mL of organic phase was removed into a new borosilicate glass test tube and dried under a N_2-stream at 40 °C until complete evaporation of the organic solvent. The residue was further dissolved in 200 μL of distilled water, followed by addition of 3 mL toluene. The mixture was further vortexed for 2 min and after 10 min of centrifugation at 2500*g*, the toluene layer was removed. Once again 3 mL of toluene was added, and the extraction procedure was repeated. After removing the organic phase, the mixture was dried under a N_2-stream at 40 °C. Two hundred microliters of a mobile phase was added to the residue, homogenized by vortexing for 10 s and 100 μL of this solution was injected into the HPLC-system.

2.2.4.4. Pharmacokinetic and statistical analysis. Individual HCT plasma concentrations were adjusted for the body weight of the dogs and plotted against the time. $AUC_{0\rightarrow 24}$ was calculated using the pharmacokinetic programme MW/Pharm (ver. 3.0; Mediware, Utrecht, The Netherlands), while C_{max} and t_{max} were determined from the concentration–time profiles. The relative bioavailability of the pellet formulation (F_{rel}, %) was calculated as the ratio of $AUC_{0-24\ h}$ between a pellet formulation and the immediate-release tablets. Data were statistically analysed using SPSS 14 software (SPSS, Chicago, USA). Multiple comparisons of $AUC_{0\rightarrow 24\ h}$ and C_{max} were performed by means of repeated measures univariate analysis for within-subject factors and an assumption of sphericity of covariances with Mauchly's test (P value < 0.05).

3. Results and discussion

A previous study of the same authors [18] demonstrated that adding a binder to UNI-PURE® EX starch significantly improved the yield of pellets prepared via extrusion/spheronisation. Furthermore, the addition of sorbitol increased the mechanical strength of wet extrudates and consequently improved the process yield and the surface structure of the pellets. In contrast, higher sorbitol concentrations were detrimental to pellet quality. Based on these observations sorbitol and HPMC concentrations were included as variables in an experimental design evaluating the quality of pellets containing poorly soluble drugs. Process parameters were not varied in the experimental design, but were selected based on the previous study with UNI-PURE® EX starch [18].

Fig. 1 presents the water concentrations which have been used in experimental design in order to maximize pellet yield. Water concentration was directly related to the sorbitol and HCT concentration: it was lower in formulations containing sorbitol and when the HCT load was increased. The influence of sorbitol is a consequence of its solubility in water: since it increases the volume of the liquid phase during wet massing, less water is required before pellet agglomeration occurs during spheronisation. A similar relationship between the required water amount and the concentration of water-soluble filler or a drug has been reported previously [18,22–25]. The effect of sorbitol on water concentration was higher for batches with a lower HCT concentration since the UNI-PURE® EX starch content in these formulations was higher and the sorbitol concentration was correlated with the UNI-PURE® EX starch fraction of the formulation. Substituting a part of the UNI-PURE® EX starch by HCT to increase the drug load also required less water for successful spheronisation, since the hydrophilic starch molecule is able to bind more water before overwetting occurs. In addition, increasing the HCT concentration in the powder mixture reduced the total powder surface area as HCT particles (D(v,0.5) = 104.4 μm) are smaller compared to UNI-PURE® EX starch particles (D(v,0.5) = 43 μm), thus liquid saturation was obtained at lower water levels [26]. Similar relationships between the amount of granulation liquid and the particle size were observed by Kristensen et al. [27] (granule growth by coalescence was achieved at

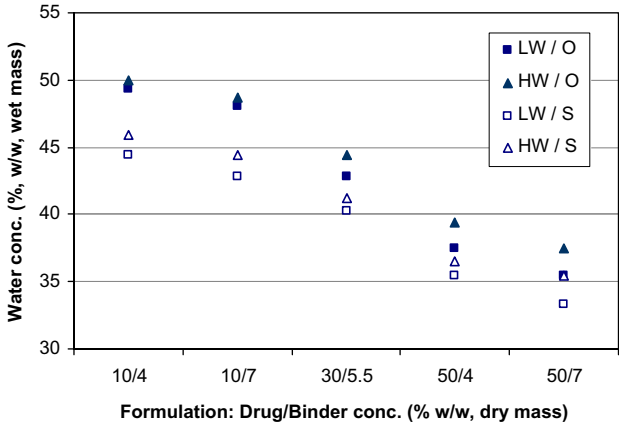

Fig. 1. Water levels (% w/w, wet mass; LW-low and HW-high) used in formulations without (O) and with sorbitol (S) for different drug (HCT) and a binder (HPMC) concentrations (% w/w, dry mass).

lower liquid saturation values when increasing the mean particle size of dicalcium phosphate powder) and Bains et al. [28] (substituting part of the MCC-fraction by the smaller sized barium sulphate increased the amount of liquid required for successful extrusion/spheronisation).

Table 3 presents the results of ANOVA for pellet yield. A factorial model was significant ($P < 0.05$) and the predicted R^2 value was in reasonable agreement with the R^2 value adjusted for the degrees of freedom, which indicated that the data can be fitted by the model. Significant curvature ($P < 0.05$) suggested non-linearity of the response plots, indicating that more points (surface response design) should be included in the design space if formulation optimisation is needed. All factors except sorbitol content were significant ($P < 0.05$) as well as the interactions between drug concentration and binder level and between drug and water level. The regression equation for pellet yield in terms of the coded values is the following:

$$\text{Yield } (\%) = 66.32 - 3.70 * A + 10.09 * B - 6.97 * D - 8.83 * A * B - 6.90 * A * D \quad (3)$$

3D diagrams of pellet yield in function of binder level and drug load are presented in Fig. 2. The weight distribution of the different pellet formulations is presented in Fig. 3, indicating that a pellet yield (710–1400 μm fraction) higher than 90% could be obtained. For formulations with a low HCT load, maximal yield was obtained at the highest binder level since the binder increased the mechanical strength of wet extrudates and consequently fewer fines were formed during spheronisation. Similar results were reported in our previous study with UNI-PURE® EX starch-based pellets [18] and by Agrawal et al. (1994), who used HPMC as a binder in chitosan-based pellets [29]. Using a higher water level did not influence pellet yield, but the amount of fines was reduced in favour of the larger pellet fraction (agglomerates). At the highest HCT load and low water level, pellet yield was high, irrespective of the binder level. Furthermore, at the same (low) binder concentration, higher HCT amounts significantly increased pellet yield due to the difference in morphology between HCT and starch particles: spherical UNI-PURE® EX starch particles (Fig. 4a) yielded mechanically weaker extrudates and an adhesive binder (HPMC) was required to increase the mechanical strength of wet extrudates. In contrast, the larger and irregular HCT particles (Fig. 4b) can significantly improve the mechanical strength of the wet extrudates via mechanical interlocking of particles [26]. At high water levels (Fig. 2b) pellet yield was lower for a higher HCT load due to pellet agglomeration. Previous work with UNI-PURE® EX [18] showed that sorbitol improved mechanical strength of the wet extrudates and increased pellet yield due to its interaction with starch molecules, but in this study the effect of sorbitol on pellet yield was not significant ($P > 0.05$).

A mathematical correlation between the experimental design variables and pellet sphericity (aspect ratio and shape factor) could not be established. This was not surprising because spheronisation speed (together with the amount of granulation liquid and spheronisation time) is the most important factor determining pellet sphericity [30]. Since a constant spheronisation speed was used (selected based on the previous study [18]), the formulation variables in this experimental design had a limited effect on

Table 3
ANOVA results for pellet yield and size

Source	*P* value	Source	*P* value
Yield			
Model	<0.0001	*AB*	0.0001
A: HCT	0.0384	*AD*	0.0009
B: HPMC	0.0001	Curvature	0.0009
D: water	0.0003		
R^2	0.9000		
Adjusted R^2	0.8616		
Mean Feret diameter			
Model	0.0005	*AD*	0.0259
A: HCT	0.0012	Curvature	0.2259
D: water	0.0067		
R^2	0.6794		
Adjusted R^2	0.6153		

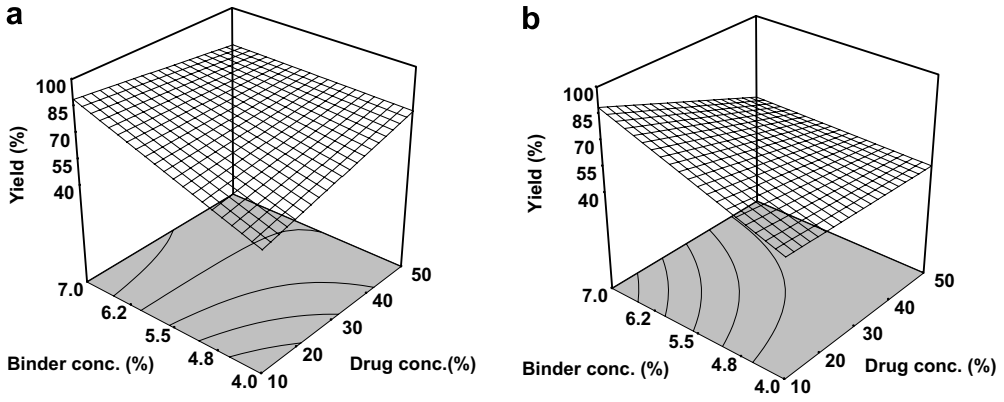

Fig. 2. 3D diagrams of pellet yield as a function of binder and drug level for formulations without sorbitol and different water levels: (a) low water level and (b) high water level.

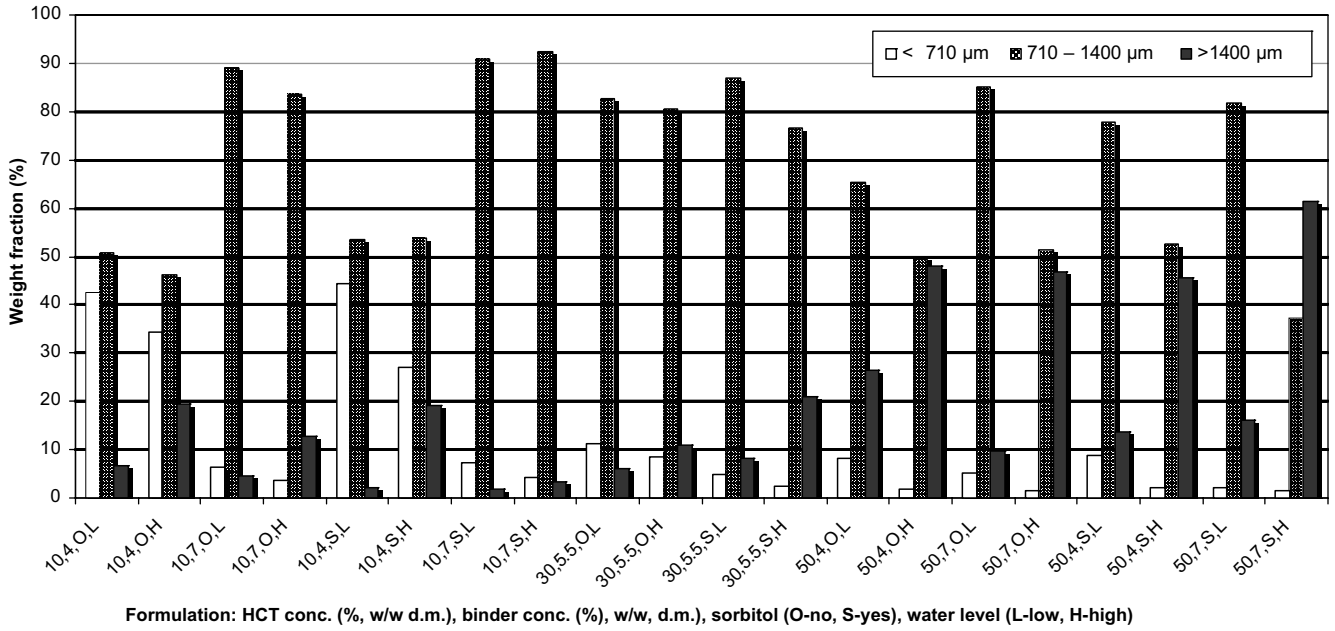

Fig. 3. Weight distribution (%) of pellet formulations containing different HCT (10%, 30% and 50% w/w, dry mass), binder (4%, 5.5% and 7%, w/w, dry mass), sorbitol (yes or no) and water (low or high) level.

pellet sphericity. All pellets had an aspect ratio between 1.1 and 1.2 and a two-dimensional shape factor >0.5, both complying with the ranges for acceptable pellet sphericity defined by Chopra et al. [31].

The results of ANOVA for pellet size are presented in Table 3. The model was significant ($P < 0.05$) and the predicted R^2 value was in reasonable agreement with the adjusted R^2 value. The curvature was not significant ($P > 0.05$), indicating the linearity of response plots. Drug and water level were significant ($P < 0.05$) as well as their interaction. The regression equation for pellet size in terms of the coded values is the following:

$$\text{Pellet size } (\mu\text{m}) = 1137.24 + 42.51 * A + 30.02 * D + 26.42 * A * D \quad (4)$$

The mean pellet diameters ranged from 1000 to 1300 μm for all batches. Interaction diagrams of pellet size (Fig. 5) showed that a higher water concentration generated larger pellets for a higher HCT load due to particle agglomeration during spheronisation.

The residual moisture content determined by Karl Fischer titration was for all pellet batches within the range from 3.6% to 7.8%. Generally, pellets with a lower HCT load had a higher residual moisture content (6.2–7.8%) than pellets with a higher HCT concentration (3.6–4.4%), irrespective of binder, sorbitol or water level. This was due to a higher starch content in formulations with low HCT load since the hydrophilic starch polymer has a higher residual moisture content (9.3% vs. 0.8% for HCT). Friability of all batches was less than 0.01% since the solid HPMC bridges formed during drying yielded pellets with high mechanical strength [32]. Due to disintegrating properties of UNI-PURE® EX starch as the main excipient, disintegration time of all batches was between 5 and 10 min.

In-vitro drug release profiles (Fig. 6) showed that more than 80% HCT was released in 30 min for all formulations, while MCC-based pellets released less than 40% HCT after 75 min. This significant difference in drug release profiles was due to disintegration of starch-based pellets, which ensures fast exposure of the poorly soluble drug to the dissolution medium. Drug release was slightly faster for formulations containing sorbitol due to its high solubility in water. Initial drug release was influenced by the binder level only for pellets loaded with lower HCT concentration. Nevertheless, immediate release of the poorly soluble drug was obtained, irrespective of the composition.

Piroxicam was used as a second poorly soluble model drug in this study. Since it was incorporated at a low concentration (2.5%, w/w, dry mass), pellet disintegration becomes even more critical to obtain fast drug release (compared to non-disintegrating MCC matrix). Pellet yield for all piroxicam formulations was around 90%, sphericity was acceptable (between 1.12 and 1.14), pellet friability was below 0.01% and pellet size (mean Feret diameter) varied between 1000 and 1100 μm. Dissolution profiles of piroxicam pellets (Fig. 7) showed that more than 90% piroxicam was released within 45 min from starch-based pellets, while only 30% piroxicam was released from MCC-based pellets at the same time. Furthermore, addition of sorbitol increased piroxicam release: more than 90% of piroxicam was released in only 30 min, since sorbitol-containing formulations had a shorter disintegration time (10 min, vs. 15 min for starch-based pellets without sorbitol). Furthermore, since piroxicam powder is a very fine

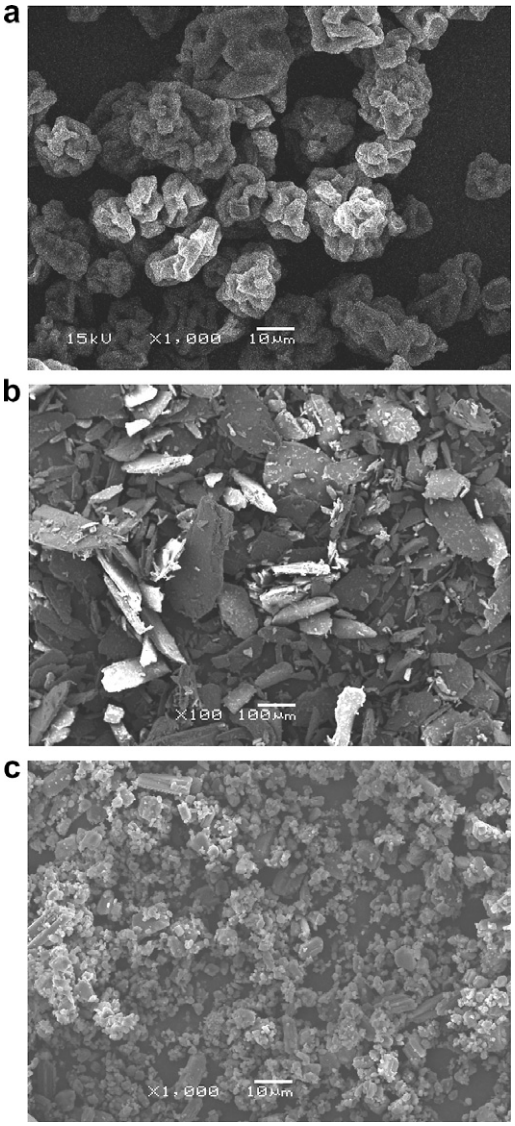

Fig. 4. Scanning electron micrographs of: (a) UNI-PURE® EX starch, (b) hydrochlorothiazide and (c) piroxicam powder.

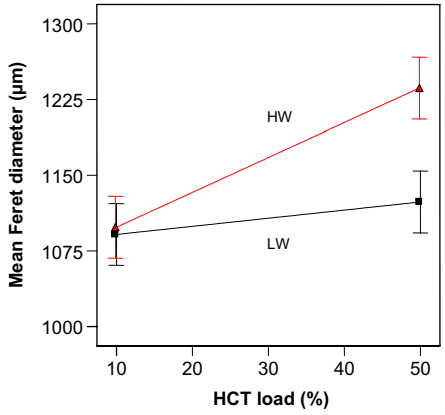

Fig. 5. Interaction diagram of pellet size (mean Feret diameter, µm) as a function of HCT load (%) and water level (LW – low water level; HW – high water level).

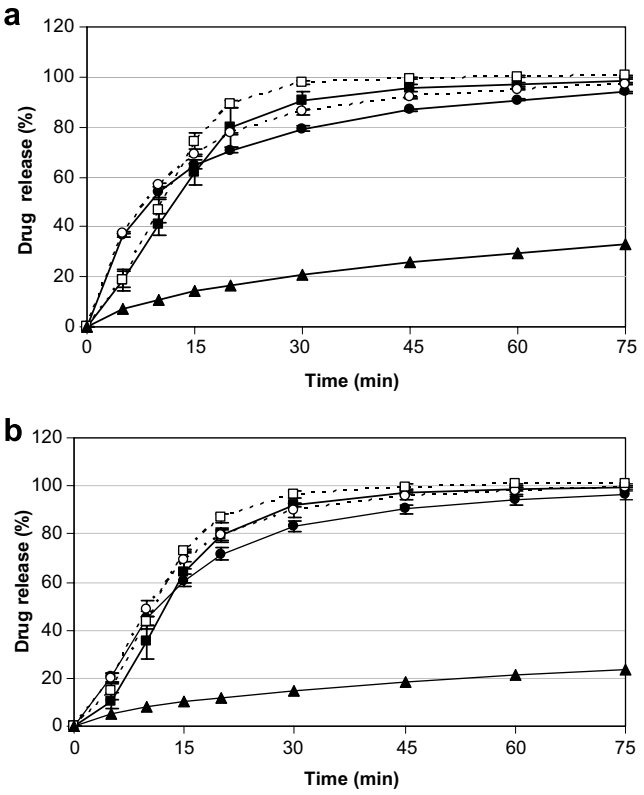

Fig. 6. Hydrochlorothiazide (HCT) release profiles of pellets containing different amounts of a binder and sorbitol (—●— 4% HPMC; —■— 7% HPMC; ---○--- 4% HPMC and sorbitol; ---□--- 7% HPMC and sorbitol, —▲— MCC pellets): (a) pellets containing 10% (w/w, dry mass) and (b) 50% HCT (w/w, dry mass).

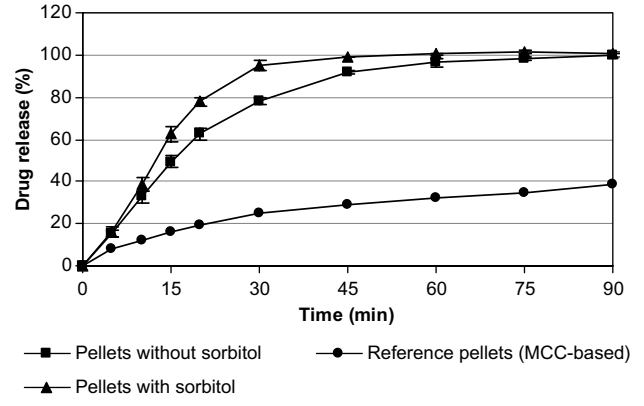

Fig. 7. Dissolution profiles of piroxicam from starch-based pellets with (▲) and without (■) sorbitol compared with MCC-based pellets (●).

(D(v,0.5) = 9.9 µm) and cohesive powder (Fig. 4c), agglomeration of these hydrophobic particles would reduce the effective surface area available for dissolution. The addition of a hydrophilic component (sorbitol) might increase the effective surface area of piroxicam particles possibly by hydration and wetting of piroxicam particles surface during granulation of the powder mixture and drying, thereby improving immediate-release properties of piroxicam [33,34].

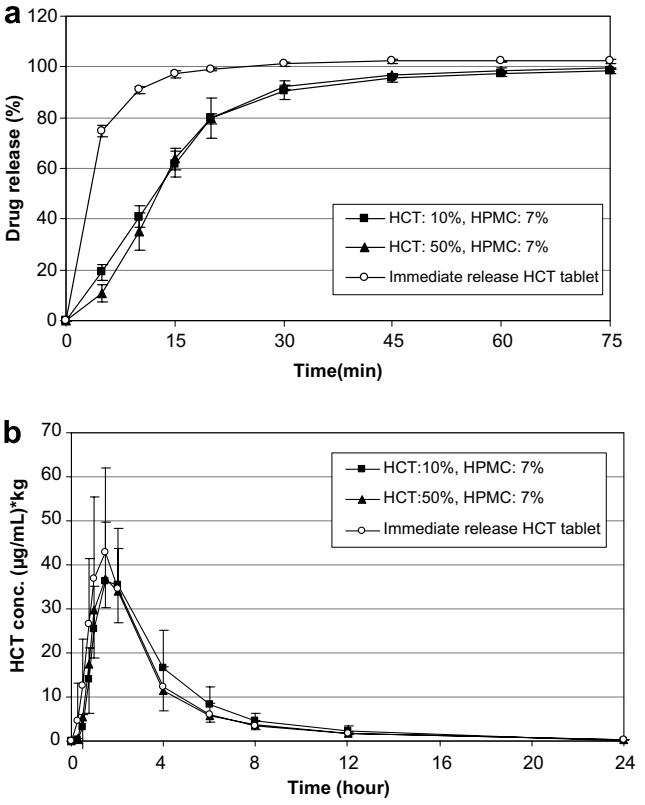

Fig. 8. In-vitro HCT release (a) and mean HCT plasma concentration–time profiles (±SD; $n = 6$) obtained after administration of an oral dose of 50 mg HCT (b) for pellets containing 10% HCT (—■—), 50% HCT (—▲—) and for immediate-release HCT tablets (—○—).

Table 4
Mean $AUC_{0\rightarrow 24\ h}$, C_{max}, t_{max} and F_{Rel} values (±SD) after oral administration of 50 mg to dogs ($n = 6$)

Formulation	$AUC_{0\rightarrow 24\ h}$ (µg h kg/mL)	C_{max} (µg kg/mL)	t_{max} (h)	F_{Rel} (%)
1. HCT: 10%	149 (±42)[a]	40 (±11)[a]	1.7 (±0.4)[a]	109 (±35)
2. HCT: 50%	131 (±21)[a]	38 (±8)[a]	1.6 (±0.4)[a]	98 (±29)
3. Immediate-release HCT tablet	141 (±39)[a]	46 (±18)[a]	1.3 (±0.5)[a]	

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

An *in-vivo* study was performed to compare the bioavailability of two hydrochlorothiazide pellet formulations against fast-disintegrating immediate-release Esidrex®-tablets as a reference (relative bioavailability, F_{Rel}). The absorption of hydrochlorothiazide is limited to the upper part of intestine (duodenum) [35], which combined with its poor solubility in water indicates possible bioavailability problems [36]. Therefore, fast dissolution of hydrochlorothiazide is essential for obtaining its maximal concentration at the absorption site. Fig. 8a presents in-vitro dissolution profiles of the formulations used in the *in-vivo* study, while Fig. 8b presents the mean ($n = 6$) plasma HCT concentration vs. time profiles of both pellet formulations and the immediate-release tablet, while Table 4 summarises the pharmacokinetic parameters. No statistically significant differences of $AUC_{0\rightarrow 24\ h}$, C_{max} and t_{max} were detected between pellet and reference formulations ($P > 0.05$, repeated measures univariate test), indicating that similar drug concentrations were available at the absorption site after administration of disintegrating pellets compared to immediate-release tablets. Relative bioavailabilities (F_{Rel}) of both pellet formulations were similar.

4. Conclusion

Due to pellet disintegration, fast dissolution of poorly soluble drugs such as hydrochlorothiazide and piroxicam was achieved (>80% drug release in 30 min) when using UNI-PURE® EX starch as the main excipient in pellet formulations prepared via extrusion/spheronisation. Pellets with a high yield and acceptable sphericity were obtained. The bioavailability in dogs of orally administered hydrochlorothiazide pellets was similar to that of fast-disintegrating immediate-release hydrochlorothiazide tablets.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2007.04.014.

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