GHENT UNIVERSITY
FACULTY OF PHARMACEUTICAL SCIENCES
LABORATORY OF PHARMACEUTICAL TECHNOLOGY

MODIFIED STARCH AS AN EXCIPIENT FOR PELLETS PREPARED BY MEANS OF EXTRUSION/SPHERONISATION

Aleksandra DUKIĆ-OTT

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Promoters:
Prof. Dr. C. VERVAET
Prof. Dr. J. P. REMON
Laboratory of Pharmaceutical Technology - Ghent University

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AIM OF THE STUDY

The interest of researchers in pellets as multiparticulate solid dosage forms derives from several important advantages of those multi-unit forms over conventional, single-unit solid dosage forms (tablets). Extrusion/spheronisation is one of the most established techniques for production of pellets with high quality. Due to the fact that it is a multi-step process, a number of process parameters should be controlled. Furthermore, formulation development is of special concern since the moistened mass needs to possess specific characteristics in order to be successfully extruded and spheronised.

Microcrystalline cellulose is the most widely used excipient for the production of pellets via extrusion/spheronisation. Due to some important disadvantages, several excipients have been already described in literature as possible alternatives.

The goal of this study was to evaluate the potential of a modified starch (high-amylose, crystalline and resistant starch) as an alternative to microcrystalline cellulose in pellet production via extrusion/spheronisation. Several issues have been addressed:

- Evaluation of formulation suitability for production of pellets with acceptable quality.
- Elucidation of the influence of process parameters on pellet properties during extrusion/spheronisation.
- Optimisation of process parameters and pellet formulation by means of surface response methodology.
- Influence of model drug solubility and concentration on in-vitro drug release from immediate-release and enteric-coated pellet formulations.
- Bioavailability of model drugs from immediate-release and enteric-coated pellet formulations after oral administration to dogs.

1

INTRODUCTION

1.1 Pellets as solid dosage forms and preparation methods

Pellets are spherical or nearly spherical, free-flowing granules with a narrow size distribution, typically varying between 500 and 1500 µm for pharmaceutical applications (Ghebre-Sellassie, 1989a). They are generally produced via a pelletisation process, whereby a powder blend consisting of an API and excipient particles is agglomerated into spherical granules. After being produced, pellets are usually filled into hard gelatine capsules or compressed into tablets. Furthermore, they can be formulated as immediate-release dosage forms or coated in order to sustain drug release over a longer period of time or to deliver a drug to a specific site of action in the gastrointestinal tract.

The multiparticulate nature of pellets offers some important pharmacological as well as technological advantages over conventional “single-unit” solid dosage forms (Bechgaard and Hagermann, 1978). Consequently, the interest of researchers in this dosage form increases continuously (Ghebre-Sellassie and Knoch, 2002). The main advantages are:

- Particles smaller than 2-3 mm are rapidly emptied from the stomach regardless of the feeding state of the patient and the influence of gastric emptying rate on the upper gastrointestinal transit time of pellets is minimised (Follonier and Doelker, 1992). Consequently, the intra- and inter-subject variability of drug plasma profiles are lower compared to single-unit formulations (Krämer and Blume, 1994).
- Uniform dispersion of a drug into small dosage units reduces the risk of high local drug concentration and their potentially irritating effect on gastric mucosa. Furthermore, drug absorption is maximised and peak plasma fluctuations are reduced (Ghebre-Sellassie, 1989a).
- In case of coated multiparticulates, every pellet acts a single drug reservoir with its own release mechanism. Any coating imperfection would therefore only affect the release of a

small drug portion, in contrast to complete “dose dumping” from a single-unit drug reservoir (Bechgaard and Hagermann, 1978).

- Pellets offer the possibility of combining several active components, incompatible drugs or drugs with different release profiles in the same dosage unit.
- Dosage forms with different dose can be produced from the same batch by adjusting the filling weight of pellets (Ghebre-Sellassie and Knoch, 2002).
- Owing to their smooth surface morphology, narrow size distribution, spherical shape, low friability and improved hardness pellets can be easily coated; they have good flow properties which ensure reproducible die or capsule filling and consequently good content uniformity; dust formation is minimised and therefore all processing operations are facilitated (Erkoboni, 2003).

Several methods are used for pellet preparation. The most widely used techniques can be classified based on the equipment type or incorporation method of active ingredient (Table 1.1). Those and other pelletisation techniques are reviewed in detail by Ghebre-Sellassie and Knoch (2002).

- **Solution/suspension layering** involves the application of a drug/binder solution or suspension to solid cores which can be either inert materials (e.g. sugars) or granules/crystals of the same drug (Ghebre-Sellassie and Knoch, 2002). Next to the more traditional drum/pan coaters (Zhang et al., 1990, 1991) fluidised bed equipment with conventional top spray, Würster bottom spray or rotor tangential spray is used to produce pellets (Jones, 1994).

Table 1.1 *Pelletisation techniques according to equipment type and incorporation method of active ingredient (Adapted from Pišek, 2002).*

PELLETISATION TECHNIQUES			
→ incorporation method of active ingredient		*→ equipment type*	
Layer	*Powder layering* *Suspension/solution layering*	***Fluidised bed***	*Powder layering* *Suspension/solution layering* *Direct pelletisation*
Matrix	*Direct pelletisation* *Extrusion/spheronisation* *High-shear pelletisation*	***Drum/pan coater***	*Powder layering* *Suspension/solution layering*
		High-shear mixer	*High-shear pelletisation*
		Extruder and spheroniser	*Extrusion/spheronisation*

- **Powder layering** comprises the deposition of successive layers of dry powder (drug and excipients) on inert materials with the help of a binding liquid (Ghebre-Sellassie and Knoch, 2002). The equipment mostly used is a rotor tangential spray fluid-bed (Vupppala et al. 1997), although traditionally drum/pan coaters were also used.
- **Direct pelletisation** is usually performed using rotor tangential spray fluid-bed equipment (direct rotor pelletisation), but conventional fluid-bed granulators can be also used (Kleinebudde and Knop, 2007). No seeding material is necessary, since a powder mixture of a drug and excipients is wetted with an agglomeration liquid, followed by pelletisation by means of a rotating disc (Pišek et al. 2001; Kristensen et al., 2002; Liew et al. 2007). According to the nature of the liquid binder, direct pelletisation can be described as wet (a binder is added in liquid phase at room temperature) or melt (a molten binder is added) pelletisation (Kleinebudde, 2007). Direct pelletisation in fluid-bed processes has been recently described in detail by Kleinebudde and Knop (2007).
- **High-shear pelletisation** comprises agglomeration of powdered material using a high-shear mixer. A binder can be added as a liquid (wet pelletisation) or melted before or during the process (melt pelletisation) (Zhou et al. 1996, 1997).
- **Extrusion/spheronisation** method involves several distinct preparation phases: a uniform powder mixture of drug and excipient(s) is initially wet massed by addition of a liquid binder, followed by pressing of the moistened mass through an extrusion screen (extrusion) to form cylindrical extrudates, which are subsequently broken into smaller cylindrical rods and rounded into spherical granules by means of a fast-rotating friction plate (spheronisation) and finally dried.

1.2 Extrusion and spheronisation process

In the pharmaceutical industry, production of pellets via extrusion/spheronisation was first described by Conine and Hadley (1970) and Reynolds (1970). Compared to other pelletisation methods, extrusion/spheronisation is especially suitable in cases where the active component is highly dosed (Hileman et al., 1993a,b; Lambert et al., 1995; Sonaglio et al., 1997; Podczeck and Knight, 2006) or when a high process efficiency is needed since the production time is relatively short. The pellets manufactured via extrusion/spheronisation have a high process yield, narrow size distribution, good sphericity and low friability.

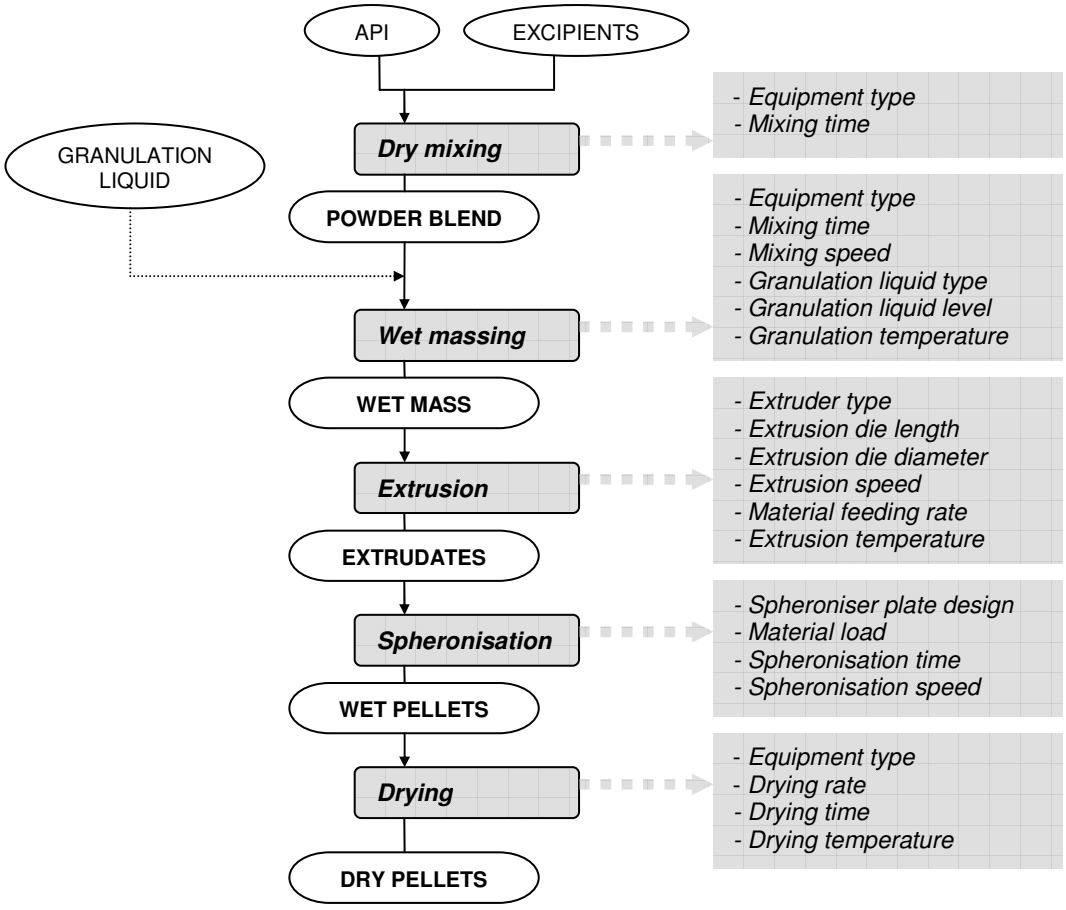

Figure 1.1 *Extrusion/spheronisation flow chart with the corresponding process parameters.*

As mentioned previously extrusion/spheronisation is a multi-step process (Fig. 1.1). Each phase of the process (except for dry mixing and wet massing which are often performed in

the same equipment type) requires highly specialized equipment, which can be a disadvantage in terms of expenses. Furthermore, each production step is a distinct process and involves control over a number of process parameters in order to obtain pellets of required quality. In recent years a lot of research was also dedicated to the influence of formulation variables on the success of extrusion/spheronisation.

1.2.1 Dry mixing and wet massing

Obtaining a uniformly blended dry powder mix of active ingredient(s) and excipient(s) is the first step in any process involving agglomeration of particles. Dry mixing, followed by wet massing or granulation is usually performed in the same equipment (batch-type mixer/granulators). Commonly used types reported in the literature are: planetary mixers (reviewed by Vervaet et al., 1995), high-shear mixers (Lövgren and Lundberg, 1989; Baert et al. 1991; Elbers et al., 1992) and sigma blade mixers (Woodruff and Nuessle, 1972).

The wet massing step of extrusion/spheronisation involves the addition of a granulation liquid in much higher amounts than those required for conventional granulation (Newton, 2002). The amount of granulation liquid has a crucial role in the success of extrusion and spheronisation and should therefore be included as a variable during formulation development (Kleinebudde, 1995). In addition, temperature generation during wet massing can promote water evaporation and significantly influence pellet properties (Baert et al., 1991).

Wet massing by means of a continuous granulator was described by Hellén et al. (1993a,b) and Hellén and Yliruusi (1993). Moreover, a twin-screw extruder, where granulation and extrusion is performed in a single step was described by several authors (Gamlen and Eardley, 1986; Lindberg et al., 1987a,b, 1988; Kleinebudde and Lindner, 1993; Kleinebudde et al., 1994; Kleinebudde, 1995). Schmidt and Kleinebudde (1999) evaluated the influence of granulation on pellet quality by comparing different equipment used for wet massing.

1.2.2 Extrusion

The extrusion phase comprises forcing of the wet plastic mass through a small orifice (extrusion die), thus forming cylinders or strands with a breadth corresponding to the die

diameter and a length which depends on material properties and extruder type (Hicks and Freese, 1989).

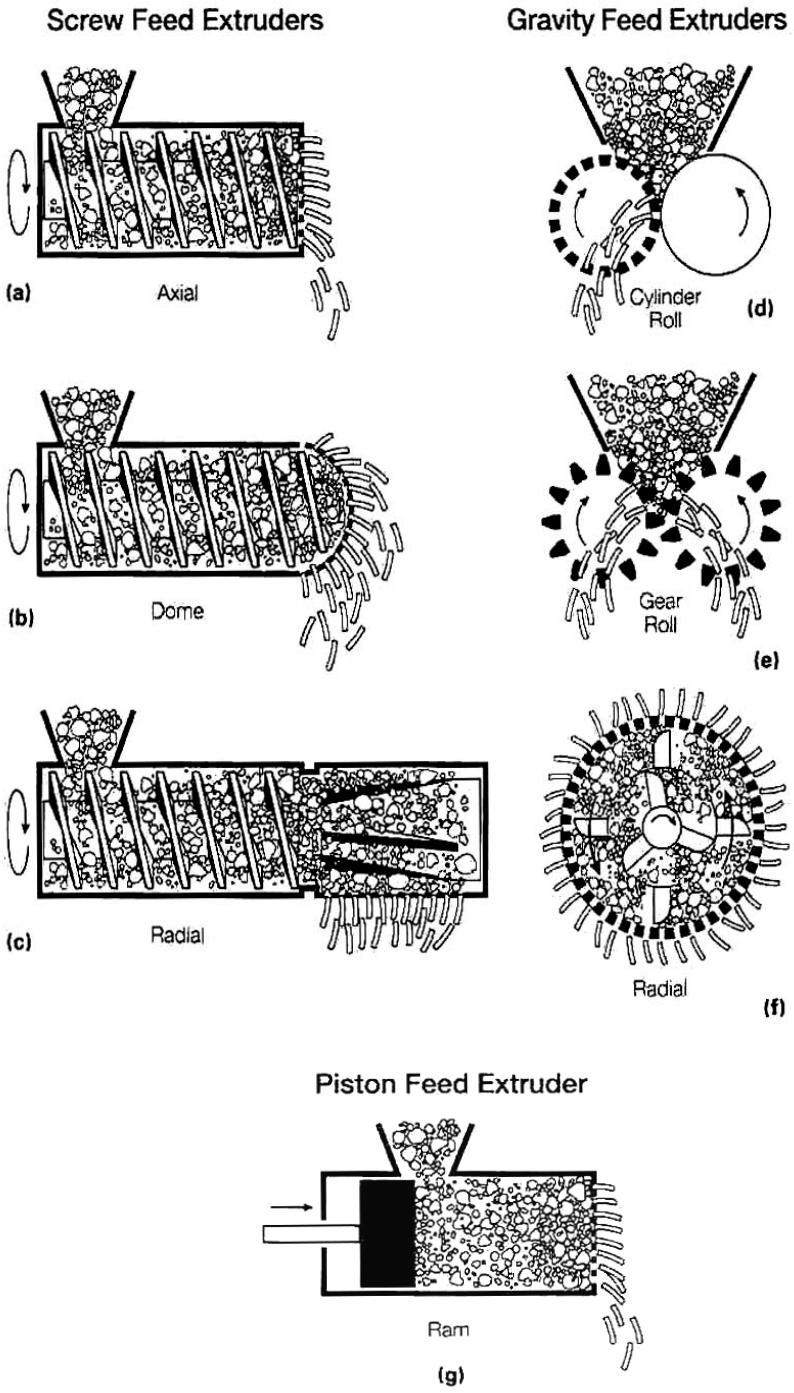

Figure 1.2 *Schematic diagram of extruder types used in extrusion/spheronisation: screw feed (**a.** axial-, **b.** dome- and **c.** radial- type), gravity feed (**d.** cylinder-, **e.** gear- and **f.** radial- type) and piston feed (**g.** ram) extruders (Erkoboni, 2003).*

Extruders have a part which transports the wet mass towards the extrusion screen and a die which shapes the extruded material (Newton, 2002). Several extruder types are used. Different authors classified extruders based on several criteria: (a) Rowe (1985) classified them into screw-, gravity- and piston-type extruders (Figure 1.2) based on the material feeding mechanism; (b) Hicks and Freese (1989) grouped them into four types (screw; sieve and basket; roll and ram extruders) and (c) recently Wilson and Rough (2007) classified them into extruders with pumping (ram, axial screw) and wiping action (sieve and basket, roll, radial screen).

- **Screw feed extruders** consist of one or two rotating screws which push the moistened mass from the material feeding zone towards the extrusion screen. Based on the extrusion screen design, screw feed extruders are classified into axial-, dome- or radial-types. The major advantages of screw feed extruders are a higher throughput rate, ease of changing different screen types and ease of cleaning (Trivedi et al., 2007).
- In **gravity feed extruders** the wet mass is transported towards the extrusion screen by means of gravitational force and several types are in use: rotary cylinder, rotary gear and radial.
- **Piston feed extruders** or ram extruders are mainly used as laboratory extruder (Erkoboni, 2003) or for extrusion of specialized materials which require strict in-process control (Trivedi et al., 2007).

The influence of the extruder type, extrusion screen geometry and extrusion speed on extrudate and pellet properties has been reviewed extensively by several authors (Hicks and Freese, 1989; Vervaet et al., 1995; Newton, 2002; Erkoboni, 2003; Wilson and Rough, 2007).

1.2.3 Spheronisation

A spheroniser consists of a bowl with a stationary cylindrical wall and a fast-rotating bottom plate with grooved surface to increase the friction. During the initial stage of spheronisation extrudates are broken into small cylinders and after a relatively short period of time spherical pellets are formed. The spheronised material moves outwards to the wall due to centrifugal forces, followed by collision and climbing up the stationary wall. Then the particles fall back onto the rotating disk which due to its angular motion pushes the mass again towards the wall, creating a typical “rope-like” formation (Fig. 1.3) which is considered crucial for successful spheronisation (Reynolds, 1970).

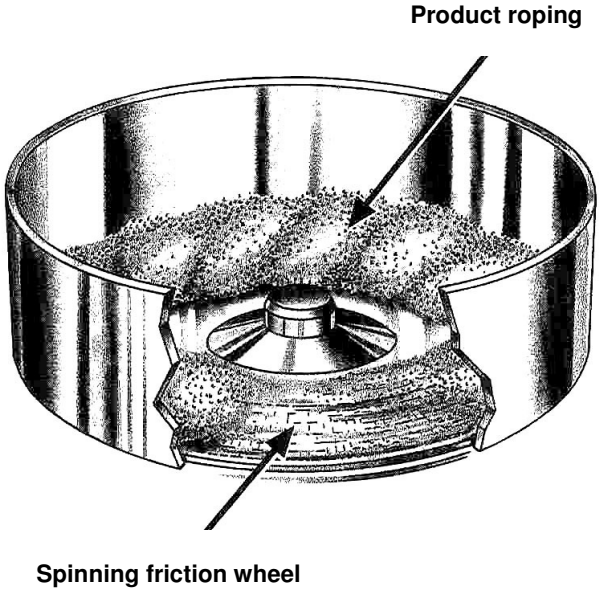

Figure 1.3 *Schematic representation of the "rope-like" motion during spheronisation (Erkoboni, 2003).*

The transition of cylindrical into spherical shape occurs via several stages, as proposed by two models: Rowe (1985) suggested that after the initial breaking of the extrudates, cylinders are initially rounded at the edges, followed by formation of dumbbell-like granules and finally spherical pellets are formed (Fig. 1.4 a), while Baert and Remon (1993) described an alternative model where cylinders are rounded at the edges but additionally bent, followed by twisted dumbbell formation which initiates particle breaking into two parts with a cavity on their flat side and further rounding into spheres (Fig. 1.4 b). Which mechanism will dominate most likely depends on the formulation, while granulation liquid level and spheronisation process parameters influence whether the spheronisation step will result in pellets with a broad size distribution, dumbbells, agglomerated material or spherical granules with a narrow size distribution (Erkoboni, 2003).

The main spheronisation variables affecting pellet characteristics are: material load, residence time, spheroniser type, geometry of spheronisation plate, peripheral velocity (rotational speed of the friction plate combined with the plate diameter). Their influence has been reviewed in detail by Hicks and Freese (1989), Vervaet et al. (1995) and Erkoboni (2003).

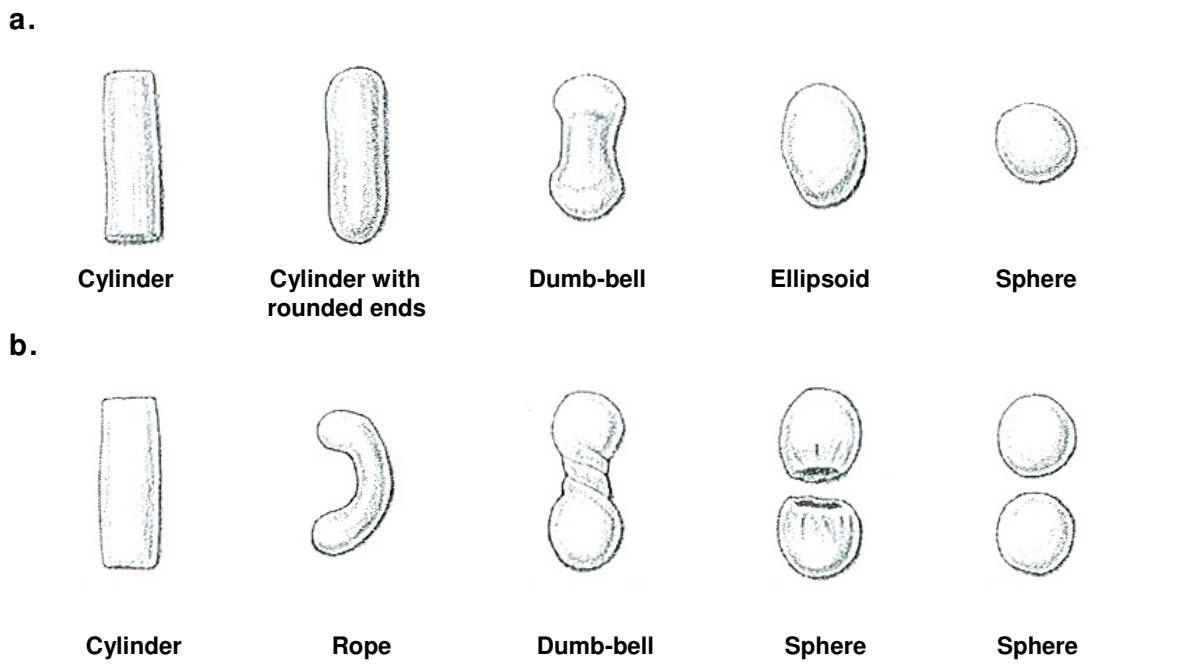

Figure 1.4 *Schematic representation of different pellet formation stages during spheronisation according to two models proposed by:* ***a****. Rowe (1985) and* ***b****. Baert and Remon (1993) - adapted from Erkoboni (2003).*

1.2.4 Drying

Wet pellets are mostly dried in an oven or fluid-bed, although micro-wave and freeze-drying have been also used to study the influence of drying method on pellet properties. The main differences between oven and fluid-bed drying are the rate of granulation liquid evaporation and the way how the material is handled during drying: during oven drying in a static bed liquid evaporates from the material over longer period of time, while during fluid-bed drying the turbulent motion of dried material in a heated air stream promotes significantly faster drying (Lieberman and Rankell, 1970). Several authors studied the influence of different drying techniques on pellet characteristics (Bataille et al., 1993; Dyer et al., 1994; Kleinebudde, 1994; Sousa et al., 1996; Berggren and Alderborn, 2001a,b; Pérez and Rabišková, 2002; Bashaiwoldu et al., 2004; Lutchman et al., 2005; Song et al., 2007).

1.2.5 Binding forces in relation to pellet formation

Since pelletisation is an agglomeration process, there are several binding mechanisms which can contribute to pellet formation (Ghebre-Sellassie, 1989b). Those mechanisms, as defined by Rumpf (1962) can be divided into five groups:

- **Solid bridges** (formed by crystallization of dissolved particles or binder hardening) which determine the strength of the dried pellets.
- **Adhesion and cohesion forces in immobile liquid bridges**, formed by viscous binders.
- **Interfacial forces and capillary pressure in movable liquid surfaces**, created after addition of the granulation liquid. When increasing the liquid saturation level in interparticulate voids, the agglomerates transform over several stages: (a) *pendular* stage where adhesion originates from surface tension of the liquid and the negative suction pressure in the liquid bridges (liquid saturation is below 25%); (b) intermediary *funicular* stage*;* (c) *capillary* stage where all the void space is filled with liquid (liquid saturation is above 80%), granule strength is maximal and the bonding forces mainly originate from negative capillary pressure; (d) the *droplet* stage where the liquid completely envelopes the agglomerate (Ghebre-Sellassie, 1989b).
- **Mechanical interlocking bonds**, originating from fibrous, lamellar (flat-shaped) or bulky materials. This kind of bonds can occur if compression and shear forces are acting in the system (Pietsch, 1991).
- **Attraction forces between solid particles** (molecular – van der Waals' forces, electrostatic and magnetic forces).

During pelletisation, a uniformly blended powder mixture is granulated with a liquid and the strength of the agglomerates depends on the liquid saturation level, the granule strength being maximal in the capillary state, as described previously. The granulate strength can be additionally increased using more adhesive (viscous) binders. The main wet mass densification occurs via extrusion and the resulting extrudates are brought together by capillary forces, mechanical interlocking (due to irregularities in particle shape) solid bridge formation (via solvent evaporation) and molecular forces (Ghebre-Sellassie, 1989b). During spheronisation moisture migrates towards the surface of the particles, thereby providing additional plasticity for rounding of the pellets. Additionally, crystallization of dissolved particles due to solvent evaporation can contribute to solid bridge formation. Drying is the final phase where solvent is completely removed via evaporation and the pellet strength is mainly related to solid bridge formation.

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2

EXCIPIENTS IN EXTRUSION/SPHERONISATION

2.1 Introduction

Not every moistened powder mixture can be successfully extruded and spheronised. Newton (2002) defined the requirements for a wet mass suitable for extrusion and spheronisation in the following way:

"*Extrusion mixtures are formulated to produce a cohesive plastic mass that remains homogeneous during extrusion. The mass must possess inherent fluidity, permitting flow during the process and self-lubricating properties as it passes through the die. The resultant extrudate must remain nonadhesive to itself and retain the degree of rigidity so that the shape imposed by the die is retained. (…)*

The requirements for spheronisation of the cylindrical extrudate are as follows:

1. The extrudate must possess sufficient mechanical strength when wet, yet it must be brittle enough to be broken down to short lengths in the spheroniser, but not so friable that it disintegrates completely. (…)

2. The extrudate must be sufficiently plastic to enable the cylindrical rods to be rolled into spheres by the action of the friction plate in the spheroniser.

3. The extrudate must be nonadhesive to itself in order that each spherical granule remains discrete throughout the process."

In relation to the above mentioned requirements, formulation variables like the granulation liquid type and concentration, as well as the API and excipients properties (including concentration, solubility and particle size distribution) significantly influence the pellet properties. In the following paragraphs, an overview of the commonly used excipients and their influence on pellet properties like sphericity, size distribution, mechanical strength and drug release will be presented.

2.2 Granulation liquid level and type

As mentioned previously, granulation liquid is an extremely important formulation parameter during extrusion/spheronisation and for a given formulation only a relatively narrow concentration range is optimal for the process. A sufficiently wetted mass possesses the plasticity and cohesiveness required to obtain spherical pellets with a narrow size distribution. On the one hand, insufficient moisture gives rise to generation of fines and insufficiently round pellets (dumb-bell formation), while a too high moisture content promotes uncontrolled pellet agglomeration and broadening of the pellet size distribution (Newton, 2002; Erkoboni, 2003). Water is the most often used granulation liquid in extrusion/spheronisation. Next to its role as binder during wet massing, water acts as a lubricant during the extrusion phase and provides sufficient plasticity of the extrudates for successful rounding during spheronisation (Hileman et al., 1993b).

Many authors reported on the influence of water level on pellet properties. Within the acceptable liquid concentration range for extrusion/spheronisation, a higher water level improved sphericity, narrowed pellet size distribution and increased mean pellet diameter (Malinowski and Smith, 1975; Lövgren and Lundberg, 1989; Bains et al., 1991; Pinto et al., 1992; Hasznos et al., 1992; Baert et al., 1993; Wan et al., 1993; Hileman et al., 1993b; Otsuka et al., 1994; Sognalio et al., 1995; Umprayn et al., 1995; Sousa et al., 1996; Varshosaz et al., 1997), reduced pellet friability (Reynolds, 1970; Malinowski and Smith, 1975; Otsuka et al., 1994 Varshosaz et al., 1997), improved pellet surface properties (Hellén et al., 1993a) and prolonged drug release due to increased pellet density and hardness (Baert and Remon, 1993; Varshosaz et al., 1997). Water level in relation to extrusion/spheronisation was studied by several authors: Harison et al. (1984, 1985a,b) used a ram extruder to study the mass flow during extrusion and its relation to water content, formulation and extrusion process variables, while Baert et al. (1991,1992), Elbers et al. (1992), Kleinebudde and Lindner (1993) and Kleinebudde et al. (1994) reported a reduction of torque or power consumption of the extruder with increasing water level.

The optimal water level is also related to drug and excipient properties like solubility, particle size and concentration in the powder mixture. Elbers et al. (1992), Wan et al. (1993) and Hileman et al. (1993a,b) reported that the optimal water level was proportional to the MCC concentration due to its high water retaining capacity (Fielden et al., 1988, 1992a). In contrast to these authors, Bains et al. (1991) reported a higher optimal water level for barium sulphate/MCC mixtures with a lower MCC content due to the poor water

solubility, particle size and morphology of barium sulphate powder. Furthermore, including water soluble excipients (Baert et al, 1992) or active ingredients (Hileman et al. 1997; Lustig-Gustafsson et al., 1999) in the powder mixture reduces the optimal water level due the contribution of the dissolved fraction to the liquid phase. Fielden et al. (1989, 1992b,c 1993) evaluated the influence of lactose particle size in lactose/MCC-mixtures on extrusion/spheronisation. The authors reported that more water was needed when using a finer lactose particle size. Wan et al. (1993) also concluded that using coarser lactose increased the mean pellet size when the same water level used.

Millili and Schwartz (1990) studied the influence of water/ethanol mixtures as granulation liquid: with an increase of ethanol concentration in the mixture, the mean pellet diameter decreased, while their higher pellet friability and porosity promoted a faster drug release. Schröder and Kleinebudde (1995a) reported about obtaining disintegrating pellets and therefore a faster drug release when using 2-propanol/water mixtures as granulation liquid.

2.3 Microcrystalline cellulose as extrusion/spheronisation aid

Microcrystalline cellulose (MCC) is commonly used as excipient in extrusion/spheronisation (Newton, 2002). In relation to the previously mentioned wet mass requirements, the rheological properties of an MCC-based wet mass are suitable for successful extrusion and spheronisation (Shah et al. 1995) and it is regarded as a spheronisation aid. MCC has good binding properties and provides cohesiveness to the wet mass. Furthermore, it is able to absorb and retain a large quantity of water due to its large surface area and high internal porosity (Sognalio et al. 1995a), thus facilitating extrusion, improving wet mass plasticity and enhancing spheronisation. Moreover, by controlling the movement of water through the plastic mass, it prevents phase separation during extrusion or spheronisation (Fielden et al., 1992b).

Two models have been proposed to explain the behaviour of MCC during extrusion/ spheronisation process:

- In the first model MCC is described as a “molecular sponge” (Fielden et al., 1988; Ek and Newton, 1998). The MCC particles are able to retain water in a similar way as a sponge. During extrusion these sponges are compressed and the water which is squeezed from the internal structures acts as a lubricant. After extrusion, the volume of the sponges increases and they appear dry and brittle, which facilitates the breaking of the extrudates during the initial phase of spheronisation. During the spheronisation phase, the sponges are densified, and water facilitates spheronisation of pellets.
- According to the “crystallite-gel” model, during granulation and extrusion in the presence of water MCC particles are broken down into smaller units and even partly into single crystals of colloidal size. The resulting crystallites and porous particles form a coherent gel-like network (with a high fraction of an insoluble solid phase) and immobilize the granulation liquid. At a specific water content, which relates to a certain gel strength, extrusion and spheronisation becomes possible (Kleinebudde, 1997).

Based on the extensive literature about the use of MCC in extrusion/spheronisation, it can be observed that pellets produced with MCC possess good sphericity, low friability, high density and smooth surface properties. Furthermore, from a processing viewpoint, relatively wide ranges of water content and processing parameters can be employed to provide pellets with acceptable quality, indicating the robustness of the formulations.

In spite of its excellent characteristics as an extrusion/spheronisation aid, in several cases MCC is not considered as the excipient of choice in the production of pellets via

extrusion/spheronisation:

- Drug decomposition in the presence of MCC (Basit et al., 1999) as well as drug adsorption onto the surface of MCC fibres has been reported (Okada et al., 1987; Rivera and Ghodbane, 1994; Al-Nimry et al., 1997).
- Several authors reported about the chemical incompatibility of MCC with a number of drugs (Carstensen et al, 1969; Signoretti et al., 1986; Patel et al., 1988; George et al., 1994; Torres and Camacho, 1994; Brandl et al., 1995).
- O'Connor and Schwartz (1985) reported a prolonged drug release when using poorly soluble drugs in a mixture with MCC. This was attributed to the lack of disintegration of MCC-based pellets. Diffusion through an insoluble inert matrix was therefore proposed as drug release mechanism (O'Connor and Schwartz, 1993; Zimm et al., 1996). Other authors also reported about the influence of drug water solubility on the release from MCC-based pellets (Baert and Remon, 1993; Blanqué et al., 1995; Hileman et al., 1997; Lustig-Gustafsson et al., 1999; Sousa et al., 2002).
- Drug/MCC ratio in the powder mixture also influenced the release of poorly water soluble drugs, being prolonged if the MCC level was higher (Pinto et al., 1982; O'Connor and Schwartz, 1985).
- Pellet properties were influenced by batch-to-batch variability of MCC powders and adjustment of the optimal water content was needed. Furthermore, an effect of MCC powders originating from different supplies on pellet properties has been reported (Sognalio et al., 1995b; Bataille et al., 1997).
- Due to its smooth surface properties and optimal sphericity, MCC-based pellets are suitable for subsequent coating in order to sustain drug release. However, if the goal is to minimize the number production steps, matrix formulations would be more suitable for controlled release applications (Tapia et al., 1993; Jess and Steckel, 2007).
- There is a constant need for reducing the cost of raw materials in pharmaceutical industry.

Taking into consideration the above mentioned limitations of MCC, additional excipients are often included in an MCC-based formulation (Section 2.4). However, in recent years, research has been directed towards the evaluation of potential excipients as spheronisation aids, which would partially or completely substitute MCC in formulations used for extrusion/spheronisation (Section 2.5).

2.4 Excipients used in combination with MCC

Table 2.1 lists the excipients according to their function in MCC-based pellet formulations and the references to literature sources. These materials will be briefly discussed in this section.

Fillers are mainly used in pellet formulations to add bulk, but sometimes they can facilitate extrusion/spheronisation (Harris and Ghebre-Sellassie, 1989). As mentioned previously, water solubility and particle size of the filler influence the optimal water range and pellet properties. The influence of aqueous solubility of the filler on drug release from pellet formulations has also been discussed earlier.

Binders are added to pellet formulations to assure wet mass cohesiveness throughout the pelletisation process and to maintain pellet integrity after production. Although MCC has a binding function, additional binders might be necessary when an active component of unfavorable rheology is highly dosed in the pellet formulation. Funck et al. (1991) successfully used several binders (sodium carboxymethylcellulose, methylcellulose, hydroxypropylcellulose, polyvinylpyrrolidone, carbomer and pregelatinized starch) at 2% (w/w) level in MCC-based formulations with 80% (w/w) of a model drug. Pellet sphericity, friability and ease of processing depended on the binder type, while the drug release was similar for all formulations due to the low binder concentration. Several authors reported on the influence of binder type and concentration on pellet size distribution, sphericity and drug release (Umprayn et al., 1995; Varshosaz et al., 1997; Law and Deasy, 1997; Deasy and Law, 1997; Luchtman et al., 2005). In addition, a mixture of MCC and sodium carboxymethylcellulose was reported in a number of studies as very useful in formulations containing a high drug concentration (Malinowski and Smith, 1975; O'Connor et al., 1984; O'Connor and Schwartz, 1985; Funck et al., 1991; Elbers et al., 1992; Hileman et al., 1993a,b; Lambert et al., 1995).

Mesiha and Vallés (1993) evaluated the usefulness of lubricants, glidants and surface active agents in reducing surface defects, energy consumption and heat generation during extrusion of a highly dosed drug. Surface active agents were the most successful in enhancing extrudability of wet mass.

Table 2.1 *Overview of different classes of excipients combined* ***with MCC*** *in formulations intended for extrusion/spheronisation and references to literature sources.*

Fillers	
Lactose	*Wan et al. (1993), Otsuka et al. (1994), Umprayn et al. (1995), Blanqué et al. (1995), Santos et al. (2002), Sinha et al. (2005)*
Dicalcium diphosphate	*Rodrigues et al. (2001), Sousa et al. (1996, 2002), Santos et al. (2002), Sinha et al. (2005)*
Mannitol	*Hellén et al. (1993a,b), Hellén and Yliruusi (1993), Sousa et al. (2002)*
Starch and derivatives	*Otsuka et al. (1994), Junnila et al. (1998, 2000)*
Glucose	*Sousa et al. (2002)*
β-Cyclodextrine	*Gazzaniga et al. (1998), Santos et al. (2002)*
Binders	
Sodium carboxymethylcellulose	*Malinowski and Smith (1975), O'Connor et al. (1984), O'Connor and Schwartz (1985), Funck et al. (1991), Elbers et al. (1992), Hileman et al. (1993a,b), Lambert et al. (1995)*
Polyvinylpyrrolidone	*Funck et al. (1991), Deasy and Law (1997), Law and Deasy (1997a), Varshosaz et al. (1997), Santos et al. (2002)*
Hydroxypropylmethylcellulose	*Umprayn et al. (1995), Luchtman et al. (2005)*
Methylcellulose	*Funck et al. (1991), Umprayn et al. (1995)*
Hydroxypropylcellulose	*Funck et al. (1991), Mesiha and Vallés (1993), Otsuka et al. (1994), Umprayn et al. (1995)*
Gelatine/starch, gelatine	*Varshosaz et al. (1997), Rodrigues et al. (2001)*
Starch	*Funck et al. (1991), Mesiha and Vallés (1993)*
Silicates	*Law and Deasy (1997b)*
Carbomer	*Funck et al. (1991)*
Chitosan	*Goskonda and Upadrashta (1993), Santos et al. (2002)*
Lubricants / glidants	
Light mineral oil, sodium stearyl fumarate, colloidal silicon dioxide	*Mesiha and Vallés (1993)*
Hydrogenated castor oil	*Law and Deasy (1997b)*
Glycerol behenate (Compritol®)	*Iloanusi and Schwartz (1996)*
Precirol® ato 5, Gelucire® 50/02	*Edimo et al. (1993), Law and Deasy (1997b)*
Disintegrants	
Croscarmellose sodium, sodium starch glycolate	*Souto et al. (2005), Schröder and Kleinebudde (1995b)*
Surface active agents	
Sodium lauryl sulphate	*Mesiha and Vallés (1993), Edimo et al. (1993), Law and Deasy (1997a,b), Deasy and Law (1997)*
Polysorbate 80, glyceryl and sorbitan mono-oleate, sorbitan mono-palmitate	*Mesiha and Vallés (1993), Junnila et al. (1998)*
Glycerol monostearate	*Blanqué et al. (1995)*
Self-emulsifying systems	*Newton et al. (2001, 2005), Tuleu et al. (2004)*
Miscellaneous	
pH adjusters	*Bianchini et al. (1992), Law and Deasy (1997b)*
Release modifiers	*Bianchini et al. (1992), Gouldson and Deasy (1997)*
MCC co-processed with hydrophilic polymers	*Law and Deasy (1998)*
Cosolvents (PEG)	*Vervaet et al. (1994), Blanqué et al. (1995)*

Iloanusi and Schwartz (1996) reported a reduction of extrusion force after addition of glycerol behenate to a MCC-based formulation. Edimo et al. (1993) used lipophilic substances (Precirol® ato 5, Gelucire® 50/02) to prepare pellets via extrusion/spheronisation. However, addition of sodium lauryl sulphate (as a wetting agent) and MCC (to provide plasticity and cohesiveness) were needed. Furthermore, up to 40% w/w self-emulsifying systems (mixtures of oil, surfactant and water) were used as granulation liquid in pellet formulations in order to increase the release of poorly soluble drugs (Newton et al., 2001, 2005; Tuleu et al., 2004).

Superdisintegrants (croscarmellose sodium and sodium starch glycolate) were not successful to increase drug release from MCC-based pellets containing a poorly water soluble model drug (Souto et al., 2005). Previously Schröder and Kleinebudde (1995b) reported about the inefficiency of sodium starch glycolate to promote disintegration and therefore increase the dissolution of propyphenasone in MCC-based pellets, while it was achieved in another study (Schröder and Kleinebudde, 1995a) when using different 2-propanol/water mixtures as granulation liquid.

Surface active agents were also used either to increase the release of poorly soluble drugs (Law and Deasy, 1997a; Deasy and Law, 1997) or to improve the plasticity of wet mass (Law and Deasy, 1997b; Junnila et al., 1998).

Law and Deasy (1998) reported on co-processing of hydrophilic polymers with MCC by spray-drying. Compared to the physical mixture, pellets obtained after extrusion/spheronisation of the mixture containing this co-processed excipient and 80% of lactose, were of superior sphericity and yield. Furthermore, the optimal water range was broader.

2.5 Excipients alternative to MCC

Liew et al. (2005) proposed the following properties as being important for an excipient intended for production of pellets via extrusion/spheronisation:

- water insolubility
- large water absorption and retention capacity
- binding properties
- sufficiently large surface area for interaction with water and other ingredients in the powder mixture
- ability to enhance drug release.

Several excipients were evaluated as alternatives to MCC in extrusion/spheronisation. They have been used as spheronisation aids or release modifiers either alone or in combination with MCC (Table 2.2) or as complete substitutes for MCC (Table 2.3). Nevertheless, none of them succeeded to provide the flexibility in formulation and processing which MCC as an excipient can offer. These excipients will be briefly reviewed in the following sections.

Table 2.2 *Overview of excipients used* ***as spheronisation aid*** *in extrusion/spheronisation with or without MCC.*

Excipient	Physical properties	Formulation	Process requirements	Pellet properties	Drug release	References
Chitosan	- Soluble in acidic medium - Insoluble in basic medium -Degr. of deacetyl. and mol. weight influenced performance	- Used as binder and release modifier (with MCC) and as spheronisation aid (without MCC and/ or with sodium alginate) - Additional binder necessary or acetic acid solution as granulation liquid	- Takes up large quantity of water - Processing parameters influenced pellet yield, size, sphericity and friability	- Pellets were produced with acceptable size, size distribution and sphericity, low friability, high crushing strength and smooth surface properties	- Additional binder promoted immediate drug release (HPMC) or sustained drug release (PVP) - Chitosan (MCC-free) pellets obtained with acetic acid solution as granulation liquid showed sustained drug release - Citosan-alginate pellets showed fast drug release	*Goskonda and Upadrashta (1993), Tapia et al. (1993), Agrawal et al. (2004), Chatchawalsaisin et al. (2004), Santos et al. (2004), Steckel and Mindermann-Nogly (2004), Jess and Stckel (2007), Charoenthai et al. (2007a,b).*
Low substituted hydroxylpropylcellulose (L-HPC)	- Insoluble in water - Swells in water	- Binder may be needed. - Used with MCC	- Wider optimal water content range	- MCC pellets had better sphericity - L-HPC type influenced pellet properties	- Pellets do not disintegrate, but swell in water - Drug release faster due to higher pellet porosity	*Kleinebudde (1993; 1994a,b)*
Glyceryl monostearate (GMS)	- Insoluble in water	- Depending on drug properties, used with and without MCC - It was possible to disperse drug in molten GMS, to grind it and process with MCC and water	- Less water required	- Sphericity acceptable - Pellets are larger compared to MCC pellets - Increased GMS level gave larger pellets - Porosity depended on other materials properties, water and GMS level	- It was considered as spheronisation enhancer rather than controlled release additive - Drug solubility influenced the release in a similar manner as in MCC-based pellets	*Basit et al. (1999), Newton et al. (2004), Chatchawalsaisin et al. (2005)*
Carbopol® resins	- Swells in water	- Addition of aqueous solution of a strong electrolyte ($CaCl_2$) reduced tackiness of the wet mass - Used up to 55% with MCC as the main spheronisation aid	- Optimal water level depended on MCC and $CaCl_2$ content - Processing parameters influenced pellet properties in a complex manner	- Using $CaCl_2$ yielded pellets with the best sphericity and smoothest surface	- Used to sustain the drug release - Drug release was prolonged with increasing Carbopol level - Ionic interactions between Carbopol and drugs rather than drug solubility influenced the release	*Neau et al. (1996, 2000), Gómez-Carracedo et al. (2001), Bommareddy et al. (2006)*
Starch and starch derivatives	- Swells in water, ratio of amylose and amylopectine determines solubility	- Native starches up to 30% or waxy maize starch up to 50% combined with MCC - Native starches were combined with waxy maize starch, white or yellow dextrin (without MCC)		- Pellet sphericity was generally not satisfactory, except for pellets containing native starch and 20% of white dextrin	- Model drug was not used in pellets prepared without MCC - Drug release was not tested from pellets with MCC	*Junnila et al. (1998, 2000), Almeida Prieto et al. (2005)*

Table 2.3 *Overview of excipients used* ***as spheronisation aid*** *in extrusion/spheronisation without MCC.*

Excipient	Physical properties	Formulation	Process requirements	Pellet properties	Drug release	References
Powdered cellulose	- Insoluble in water	- Binder necessary		- Smaller mean size, broader size distribution, rougher surface, higher friability and porosity compared to MCC pellets	- Pellets do not disintegrate - Drug release faster due to higher porosity	*Lindner and Kleinebudde (1994), Alvarez et al. (2003)*
Cross-linked polyvinyl-pyrrolidone	- Insoluble in water	- Binder is not necessary	- Higher water levels required - Lower spheronisation speed needed	-Narrow size distribution and good sphericity - Higher mean diameter	- Model drug was not used	*Liew et al. (2005)*
Kappa-carrageenan	- Insoluble in cold water - Swells in water	- Binder is not necessary - Effective in concentrations from 5 to 98 %	- Higher water levels required and therefore longer drying times are needed - Optimal water level range broader	- Acceptable pellet size distribution and sphericity - Pellets are larger and more porous, compared to MCC	- Drug release was faster due to pellet disintegration - Immediate drug release achieved, irrespective of drug and filler solubility	*Bornhöft et al. (2005), Thommes and Kleinebudde (2006a,b)*
Hydroxypro-pylmethyl-cellulose (HPMC), Hydroxyethyl-cellulose (HEC)	- Water soluble - Insoluble in alcohols	- Binder necessary - Non-aqueous granulation liquid used	- Higher liquid levels required	- Rougher surface properties, higher friability and poor sphericity compared to MCC	- HPMC pellets dissolve into gel-like structure, while HEC pellets erode - Release properties can be modified by using different HPMC/HEC types	*Chatlapalli and Rohera (1998a,b)*
Polyethylene oxide (PEO) with methoxypoly-ethylene glycol (MPEG)	- Water soluble	- PEO provides wet mass plasticity, MPEG self-lubrication - Optimal PEO/MPEG/ water ratio was 2:1:1	- Processing parameters influenced pellet yield, sphericity and friability in a complex manner	- Pellets with acceptable yield, sphericity and friability were produced	- High drug load (>80%) - Immediate release obtained	*Howard et al. (2006)*
Pectinic acid	- Insoluble in water	- Binder is not necessary - Effectiveness in producing pellets depends on drug properties - Drug load up to 80%	- Less water is required, but optimal water range is narrow compared to MCC - Spheronisation performed at 45°C to improve sphericity	- Acceptable pellet sphericity and mechanical strength - Larger pellets and rougher surface properties compared to MCC	- Pellets disintegrate and therefore the release of poorly water soluble drugs is faster compared to drug release from MCC-based pellets	*Tho et al. (2002, 2003)*

2.5.1 Chitosan

Chitosan is a polycationic copolymer, consisting of glucosamine and N-acetylglucosamine. It is obtained by N-deacetylation of a natural polysaccharide, chitin. Due to its cationic character, chitosan has a pH-dependent solubility in water: it is soluble in acidic medium and insoluble in basic medium (Steckel and Mindermann-Nogly, 2004). Use of chitosan as a release modifier and/or spheronisation aid in extrusion/spheronisation has been reported by several authors.

Pellets with sustained drug release were obtained by Tapia et al. (1993) who added low concentrations of chitosan to an acetic acid solution used as granulation liquid and produced pellets with MCC as spheronisation aid. Goskonda and Upadrashta (1993) also obtained pellets with sustained drug release after investigating different viscosity grades of chitosan in concentrations up to 40 % (w/w) in mixtures with MCC and sodium carboxymethylcellulose as binder. Santos et al. (2004) used chitosan in combination with PVP as binder, MCC as spheronisation aid and different fillers to obtain pellets with immediate release of a model drug. The drug release was not retarded due to low concentration of chitosan.

Above mentioned authors used chitosan in combination with MCC. In addition, a binder was necessary to provide wet mass cohesiveness. Agrawal et al. (2004) prepared MCC-free pellets using up to 15% (w/w) chitosan and up to 10% (w/w) HPMC as additional binder. Pellets disintegrated and the drug release was not sustained. Pellet properties depended on the formulation (chitosan, HPMC and water concentration) and processing variables (extrusion and spheronisation speed). In general, pellets with acceptable yield, size and sphericity, low friability and high density were obtained.

In contrast to Goskonda and Upadrashta (1993), Steckel and Mindermann-Nogly (2004) produced pellets comprising equal amounts of MCC and chitosan without additional binder. Furthermore, they succeeded to produce pellets with acceptable quality with increased chitosan concentration using a 0.1N acetic acid solution as granulation liquid. The authors postulated that partial dissolution of chitosan at the particle surface increased the wet mass plasticity and cohesiveness. Furthermore, with increasing amount of chitosan in the mixture, a higher amount of granulation liquid was needed for successful extrusion/spheronisation. In a recent study, Jess and Steckel (2007) investigated the influence of the degree of deacetylation of chitosan on the properties of pure chitosan pellets. It was concluded that chitosan with the highest degree of deacetylation (99.9 %)

and wetted with 0.2N acetic acid provided the best wet mass plasticity to obtain pellets with adequate size, sphericity, friability, mechanical strength and surface properties. Furthermore, the drug release of a model drug (0.6% budesonide) was sustained according to a zero-order model.

Recently, Charoenthai et al. (2007a) investigated the influence of chitosan molecular weight on the quality of pellets produced via extrusion/spheronisation. Acetaminophen (10 and 20 %, w/w) was used as model drug and chitosan level was up to 60 % (w/w). Formulations also contained MCC (up to 30 %, w/w) and dibasic calcium phosphate as filler. In general, low molecular weight chitosan produced pellets with better sphericity and a higher crushing strength. Furthermore, pellet properties were improved when adding 2.5 % (w/w) of sodium alginate to the pellet formation. Drug release depended on the chitosan molecular weight, sodium alginate addition and the pH of dissolution medium. The same authors in another study (Charoenthai et al., 2007b) further investigated the influence of formation of polyelectrolyte complex between polycationic chitosan and polyanionic sodium alginate on the quality of MCC-free pellets. The same model drug was used, while lactose monohydrate was used as filler. It was possible to produce pellets with fast drug release. Similarly as in the previous study of the same authors, pellet properties and drug release depended on chitosan molecular weight, addition of sodium-alginate, filler properties and dissolution medium.

2.5.2 Low-substituted hydroxypropylcellulose (L-HPC)

In low-substituted hydroxypropylcellulose a small fraction of free hydroxyl groups of glucose subunits is substituted with hydroxypropyl ether groups. It is insoluble in water and alcohols, but swells in water. Kleinebudde (1993) evaluated pellets containing several L-HPC types with different hydroxypropyl content and particle size in concentrations up to 20% (w/w). In all formulations acetaminophen (30%, w/w) was used as model drug and MCC was used as additional spheronisation aid. MCC-based pellets were also produced for comparison reasons. Compared to L-HPC free pellets, formulations with L-HPC had a higher optimal water content and were less sensitive to water content. However, MCC-based pellets had a better sphericity compared to pellets containing L-HPC, since the addition of L-HPC increased the elasticity of the wet mass, thus reducing the brittleness of the extrudates and increasing the pellet length. Furthermore, for the same water content, pellet sphericity decreased with increasing particle size of L-HPC. L-HPC based pellets did not disintegrate during dissolution tests, but swelled and softened. Drug release was

related to pellet porosity: MCC-based pellets had the lowest porosity, and the drug release was the slowest. With an increase of hydroxypropyl content in the formulations, pellet porosity increased and consequently drug release was faster. Moreover, using a higher water level in the formulations reduced the porosity, which also prolonged drug release. It was concluded that L-HPC grades with lower particle size and higher hydroxypropyl content were of interest to produce pellets with fast drug release.

2.5.3 Glyceryl monostearate (GMS)

Glyceryl monostearate and barium sulphate were proposed as excipients alternative to MCC for the production of ranitidine pellets due to the chemical degradation of ranitidine by means of a complex three-way interaction between drug, MCC and water (Basit et al., 1999). It was possible to obtain good pellets by completely replacing MCC by a mixture of barium sulphate and GMS.

Newton et al. (2004) used barium sulphate and diclofenac sodium as model drugs to prepare MCC-free pellets. The optimal water content decreased with an increase of GMS in diclofenac sodium-containing formulations, while the opposite trend was observed for barium sulphate formulations. Nevertheless, compared to MCC-based formulations, the optimal water contents were two times lower, which is a considerable advantage when using water-sensitive drugs. Furthermore, GMS-based pellets were larger compared to MCC pellets and sphericity was acceptable. Drug release was not sustained and GMS was rather considered a spheronisation aid than controlled release additive. Chatchawalsaisin et al. (2005) further investigated the potential of GMS as spheronisation aid by using several model drugs with varying solubility (drug concentration: 10% w/w). None of the model drugs (except diclofenac sodium) could be processed without addition of at least 30% (w/w) MCC. With increasing GMS content in the formulations, the optimal water level decreased and pellet size increased. Pellet sphericity was acceptable. Drug release depended on drug solubility, being slower if a poor water soluble drug was used in the formulation.

2.5.4 Carbopol® resins

Carbopol® resins are synthetic, cross-linked acrylic acid polymers. They swell in alkaline or neutral media and form a hydrogel due to strong repulsion of negatively charged

carboxylic groups. Carbopol® 974P was firstly described by Neau et al. (1996) as a release modifier in pellets manufactured via extrusion/spheronisation. Using water as a granulation liquid was not successful since the material turned into a tacky mass, making it difficult to extrude. However, extrusion and spheronisation was possible when an aqueous solution of a strong electrolyte was used as granulation liquid. Neau et al. (1996) evaluated the effect of several types and concentrations of inorganic salt aqueous solutions on the extrusion and spheronisation of mixtures containing Carbopol® 974P (up to 55%, w/w), MCC (as main spheronisation aid) and 5% (w/w) of highly water soluble model drug. The electrolyte efficiency in reducing tackiness depended on the type of salt and its concentration. In general, when using an aqueous solution of $CaCl_2$, pellets had the lowest roundness score (~1.15) and the smoothest surface properties. The drug release was prolonged with an increase of Carbopol concentration in the formulation. Furthermore, the release of the model drug was also influenced by pH and ionic strength of the dissolution medium. In another study the same authors (Neau et al., 2000) investigated the influence of formulation (water, Carbopol and $CaCl_2$ concentration) and process (spheronisation time and load) variables on pellet properties and drug release. The optimal water level depended on Carbopol/MCC ratio and $CaCl_2$ level in the formulation. In general, after process optimization, pellets with acceptable quality and prolonged drug release were produced. Bommarenddy et al. (2006) used Carbopol® 974P/ MCC mixtures granulated with the same granulation liquid to test drug release from pellets containing four drugs with different water solubilities. Two of them were non-electrolytes (caffeine and dyphylline) while two were salts of weakly basic drugs (chlorpheniramine and dipheniramine maleate). The drug release studies showed that drug solubility was not the major factor influencing drug release, but the ionic nature of the drug: the release of non-ionic drugs was much faster than the release of weakly basic salts, irrespective of their water solubility. It was proposed that in the latter case, ionic interactions between the protonated amines of the salts and carboxylic groups of Carbopol are responsible for a slower drug release.

2.5.5 Starch and starch derivatives

O'Connor et al. (1984) reported about the unsuccessful production of pellets via extrusion/spheronisation with starch (native and pregelatinized) as the main excipient in the formulation. Erikäinen and Lindqvist (1991) produced starch-based pellets containing 20% (w/w) of a slightly water soluble drug and gelatine as binder. Drug release was fast, but no data on pellet properties were provided. Several authors reported on the use of

starch as binder (Funck et al., 1991; Mesiha and Vallés, 1993; Varshosaz et al., 1997) in formulations with MCC. Otsuka et al. (1994) used a mixture of lactose (63%, w/w) and starch (27 %, w/w) to produce pellets with 10% (w/w) of theophylline as model drug. Furthermore, Junnila et al. (1998) reported on using up to 30% (w/w) native starch, combined with MCC and 2.5 % (w/w) of anhydrous theophylline. However, addition of polysorbate 80 as surface-active agent was needed to improve wetting and plasticity. The same authors (Junnila et al., 2000) introduced waxy maize starch as a co-filler in pellets containing MCC and anhydrous theophylline. It was possible to produce pellets containing up to 50% waxy maize starch. However, pellet sphericity was at the limits of acceptability and data on drug release were not provided. Almeida Prieto et al. (2005) reported on using native maize and wheat starch to prepare pellets without MCC. It was possible to produce starch-based pellets only after addition of waxy maize starch, white or yellow dextrin in concentration up to 20% w/w. However, no model drug was used and pellet sphericity was poor, except the ones prepared from mixtures of starch and white dextrin.

2.5.6 Powdered cellulose (PC)

Lindner and Kleinebudde (1994) compared the properties of PC-based pellets to MCC-based pellets containing 30% (w/w) of paracetamol as a model drug. In contrast to MCC-based pellets, a binder (sodium carboxymethylcellulose) was necessary to prepare pellets with powdered cellulose. All pellets had a low friability and did not disintegrate during dissolution testing. Furthermore, a faster drug release from PC-based pellets was attributed to their higher porosity (22-36%, compared to 3% for MCC-based pellets).

Alvarez et al. (2003) prepared PC-based pellets with furosemide (25 and 50%, w/w) as hydrophobic and cohesive model drug, but without additional binder. Compared to the reference MCC-based pellets, powdered cellulose pellets had a smaller mean size, broader size distribution, rougher surface and higher friability. Moreover, faster dissolution was related to a markedly higher micropore volume of PC-based pellets.

In general, differences in porosity between powdered cellulose- and MCC-based pellets were explained by the differences in drying behaviour: while MCC pellets shrink during drying, the structure of the PC pellets was preserved.

2.5.7 Cross-linked polyvinylpyrrolidone (crospovidone)

Liew et al. (2005) evaluated crospovidone (cross-linked polymer of N-vinyl-2-pyrrolidone) as spheronisation aid. Three grades of this water-insoluble synthetic polymer, differing in particle size, were mixed with lactose in a 1:3 ratio. No model drug was used and pellet quality was compared to pellets produced from a mixture containing MCC and lactose. Two finer grades of crospovidone were successfully extruded and spheronised using water as granulation liquid. Compared to MCC, crospovidone was able to absorb and retain higher amounts of water. Favorable properties of this excipient for use in extrusion/spheronisation were related to the cross-linked arrangement which formed of a mesh-like structure with water retention ability. The pellets had a narrow size distribution, good sphericity and high mean diameter. However, a lower spheronisation speed combined with longer spheronisation time was required during processing, since the wet mass had a low cohesiveness and binding ability. This difference in wet mass consistency between MCC and crospovidone mixtures was attributed to differences in powder morphology: the fibrous and irregular shape of MCC particles provided extra strength via mechanical interlocking, in contrast to the granular structure of crospovidone particles. Nevertheless, a binder was not needed and pellets were produced with acceptable quality and good process reproducibility.

2.5.8 Kappa(κ)-carrageenan

Carrageenans are a group of acid polysaccharides, consisting of mainly potassium, sodium, calcium, magnesium and ammonium sulfate esters of galactose and 3,6-anhydrogalactose, which are alternately linked with α-1,3 and β-1,4 linkages in the polymer. There are several carrageenan types (λ-, κ- and τ- type) differing in the amount and position of the sulfate group (Bornhöft et al., 2005). After preliminary screening, Bornhöft et al. (2005) reported κ-carrageenan as the most suitable for preparation of pellets via extrusion/spheronisation. κ-Carrageenan is not soluble in cold water, but swells into strong and rigid gels (Thommes and Kleinebudde, 2006a). Compared to MCC, κ-carrageenan required a higher water content for successful extrusion and spheronisation, which might be a disadvantage if water-sensitive drugs are processed. However, the optimal water content range was much broader, indicating the robustness of the formulation (Bornhöft et al., 2005). The same authors obtained nearly spherical pellets in a κ-carrageenan concentration range from 5 to 98% (w/w). Thommes and Kleinebudde (2006a) evaluated the influence of filler type and load on the properties of pellets

containing 20% (w/w) of κ-carrageenan and water-soluble acetaminophen as model drug. The optimal water content was related to water solubility and concentration of the filler: using a higher amount of water soluble filler required a lower water content, while addition of less soluble fillers required a higher water level. The exception was insoluble, but swellable starch, which had a higher water binding capacity. In general, pellets with acceptable size distribution, yield and sphericity were obtained, irrespective of the filler type and load. Compared to MCC pellets, κ-carrageenan-based pellets were larger and had a lower crushing strength due a higher porosity. However, in contrast to MCC-based pellets, κ-carrageenan-based pellets disintegrated and an immediate drug release was achieved. Furthermore, only a minor influence of filler solubility and load on drug release was observed. In a subsequent study, Thommes and Kleinebudde (2006b) prepared pellets using four model drugs (acethaminophen, theophylline, mesalamine and hydrochlorothiazide) with different water solubility, alone or mixed with an equal amount of a filler (lactose, mannitol, maize starch and dicalcium phosphate). In addition, each formulation contained 20% of κ-carrageenan as spheronisation aid. The optimal water content was influenced on one hand by filler solubility and content as described previously, and on the other hand by the solubility of the model drug and the type of spheronisation aid. Similarly as in the previous study, all pellets had an acceptable sphericity, high yield and compared to MCC-based pellets, a larger size and higher porosity. The influence of solubility and load of drug and filler on pellet properties was negligible. Furthermore, due to pellet disintegration, the drug release from all κ-carrageenan-based pellets was immediate, irrespective of drug solubility, which was in contrast to MCC-based pellets.

2.5.9 Hydroxypropylmethylcellulose (HPMC) and hydroxyethylcellulose (HEC)

HPMC and HEC were evaluated as spheronisation aids by Chatlapalli and Rohera (1998a). Pellets were prepared without model drug and the properties were compared to MCC pellets. It was not possible to use water as granulation liquid, since HPMC and HEC are water soluble polymers and the formation of tacky mass did not allow further processing. However, it was possible to prepare pellets with isopropylalcohol (IPA) as non-dissolving granulation liquid. Furthermore, due to the low mechanical strength of the dried pellets, it was necessary to include a binder (hydroxypropylcellulose dissolved in IPA) in the formulation. All pellets were of acceptable quality although some differences were observed: MCC pellets had smooth surface properties and low friability, while HEC

pellets had the roughest surface and highest friability. HEC pellets were also the worst in terms of sphericity. The pellets showed a different behaviour when immersed in water: MCC pellets did not disintegrate and stayed intact; HPMC pellets absorbed water and turned into gel-like structure; and HEC pellets swelled significantly without adhering to each other and eroded slowly. These differences were attributed to differences in water solubility and viscosity.

2.5.10 Polyethylene oxide (PEO) with methoxypolyethylene glycol (MPEG)

Polyethylene oxide has been recently suggested (Howard et al., 2006) as spheronisation aid in a formulation containing more than 80% of pseudoephedrine hydrochloride as water-soluble model drug. Polyethylene oxide, a highly water soluble polymer, provided sufficient plasticity to the wet mass. However, methoxypolyethylene glycol was needed to improve the self-lubricating properties of the wet mass. A mass ratio of 2:1:1 for PEO/MPEG/water was used in an experimental design which studied the influence of drug load (all above 80%) and process variables (feeder, extrusion rate, spheronisation speed and spheronisation time) on pellet yield, sphericity and friability. The processing parameters highly influenced pellet properties: pellet yield ranged from about 56 to 78%, friability from 1.1 to 29.1%, while the roundness score ranged from 1.15 to 1.36 (with 1 representing a perfect sphere). Drug release was immediate. PEO/MPEG mixture was proposed to be useful in cases when high drug loads are required and the use of MCC is not possible due to incompatibility or incomplete drug release.

2.5.11 Pectinic acid

Pectin is a natural polysaccharide with a backbone of polygalacturonic acid. Different types of pectin, differing in degree of methoxylation and amide substitution, were evaluated as potential excipients for extrusion/spheronisation (Tho et al., 2001a,b). Due to polymer swelling and partial water-solubility, extrusion/spheronisation was not possible with pure water as granulation liquid. Pectinic acid is a water insoluble pectin derivative with a degree of methoxylation of less than 10 and it has been reported by the same authors as suitable alternative to MCC in extrusion/spheronisation (Tho et al. 2002, 2003). Compared to MCC-based formulations, using pectinic acid as the main excipient required less water, but the optimal water content range was narrower. Furthermore, in order to

obtain pellets with acceptable sphericity, a double-jacket heated (45°C) spheroniser was utilized. The manufactured pellets were larger compared to pellets containing MCC and had a slightly lower mechanical strength. Three drugs (riboflavin, paracetamol and theophylline) with differing solubility in water and at different concentrations were used as model drugs. In contrast to MCC, pectinic acid was more sensitive to drug load. Nevertheless, it was possible to successfully extrude and spheronise formulations containing up to 80% of paracetamol. In addition, due to disintegration of pellets containing pectinic acid as the main excipient, drug release was faster compared to MCC-based pellets.

2.6 Enzyme resistant starch - type III (UNI-PURE® EX starch)

According to EURESTA (European Flair Concerted Action on Resistant Starch), **resistant starch** is defined as the total amount of starch and products of starch degradation which are not absorbed in the small intestine of healthy individuals. In other words, it is the starch fraction which is not digested by body enzymes. However, these starches pass into the colon where they are fermented by the colonic microflora, releasing short-chain fatty acids (acetate, propionate, butyrate) and gases (CO_2, CH_4 and H_2) (Eerlingen and Delcour, 1995; Shi and Jeffcoat, 2001).

Resistant starches (RS) are divided into four categories or types (Englyst et al., 1992):

- **RS_1 (type I)** represents starch which is physical inaccessible to digestion, due to its entrapment in a plant cell. This type of RS can be found in legumes and foodstuffs which contain partly milled seeds and grains.
- **RS_2 (type II)** is a native starch which is resistant to digestion due to its compact and dense structure. It is also called granular or ungelatinised starch.
- **RS_3 (type III)** or retrograded starch is the indigestible starch fraction mainly consisting of retrograded amylose which is formed during cooling of gelatinised starch.
- **RS_4 (type IV)** are starches obtained after chemical treatment, where the formation of glycosidic bonds other than (1→4)α and (1→6)α provides resistance to digestion.

UNI-PURE® EX starch is a resistant starch type III. Prior to description of its structure, properties and method of preparation (Section 2.6.2), the structure of native starch will be shortly presented (Section 2.6.1).

2.6.1 Starch structure

Next to cellulose and chitin, starch is the most frequently occurring carbohydrate in nature (Tharanathan, 2005). It is the major reserve polysaccharide in plants: it is stored in the chloroplast of green leaves and the amyloplast of seeds, pulses and tubers (Ellis et al., 1998).

Starch is a polysaccharide, composed of two types of *alpha*-glucan: amylose and amylopectin. The amylose/amylopectin ratio depends on the botanical origin of starch: most naturally occurring starches have between 20 and 35% of amylose, while “waxy” and “high-amylose” starches have less than 15 and more than 40 % of amylose, respectively

(Tester et al., 2004).

Amylose is a long, mostly linear polymer, containing α-D-glucose units linked together with α(1→4)-D-glycosidic bonds (Fig. 2.1). Although most of the amylose polymer is linear, a small fraction was reported to be slightly branched (Parker and Ring, 2001). Amylose has a degree of polymerisation (DP) in the range from 500 to 5000 of glucose units (Hizukuri et al., 1981).

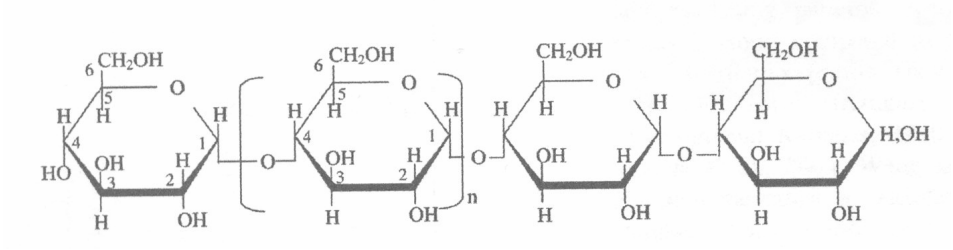

Figure 2.1 *Structure of amylose.*

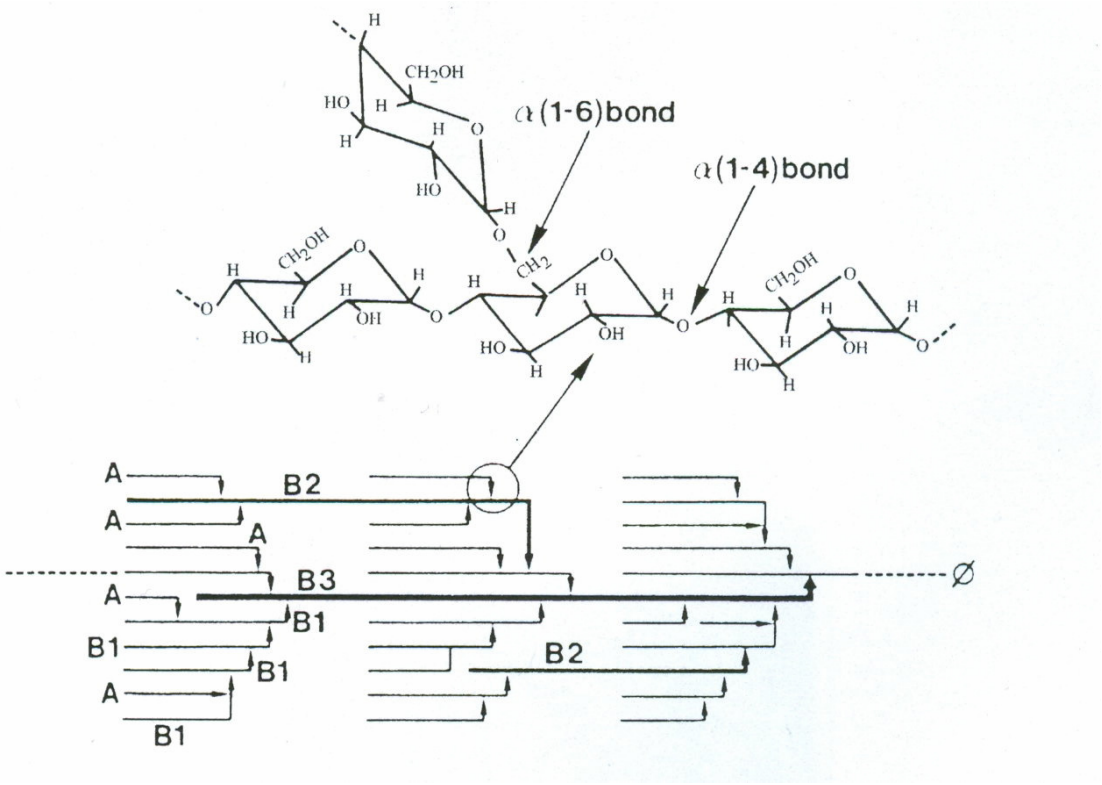

Figure 2.2 *Structure of amylopectin (adapted from Jacobs, 1998).*

Amylopectin is a highly branched polymer, containing about 95 % of α(1→4)-D bonds and about 5% of branching α(1→6)-D bonds (Tester et al., 2004). Compared to amylose, it is a larger molecule, with a degree of polymerisation between 3.10^5 and 3.10^6 (Zobel, 1988). The cluster model is often used to describe the amylopectin structure (Fig. 2.2). According to this model, several types of chains can be distinguished within the amylopectin structure, based on their length and position in the granule structure (Tester et al., 2004). α-D-glucose units, linked with α(1→4)-D bonds, form short amylopectin chains (A-chains) which are linked to the rest of the structure via α(1→6)-D bonds. Longer chains (B-chains) contain short A-chains and are connected to the other B-chains or the C-chain which contains the non-reducing end. Depending on the position in the cluster, B-chains have different numbers (from B_1 to B_4) (Hizukuri, 1986).

Macroscopically, amylose and amylopectin are organised in water-insoluble starch granules. The shape and size of the granules depend on the botanical origin of starch. In polarised light, starch granules show a dark birefringence cross, typical for crystalline materials whose index of refraction depends on the direction of the ray light (Tester et al., 2004). Based on X-ray diffraction experiments, the semicrystalline character of starch granules has been revealed. About 15 to 45 % of starch granule is regarded as crystalline, while the remaining part is amorphous (Zobel, 1988).

Exterior chains of amylopectin (A and B_1 type) as well as amylose chains can form double helices which may be organised into crystalline domains. However, the crystallinity of the starch granule is mainly attributed to amylopectin, while amylose forms double helices organised into crystalline structures during the process of starch retrogradation, which follows the gelatinisation process (Tester et al., 2004).

Based on the diffraction pattern of different starches, starches generate three types of polymorphs, which are related to the botanical source of the starch: A-, B- and C-type. The last one, C-type is a combination of A- and B- type (Tester et al., 2004). Figure 2.3 presents two polymorphic forms of starch. The double helical structures are identical for both starch types. What distinguishes them is the packing of double helices: the A-type crystalline structure is more compact, with a lower water content, while the B-type crystalline structure is more open and contains a hydrated helical core (Tester et al., 2004).

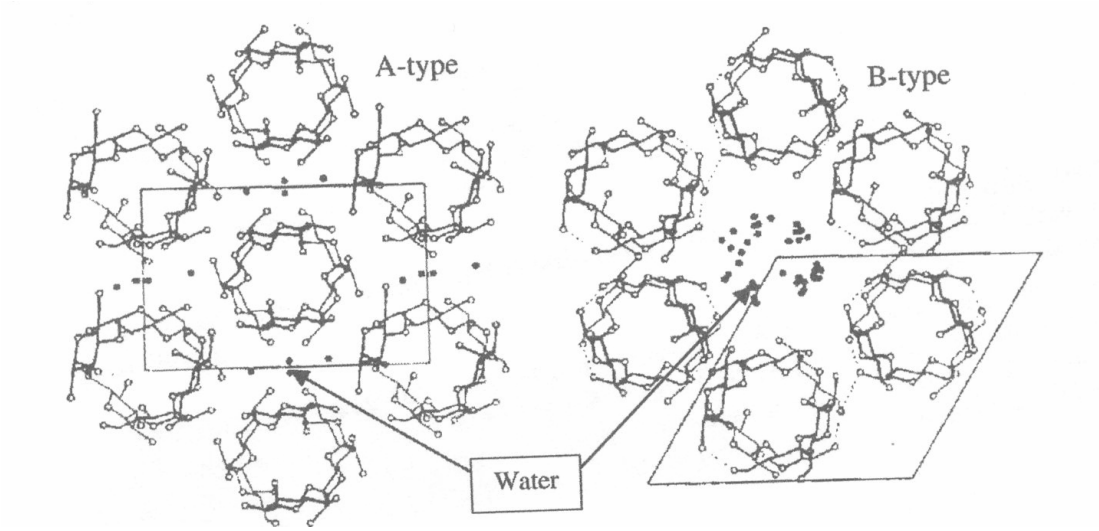

Figure 2.3 *A- and B-type crystalline structure of starch (adapted from Wu and Sarko, 1978).*

2.6.2 UNI-PURE® EX starch

UNI-PURE® EX starch is a resistant starch type III and represents a retrograded starch. Prior to retrogradation, a starch granule is gelatinised. These two processes will be briefly described.

2.6.2.1 Starch gelatinisation

During the starch gelatinisation process using excess of water (>90 %, w/w) and heat, the starch granule is gradually hydrated and irreversibly disrupted. The temperature of gelatinisation depends on the starch source and amylose content, and typically ranges from 40 to 120°C (Haralampu, 2000; Parker and Ring, 2001). At the beginning of the gelatinisation process, starch granules loose their crystalline structure, water is absorbed and the granules swell. As heating continues, granule swelling is followed by leaching of amylose molecules into the solution, the granule is disrupted and partial solubilisation is achieved. If the starch concentration was higher than 6 %, a paste is obtained (Eerlingen and Delcour, 1995).

2.6.2.2 Starch retrogradation

The retrogradation process occurs upon cooling of a starch dispersion or paste obtained during gelatinisation. During retrogradation, starch molecules re-associate and form double helical structures which are stabilised by hydrogen bonds (Wu and Sarko, 1978).

Depending on the amylose concentration, amylose aggregates or gels are formed. Amylose aggregates (amylose concentration is less than 1.5 %) consist of crystalline double helices interspersed in amorphous regions. From more concentrated dispersions, amylose gels are formed: initially a continuous network of polymer-rich phase is formed, followed by double helices formation and after a few hours aggregation into three-dimensional crystalline structure (B-type) (stabilised by hydrogen bonds) is completed (Figure 2.4). Amylose gels are very stabile and show a melting endotherm around 150°C (Eerlingen and Delcour, 1995).

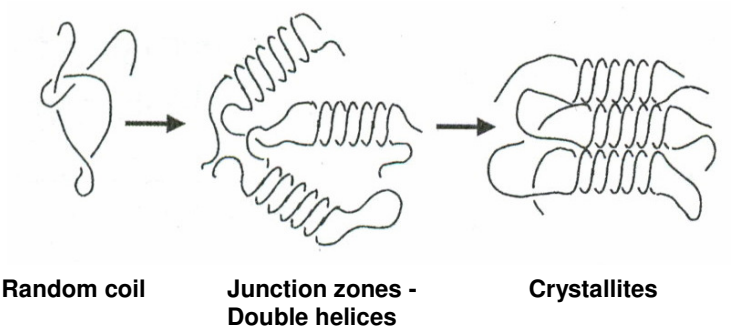

Figure 2.4 *Schematic representation of amylose retrogradation (Haralampu, 2000).*

In contrast to amylose, amylopectin retrogradation is a slow process and occurs over a period of several days or weeks. Amylopectin gels are formed from solutions with concentrations above 10 %. Since amylopectin molecules associate by crystallization of short chains, their stability is lower compared to amylose gels (melting endotherm around 60°C) (Eerlingen and Delcour, 1995).

2.6.2.3 Structure and formation of resistant starch type III

Resistant starch type III is formed by retrogradation of amylose. Two models (Figure 2.5) were proposed for the formation of resistant starch from aqueous amylose solutions. In the micellar model, double helices are ordered into a crystalline structure which is interspersed with amorphous regions. In the lamella model, lamellar structures are formed by folding of the polymer chains. The folded zones are amorphous, while the center of the lamella is crystalline (Eerlingen and Delcour, 1995).

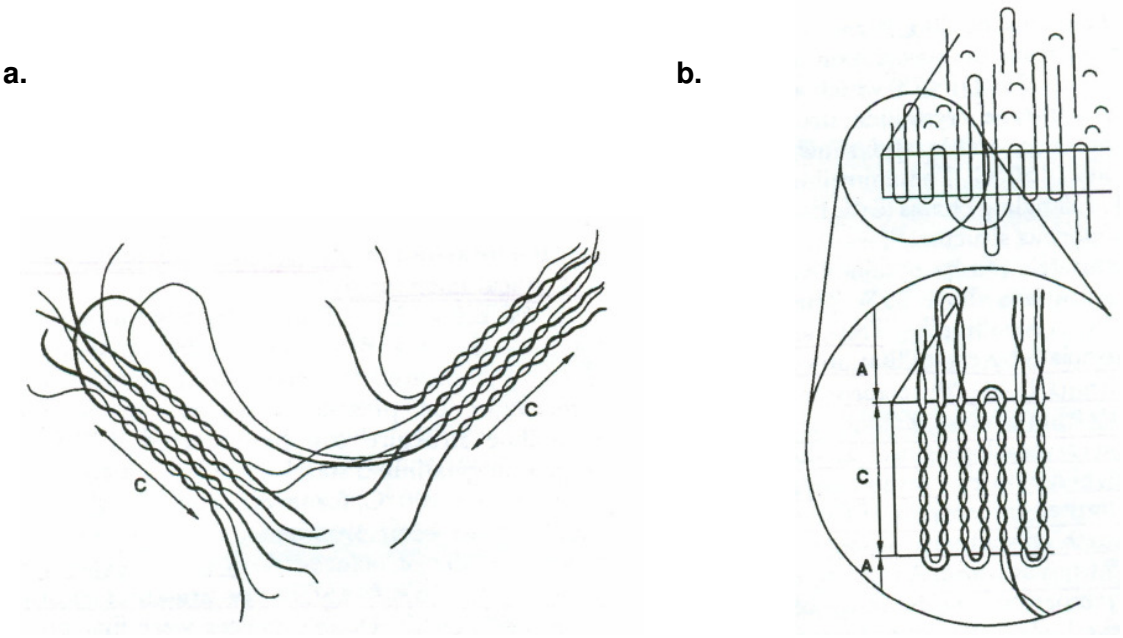

Figure 2.5 *Schematic representation of enzyme resistant starch type III formed in aqueous amylose solutions:* ***a.*** *Micellar model and* ***b****. Lamellar model (C-crystalline and A-amorphous regions) (Eerlingen and Delcour, 1995).*

UNI-PURE® EX starch is produced according to the process described by Chiu et al. (1994). The starting material is high amylose starch. The starch slurry is gelatinised and further treated with a debranching enzyme in order to remove the amylopectin fraction. After amylose retrogradation, the final product is isolated by drying or extrusion.

When viewed under polarized light, UNI-PURE® EX starch powder does not show birefringence, since the granular structure was destroyed during gelatinization process. However, UNI-PURE® EX starch powder shows the X-ray diffraction patterns typical for B-type starch crystallinity, since a crystalline structure was formed during process of retrogradation.

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3

INFLUENCE OF FORMULATION AND PROCESS VARIABLES ON THE QUALITY OF STARCH-BASED PELLETS PREPARED VIA EXTRUSION/SPHERONISATION

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A. Dukić, R. Mens, P. Adriaensens, P. Foreman, J. Gelan, J.P. Remon, C. Vervaet
"Development of starch-based pellets via extrusion/spheronisation."

3.1 Introduction

As reviewed in the previous chapter (Section 2.5), several excipients were reported as potential alternatives to MCC for production of pellets via extrusion/spheronisation. Although some of them showed superiority in immediate release of drugs with poor water solubility, none of them reached the universality of MCC in terms of formulation and processing robustness.

It has also been shown that addition of other excipients (binders, fillers, release modifiers, etc.) or using different types and concentrations of granulation liquid can influence pellet properties like process yield, size and size distribution, shape, friability, porosity, disintegration properties and drug release profile. Those properties are additionally influenced by a number of extrusion/spheronisation process variables (Fig. 1.1). Consequently, a large number of experiments would be required to fully investigate the influence of formulation and process parameters on pellet quality. The design of experiments methodology is often used in extrusion/spheronisation, which offers the possibility to obtain maximum information on the tested system while using a minimal number of experiments. In general, full (Malinowski and Smith, 1975; Pinto et al., 1982;

Chariot et al., 1987; Lindner and Kleinebudde, 1994) and half-fractional (Neau et al., 2000; Agrawal et al., 2004; Howard et al., 2006) two-level factorial designs were used to identify significant variables (factors). The central point, which relates to non-linearity of the response, was usually included to test the curvature significance. Surface response designs like central composite (Hileman et al., 1997) and Box-Behnken (Hileman et al., 1993; Liew et al., 2005) designs were used when the responses are non-linear and/or when formulation and process optimisation was needed. While above mentioned designs were used to evaluate the influence of both process and formulation variables, mixture designs (Baert et al., 1992; Schröder and Kleinebudde, 1995b; Vervaet and Remon, 1996) were used to evaluate the influence of only formulation variables on pellet properties.

In this study, the results of preliminary experiments are presented in Section 3.4.1: firstly, the influence of formulation and process variables on pellet yield and sphericity was investigated and secondly, the acceptable ranges of variables were determined. In Section 3.4.4, the most important variables were used in the experimental design for formulation and process optimisation. Furthermore, the results of wet mass consistency measurements using mixer torque rheometer and their relation to pellet yield was described in Section 3.4.2, while the solid state NMR measurements (Section 3.4.3) explored the molecular miscibility and intermolecular interactions at nano-scale for the tested formulations.

3.2 Materials

Anhydrous theophylline (25%, w/w, dry mass) was used as a model drug in this part of the study. Physicochemical properties of anhydrous theophylline are presented in Table 3.1, while Table 3.2 lists the excipients used during preliminary experiments and in the experimental design.

Table 3.1 *Physicochemical properties of anhydrous theophylline (Martindale, 2005).*

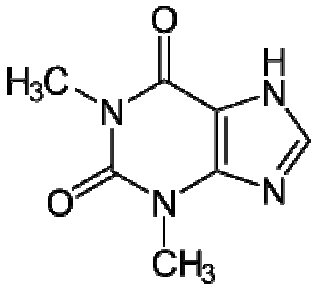
Properties		***Structural formula***
Name:	*Anhydrous theophylline*	
Chemical name:	*3,7-dihydro- 1,3-dimethylpurine - 2,6(1H)-dione*	
Producer:	*Roig Farma (Terrassa, Spain)*	
Solubility in water:	*8.3 g/L at 25°C*	
pK_a:	*0.3; 8.6*	

Table 3.2 *Excipients used during preliminary studies and in the experimental design.*

Excipient name	***Trade name and excipient type***	***Producer***
Modified (resistant) starch	** UNI-PURE® EX starch*	*National Starch and Chemical Co., Bridgewater, New Jersey, USA*
Hydroxypropylmethylcellulose	** Methocel® E15 LV EP Pharm*	*Colorcon, Dartford, UK*
	Methocel® K4M EP Pharm	
Methylcellulose	*Methocel ® A4M EP Pharm*	*Colorcon, Dartford, UK*
Hydroxypropylcellulose	*Klucel® GF Pharm*	*Aqualon, USA*
	Klucel® MF Pharm	
Polyvinylpyrrolidone	*Kollidon® 30*	*BASF, Ludwigshafen Germany*
Drum dried waxy maize starch	*National® 5730*	*National Starch and Chemical Co, Bridgewater, New Jersey, USA*
Sorbitol	** Sorbidex® P 16616*	*Cerestar, Vilvoorde, Belgium*
Erythritol	*Eridex® 16955*	*Cerestar, Vilvoorde, Belgium*
Mannitol	*Mannidex® 16700*	*Cerestar, Vilvoorde, Belgium*
Microcrystalline cellulose	*Avicel® PH 101*	*FMC, Cork, Ireland*
Demineralised water was used as granulation liquid.		

** Excipients used in the experimental design.*

3.3 Methods

3.3.1 Pellets production

The model drug and excipients were mixed (batch size: 250 g) for 15 min in a Turbula® mixer (model T2A, W.A. Bachofen, Basel, Switzerland) to obtain a uniform powder mixture. The mixture was further granulated with demineralised water for 10 min at 60 rpm by means of a planetary mixer (Kenwood Chief, Hampshire, UK) with a K-shaped mixing arm. Water was added during the first 30 seconds of the wet massing phase. To ensure uniform water distribution during wet massing, the material adhering to the mixing bowl was regularly removed. The wet mass was extruded at pre-selected extrusion speed 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: 1mm). The extrudates were spheronised for a specified time in a spheroniser having a friction plate with cross-hatched geometry (Caleva Model 15, Caleva, Sturminster Newton, Dorset, UK). To evaluate the effect of densification during extrusion, the extrudates were passed through the extruder for a second time prior to spheronisation (re-extrusion). Wet pellets were finally dried for 20 min at 60°C in a fluid-bed drier (Uniglatt, Glatt, Binzen, Germany).

3.3.2 Wet mass characterisation using mixer torque rheometer

A mixer torque rheometer (MTR) consists of a mixing bowl, which is equipped with two contra-rotating mixing blades. The mixing bowl is connected to a torque arm which presses a load cell during rotation of the mixing blades, thus generating a torque value. Torque can be described as the effectiveness of a force to produce rotation. It can be expressed by following equation (Eq.3.1):

$$M = F * r \qquad (3.1)$$

where M [Nm] is the torque, F [N] is the force applied and M [m] is the distance from the center of rotation to the force applied. The torque is generated in response to the movement of the wet powder mass mixed by the blades in the bowl. In general, the torque is a measure of the resistance of the material to the rotation of the mixing blades and is useful in comparative analysis of the consistency of wet massed material (Martin, 2003).

A mixer torque rheometer (Model MTR2, Caleva, Sturminster Newton, Dorset, UK) at a mixer speed of 50 rpm was used to measure the consistency of wet mass, extrudates and re-extruded material (35 g) as described by Parker et al. (1990) and Rowe et al. (1994). Prior to sample evaluation, an empty mixing bowl was run for 20 s in order to obtain the base-line torque. After addition of the wet mass (granulate, extruded or re-extruded material), followed by premixing for 45 s, the data were acquired during 15 s. Each sample was analysed in triplicate.

3.3.3 Experimental design and data analysis

To elucidate the influence of HPMC and sorbitol on the quality of starch-based pellets, an experimental design was set up which included binder and sorbitol concentration in combination with spheronisation speed and water as variables. The water level was determined based on preliminary tests and corresponded to the level resulting in the highest yield. In order to evaluate non-linear responses, a Box-Behnken response surface design was used. The influence of three formulation (binder, sorbitol and water level) and one process variables (spheronisation speed) was tested at three levels (Table 3.3). The total number of experiments was 29 and included 5 replicates of the central point to estimate the significance of lack-of-fit tests. Experiments were performed in randomised order. The other process parameters remained constant, with an extrusion speed of 50 rpm and a spheronisation time of 3 min.

Table 3.3 *Definition of the factors used in the experimental design.*

Factor	***Low level (-1)***	***Medium level (0)***	***High level (+1)***
A: HPMC conc. (% w/w, dry mass)	*3*	*4.5*	*6*
B: Sorbitol conc. (% w/w, dry mass)	*0*	*11.25*	*22.5*
C: Spheronisation speed (rpm)	*650*	*850*	*1050*
D: Water level[a]	*-1*	*0*	*+1*

[a] Water level is shown in coded terms as it depends on the sorbitol level.

Table 3.4 *Box-Behnken design for 4 variables (A: HPMC conc., %; B: sorbitol conc., %; C: spheronisation speed, rpm; D: water conc., %) at 3 levels and 5 replicates of the central point, presented in coded and actual terms (randomised order).*

Run	Coded values				Actual values			
	A	B	C	D	A	B	C	D
1	-1	0	0	-1	3	11.25	850	35.76
2	0	0	1	-1	4.5	11.25	1050	35.76
3	-1	0	0	1	3	11.25	850	38.76
4	0	0	0	0	4.5	11.25	850	37.26
5	0	0	0	0	4.5	11.25	850	37.26
6	0	-1	-1	0	4.5	0	650	44.47
7	-1	0	1	0	3	11.25	1050	37.26
8	-1	0	-1	0	3	11.25	650	37.26
9	1	0	0	1	6	11.25	850	38.76
10	-1	-1	0	0	3	0	850	44.47
11	0	0	-1	-1	4.5	11.25	650	35.76
12	-1	0.78	0	0	3	20	850	33.31
13	0	0.78	-1	0	4.5	20	650	33.31
14	1	0	-1	0	6	11.25	650	37.26
15	0	0.78	1	0	4.5	20	1050	33.31
16	1	0.78	0	0	6	20	850	33.31
17	0	-1	0	1	4.5	0	850	45.97
18	0	0	0	0	4.5	11.25	850	37.26
19	0	0	0	0	4.5	11.25	850	37.26
20	0	0	-1	1	4.5	11.25	650	38.76
21	0	0.78	0	-1	4.5	20	850	31.81
22	0	-1	0	-1	4.5	0	850	42.97
23	0	0	0	0	4.5	11.25	850	37.26
24	1	0	0	-1	6	11.25	850	35.76
25	1	-1	0	0	6	0	850	44.47
26	0	0	1	1	4.5	11.25	1050	38.76
27	0	-1	1	0	4.5	0	1050	44.47
28	1	0	1	0	6	11.25	1050	37.26
29	0	0.78	0	1	4.5	20	850	34.81

The selection of variables and their ranges was based on the results of preliminary experiments. Water levels are displayed in coded values because the optimal water level depended on the sorbitol concentration. At different sorbitol levels (0, 7.5, 15 and 22.5 % w/w, dry mass) the optimal water level was determined and plotted against the corresponding sorbitol level. Based on a quadratic function (Eq. 3.2) the optimal water concentration (W, % w/w, wet mass) at the sorbitol level (S, w/w, dry mass) of interest was calculated and set as the medium water level (0). The water concentration varied by ±1.5% to obtain the low (-1) and high (+1) water levels in the experimental design.

$$W(\%)=0.129*S^2-0.7865*S+44.471 \quad (R^2=0.9997) \qquad (3.2)$$

Initially the highest sorbitol level was set at 22.5% (w/w, dry mass). However, the pellet yield of these formulations was too low (<5%) due to a large fraction of fines. Therefore, the sorbitol concentration was reduced to 20% (0.78 as coded value) in order to obtain a better prediction model for process optimisation. All experiments with the coded and actual values of the variables are listed in Table 3.4.

The results were analysed using Design-Expert®, v.6.0.6. (Stat-Ease, Minneapolis, USA). Pellet yield, sphericity (aspect ratio and shape factor) and size were determined for each batch of the experimental design and those values were used as responses for modelling and process optimisation. Analysis of variance (ANOVA) with $P<0.05$ was performed for each response.

3.3.4 Pellet characterisation

3.3.4.1 Pellet yield

Pellets (100g) 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 a percentage of the total pellet weight. This size fraction was used for all further measurements.

3.3.4.2 Pellet size

Pellet size was determined using an image analysis system. 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) were used to obtain 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 from every batch. Each individual pellet was characterised by mean Feret diameter (FD) (average of 180 calliper

measurements with an angle of rotation of 1°). An average value for all pellets has been calculated as the mean pellet size (mean FD).

3.3.4.3 Pellet sphericity

Pellet shape was also determined using an image analysis system as described in the previous paragraph. Next to the mean FD, each individual pellet was characterised by aspect ratio (AR) (ratio of the longest Feret diameter and its longest perpendicular diameter) and two-dimensional shape factor (e_R), as described by Podczeck and Newton (1994) (Eq. 3.3):

$$e_r = \frac{2.\pi.r}{P_m} - \sqrt{1-\left(\frac{b}{l}\right)^2} \qquad (3.3)$$

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

3.3.4.4 Friability

A sample of pellets (F_S, 10g) was placed in an abrasion wheel together with 200 glass beads (diameter: 4mm) 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 Eq. 3.4:

$$\text{Friability}(\%) = [(F_S - F_A)/F_S]*100 \qquad (3.4)$$

3.3.4.5 Scanning electron microscopy

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

3.3.4.6 Disintegration

The pellet disintegration time 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. Discs were used to increase the mechanical stress on the pellets. Water was used as disintegration medium and the sample amount was 100 mg. Results are presented as the average of 6 determinations.

3.3.4.7 Dissolution tests

The dissolution tests were performed according to the USP basket apparatus (VK 8000, VanKel, New Jersey, USA) at a rotational speed of 50 rpm and at a temperature of 37°C. Demineralised water (900 mL) was used as dissolution medium and the sample amount used for analysis (~300 mg) was adjusted to obtain sink conditions. Samples of 5 mL were withdrawn from the dissolution vessel at 5, 10, 15, 20, 30, 45 and 60 min. The samples were spectrophotometrically analysed at 272 nm by means of a double-beam spectrophotometer (Perkin-Elmer UV/VIS λ12, Norwalk, CT, USA). Each batch was analysed in triplicate.

3.3.4.8 X-ray diffraction

X-ray diffraction patterns of pure UNI-PURE®EX starch, anhydrous theophylline, sorbitol, HPMC, as well as of pellets containing anhydrous theophylline (25%), HPMC (4.5 % w/w) combined with sorbitol (0, 11.25 and 20% w/w, dry mass) were determined using an X-ray diffractometer (D-500, Siemens, Germany) with CuK_λ radiation (0.154 nm). The angular range (2θ) varied from 10 to 60° with steps of 0.02° and the measuring time was 1s/step.

3.3.4.9 Solid state NMR

The solid-state ^{13}C CP/MAS NMR spectra of pellets containing anhydrous theophylline (25%), HPMC (4.5 %), sorbitol (0, 7.5, 11.25 and 20 % w/w, dry mass) and UNI-PURE®EX starch were recorded at room temperature on an Inova 200 Varian spectrometer operating at a static magnetic field of 4.7 T. Magic angle spinning was performed at 5 kHz using ceramic Si_3N_4 rotors. The proton spin-lattice relaxation time (T_{1H}) was measured via the chemical shift selective carbon nuclei by means of the inversion-recovery method. Because of the long T_{1H} and T_{CH} (time needed to build up the cross-polarisation) of anhydrous theophylline, two independent experiments were performed. A fast experiment was performed in order to determine the short T_{1H}'s of UNI-PURE®EX starch and sorbitol by means of a fixed contact time CT of 1 ms and a variable evolution time between 0.05 and 8 s. A longer experiment was set up to determine the extremely long T_{1H} of anhydrous theophylline by means of a fixed contact time CT of 7.5 ms (a sufficient build up of theophylline magnetisation) and a variable evolution time between 0.05 and 240 s. The recycle time was always set to 5 times T_{1H} and a spin-lock field of 50 kHz was used for cross-polarisation. The following equation (Eq. 3.5) was used for the T_{1H} analysis of the integrated signals:

$$M(t) = M_o(1-2\ exp(-t/T_{1H})) \qquad (3.5)$$

where t is the variable evolution time and M_o is the intensity of the resonance at equilibrium. The proton spin-lattice relaxation time in the rotating frame $T_{1\rho H}$ was measured by means of the variable contact time method, in which the proton magnetisation is kept in spin lock before it is cross-polarised to the carbon nuclei: CT was varied between 0.5 and 12.5 ms and a short recycle time of 4 s was used to suppress the theophylline contribution. The integrated signals were analysed according to the equation (Eq. 3.6):

$$M(CT) = M_o exp(-CT/T_{1\rho H}) \qquad (3.6)$$

3.4 Results and discussion

3.4.1 Preliminary experiments

3.4.1.1 Formulation variables

The goal of the preliminary experiments was to produce pellets with maximum yield and acceptable sphericity as visually observed. Pellets without model drug were produced in this part of the study. UNI-PURE®EX starch had a favourable extrusion/spheronisation behaviour: it could be extruded with minimal resistance (generating limited friction and heat), the extrudate fragmented evenly during spheronisation and the fragments could easily be spheronised. However, when using UNI-PURE®EX starch as the only powder component and water as granulation liquid, a large amount of fines was generated. Addition of a binder was therefore required to obtain pellets with an acceptable size distribution (Fig. 3.1).

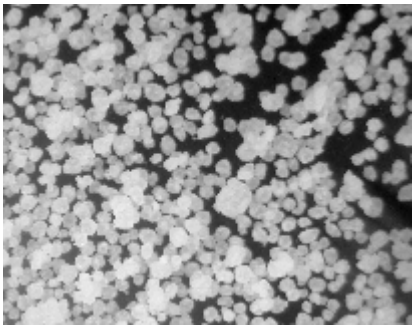
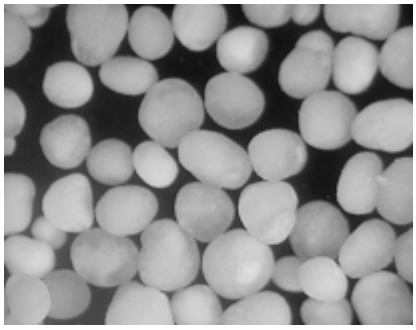
a.  **b.** 

Figure 3.1 *Photomicrographs of extruded and spheronised material containing UNI-PURE® EX starch granulated with water:* ***a.*** *formulation without a binder and* ***b.*** *formulation with a binder*

Several binders (Table 3.2) commonly used in wet granulation were tested as potential binders for UNI-PURE® EX: polyvinylpyrrolidone (PVP), drum dried waxy maize starch (DDWMS), hydroxypropylcellulose (HPC, two types with different viscosity grade), methylcellulose and hydroxypropylcellulose (HPMC, two viscosity grades). Using PVP and HPC was not beneficial, since the strength of the extruded material was reduced and the end product still contained too many fines. In contrast, HPMC, MC and DDWMS improved the binding efficiency of the extrudates, yielding sufficiently large pellets after spheronisation. However, MC (Methocel® A4M EP Pharm; 4000 mPas, 2% w/w sol. at

20°C) and HPMC of a higher viscosity grade (Methocel® K4M Pharm; 4000 mPas, 2% w/w sol. at 20°C) yielded sticky extrudates, which promoted pellet agglomeration during spheronisation. A low viscosity HPMC-grade (Methocel® E15 LV Pharm; 15 mPas, 2% w/w sol. at 20°C) was selected for further experiments, because it provided the best binding properties combined with minimal sticking of the extrudates during spheronisation. HPMC has already been used as a binder in pellet formulations containing chitosan as the main excipient (Agrawal et al., 2004). Chatlapalli et al. (1998a) even investigated the extrusion/spheronisation behaviour of formulations containing HMPC as the main excipient.

Preliminary experiments also showed that polyols increased the mechanical strength of the extruded material. In addition, pellet surface properties were improved when polyols (erythritol, sorbitol and mannitol) were added to the formulation mixture. When comparing effects of different polyols, the addition of sorbitol improved the wet mass consistency and mechanical strength to the highest extent without reducing pellet sphericity. Moreover, this beneficial effect of sorbitol was concentration dependent, which will be explained in the following sections.

3.4.1.2 Process variables

Formulations containing anhydrous theophylline (25% w/w, dry mass) as model drug and HPMC (Methocel® E15 LV, 5% w/w, dry mass) as a binder were used to evaluate the influence of the following process variables: extrusion speed, spheronisation speed, spheronisation time and spheronisation load. Furthermore, each formulation was prepared without and with 10% (w/w, dry mass) sorbitol, at two water levels (low and high) and using UNI-PURE® EX starch as the main excipient.

Extrusion was performed using extrusion speeds of 30, 50 and 70 rpm and the results showed that pellet yield (Fig. 3.2) and sphericity were not affected by extrusion speed. An extrusion speed of 50 rpm was selected for further experiments. As reviewed by Vervaet et al. (1995), the reports on the influence of extrusion speed on pellet properties were contradictory: while several authors reported about the influence of extrusion speed on pellet quality, others claimed that there was no influence.

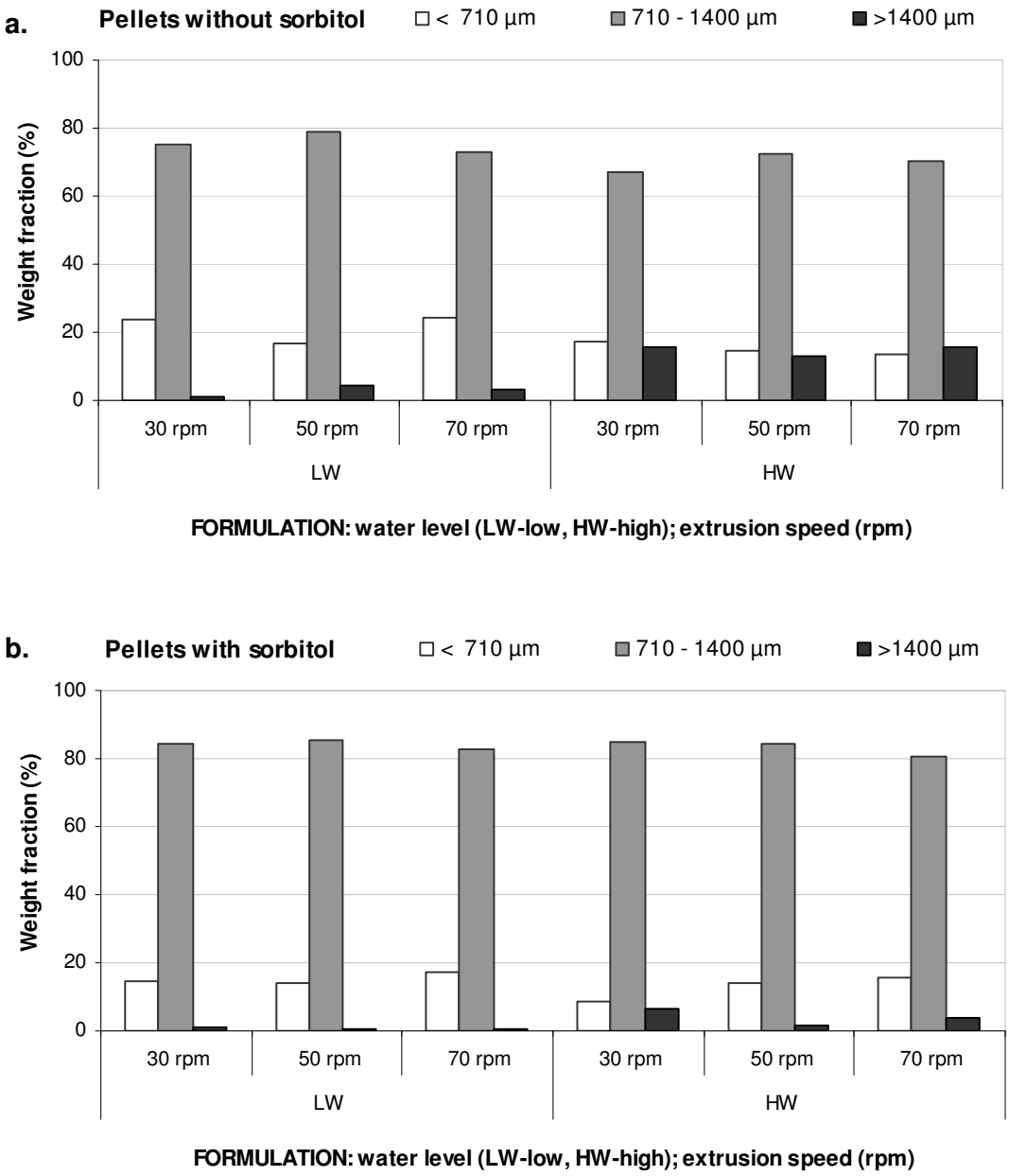


Figure 3.2 *Weight distributions (%) of pellets prepared with different water levels (**a.** formulations without sorbitol and **b.** formulations with sorbitol) and extrusion speed (30, 50 and 70 rpm). All formulations contained 4.5 % (w/w, dry mass) HPMC and 25% (w/w, dry mass) anhydrous theophylline.*

Using several spheronisation speeds during preliminary tests revealed its major influence on pellet yield and sphericity: using a lower spheronisation speed led to dumb-bell formation due to insufficient spheronisation, while a higher energy input by means of a higher spheronisation speed promoted formation of spherical pellets. However, in that case the pellet yield was lower due to excessive breaking of the extrudates. This process

variable was therefore selected for further evaluation and will be discussed in detail later in this chapter (Section 3.4.4).

Preliminary experiments also showed that a short spheronisation time (around 3 minutes) was sufficient to produce pellets with maximum yield and acceptable sphericity. Using longer spheronisation times did not improve pellet sphericity, but promoted broadening of pellet size distribution and pellet agglomeration. The spheronisation speed was therefore fixed at 3 min in further experiments.

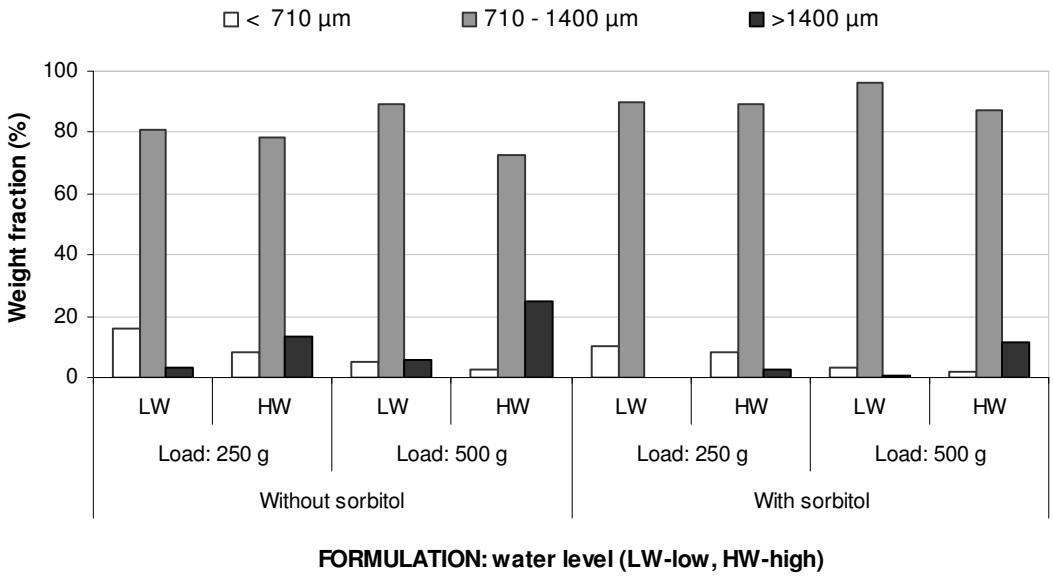


Figure 3.3 *Weight distribution (%) of pellets prepared with and without sorbitol, with different water levels (low-L and high-H) and different spheronisation load (250 and 500 g). All formulations contained 25 % (w/w, dry mass) anhydrous theophylline and 5 % (w/w, dry mass) HPMC as a binder.*

Figure 3.3 shows the pellet weight distribution of formulations with different sorbitol and water levels. In addition, the influence of spheronisation load on pellet size distribution is presented. It can be observed that in case of a higher spheronisation load, the fraction of larger pellets increased. Consequently, pellet yield was either increased mainly due to a reduction of fines (fraction of pellets with size <710 µm) or reduced due to increase of agglomerated particles (pellets with size >1400 µm). Those results are in agreement with the findings of Hasznos et al. (1992), who reported an increase of mean pellet diameter and yield with an increase of spheronisation load. Barrau et al. (1993) reported an increase of agglomerates and a reduction of fines at higher spheronisation load, while the

yield fraction remained the same. This influence of spheronisation load was explained by a lower moisture loss via evaporation when employing a higher load during spheronisation (Hasznos et al., 1992; Hellén et al., 1994) which promoted an increase of mean pellet diameter and yield as explained in Section 2.2. It can be further observed from Fig. 3.3 that introducing sorbitol in the formulation improved pellet yield.

The results of the sphericity (aspect ratio) presented in Fig. 3.4, show a reduction of pellet sphericity (a higher aspect ratio) when using a higher spheronisation load. This might be due to pellet agglomeration in case of a higher spheronisation load, as can be observed in Fig. 3.3. Newton et al. (1995) studied the influence of spheronisation load on the sphericity of MCC-based pellets and concluded that a longer spheronisation time was needed to obtain spherical pellets in case of a higher spheronisation load. In our case, prolonged spheronisation was not possible since it would lead to excessive agglomeration. For the economy reasons, a spheronisation load of 250 g was used for further experiments, even though higher loads are used for commercial applications.

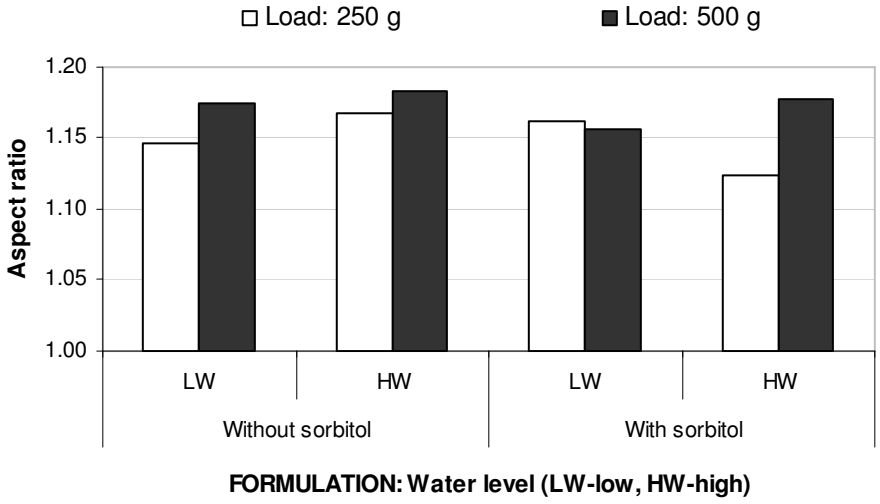


Figure 3.4 *Aspect ratio of pellets prepared with and without sorbitol, with different water levels (low-L and high-H) and different spheronisation load (250 and 500 g). All formulations contained 25 % (w/w, dry mass) anhydrous theophylline and 5 % (w/w, dry mass) of HPMC as a binder.*

3.4.2 Wet mass consistency in relation to pellet yield

Mixer torque rheometry (MTR) has been widely used to determine the optimal water level in formulations intended for extrusion and spheronisation (Chatlapalli et al., 1998b).

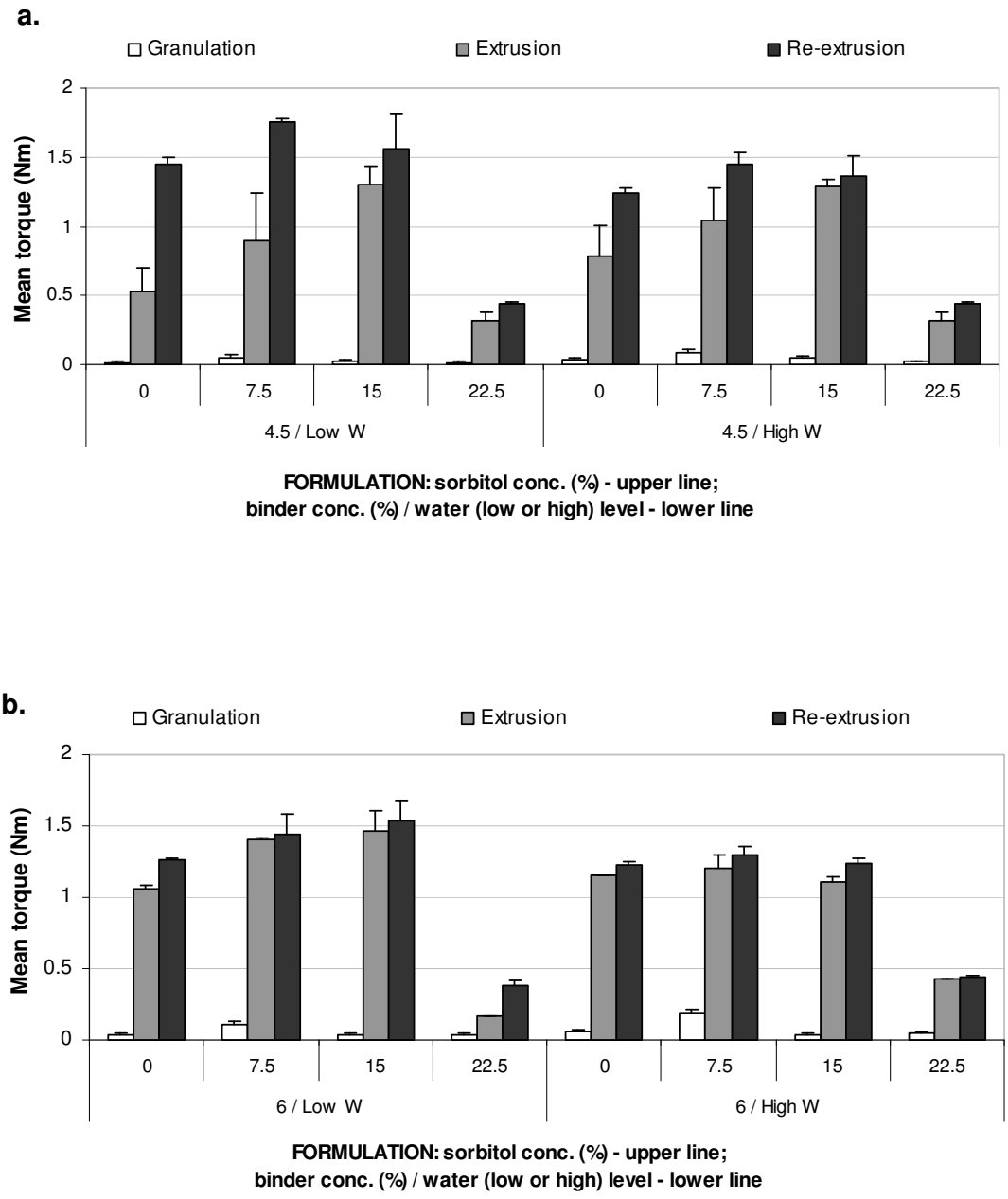


Figure 3.5 *Mean torque values (n=3) of wet mass after granulation, extrusion and re-extrusion as a function of sorbitol concentration and water level:* ***a.*** *formulations with 4.5 % and* ***b.*** *6 % (w/w, dry mass) HPMC.*

The optimal water level has been related to the maximum mean torque value which corresponds to the capillary state of wet mass, when all the pores are filled with granulation liquid and the number of capillary bridges is the largest (Parker et al., 1990; Rowe and Parker, 1994). In addition, MTR was used to measure the wet mass consistency, where a higher mean torque value indicates a higher bonding strength of the wet mass.

Since the preliminary experiments revealed that sorbitol and binder concentration affected the wet mass consistency, MTR was used to characterise the rheological properties of formulations containing varying amounts of sorbitol (0, 7.5, 15 and 22.5 % w/w, dry mass) and binder (4.5 and 6 % w/w, dry mass). In addition, each formulation was granulated with two water levels (low and high), both determined during preliminary tests as the levels closest to the optimal value for obtaining pellets with maximal yield and acceptable sphericity. All formulations contained 25% (w/w, dry mass) anhydrous theophylline.

Figure 3.5 presents the mean torque values (average of three measurements) of wet masses after granulation, extrusion and re-extrusion as a function of sorbitol, water and binder concentration.

When comparing the mean torque values for each phase of wet mass processing, it can be observed that the main densification of the wet mass occurred during extrusion. The mean torque values of wet granules containing UNIPURE® EX starch as the main excipient were lower (<0.1 Nm) compared to MCC-based wet granules (0.27-0.33 Nm, Parker et al., 1990).

Furthermore, sorbitol concentration significantly influenced the consistency of the extruded material: the mean torque value was higher when increasing the sorbitol concentration. However, at the highest concentration (22.5 % w/w, dry mass) the mean torque dropped dramatically. This wet mass behaviour correlated well with the low pellet yield after extrusion and spheronisation of formulations containing the highest sorbitol level (Fig. 3.6). Since the mean torque measures the strength of the interactions which occur between the components of the wet mass, the extrudates from the formulations with the highest sorbitol level could not resist the friction forces during spheronisation and a high amount of fines was formed.

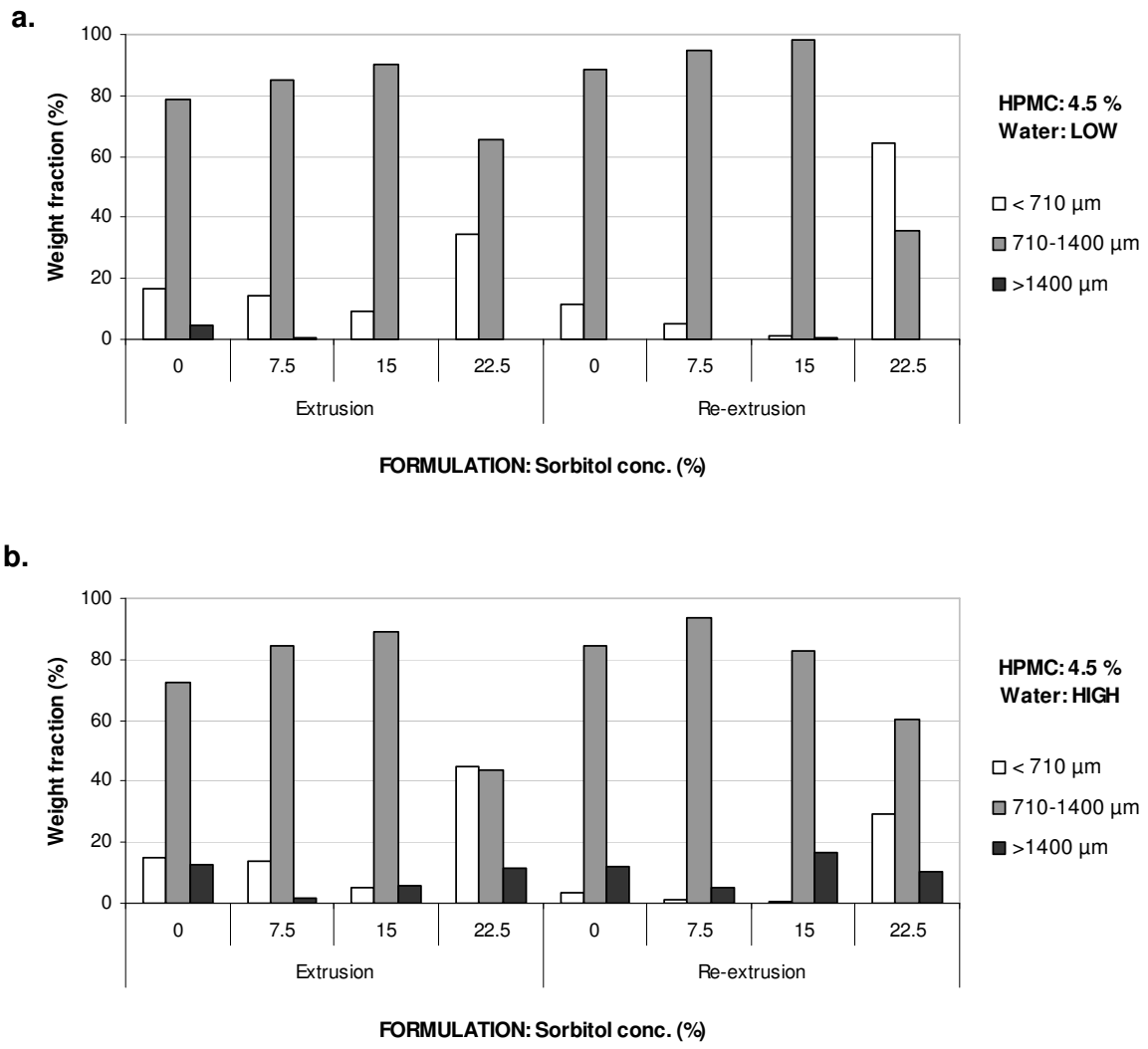


Figure 3.6 *Weight distributions (%) of pellets prepared with different sorbitol and water levels (**a.** low and **b.** high), produced after spheronisation of extruded and re-extruded wet mass. All formulations contained 4.5 % (w/w, dry mass) HPMC and 25% (w/w, dry mass) anhydrous theophylline.*

For the formulations with a low binder and water level, the consistency of extruded material increased with an increase of sorbitol level up to 15 % (Fig. 3.5a). A higher mechanical strength of extrudates correlated well with an increase of pellet yield after spheronisation due to reduction of the fines fraction (Fig. 3.6a). Furthermore, additional densification of the wet mass via re-extrusion affected the wet mass rheology since the mean torque of re-extruded material was higher compared to product extruded only once. Consequently, the yield of pellets after spheronisation of re-extruded material was higher, due to a further reduction of fines. The influence of re-extrusion could be explained by the liquid saturation (Kristensen et al. 1987): since the degree of filling of intragranular voids (liquid saturation) depends on the granulation liquid and intragranular porosity, additional

densification via re-extrusion reduced the intragranular porosity and therefore increased the liquid saturation and binding capacity. In addition, re-extrusion probably assured a better distribution of water through the wet mass and a smoother extrudate surface was obtained (Fig. 3.7). Consequently, the fragmentation of the extrudates at the beginning of the spheronisation was uniform and resulted in a reduction of fines and a higher yield.

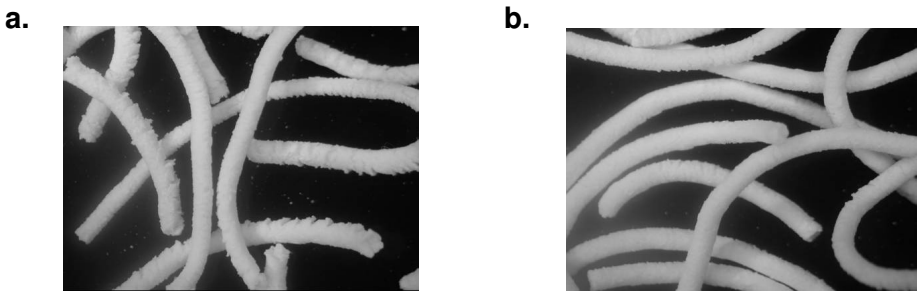
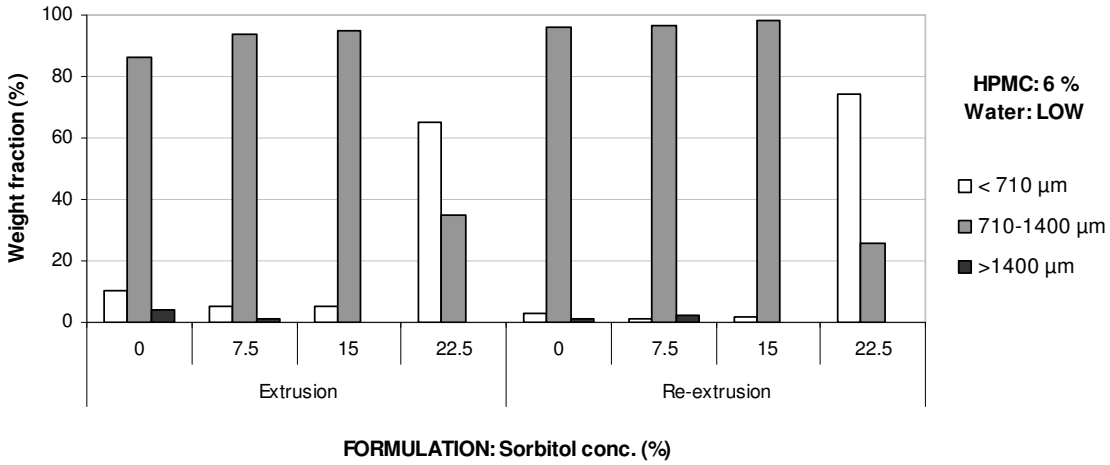


Figure 3.7 *Photomicrographs of extrudates (4.5 % HPMC, low water level, without sorbitol):* ***a.*** *after extrusion and* ***b.*** *after re-extrusion*

Using a higher water level in the formulations (Fig. 3.5a and 3.6b) only slightly increased the consistency of extruded material and pellet yield remained similar to formulations with lower water content, mainly due to an increase of agglomerates. As expected, the consistency of re-extruded material was higher compared to the extrudates, but lower compared to re-extruded material granulated with less water. This can be explained by an increase of liquid saturation when a formulation having a higher moisture level is densified during re-extrusion. If the liquid saturation corresponding to the capillary state is exceeded, pellet agglomeration occurs.

In formulations with a higher binder concentration and a lower water level (Fig. 3.5b and 3.8a), the consistency of extruded mass was increased and pellet yield was consequently higher. However, when adding a higher water level the wet mass consistency dropped due to exceeding the optimal liquid saturation. This correlated well with a lower pellet yield due to agglomeration (Fig. 3.8b). This effect of water level was even more pronounced in case of re-extruded material.

a.



b.

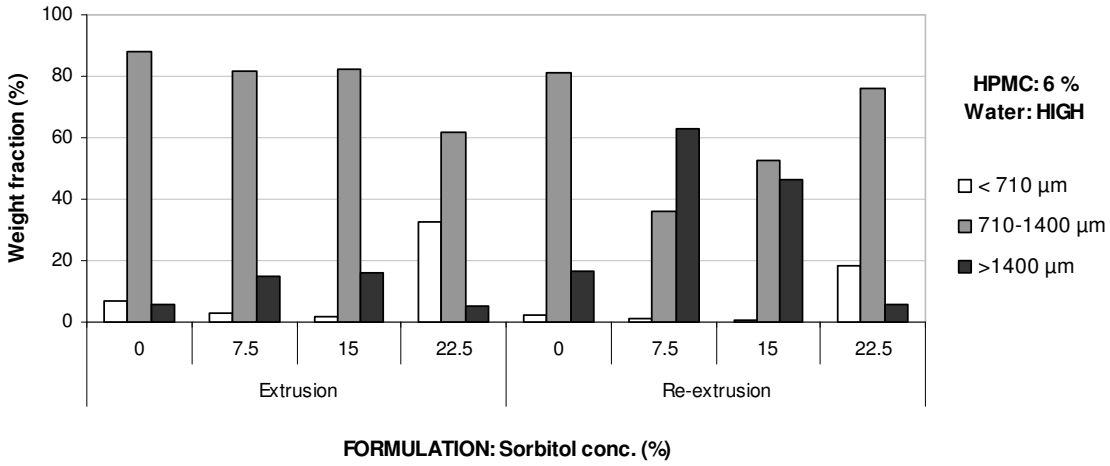


Figure 3.8 *Weight distributions (%) of pellets prepared with different sorbitol and water levels (**a.** low and **b.** high), produced after spheronisation of extruded and re-extruded wet mass. All formulations contained 6 % (w/w, dry mass) HPMC and 25% (w/w, dry mass) anhydrous theophylline.*

3.4.3 Solid state NMR

Since MTR measurements revealed the importance of sorbitol to obtain the optimal wet mass consistency for successful extrusion/spheronisation, solid state NMR spectroscopy and relaxometry was used to study the molecular miscibility at nano-scale and the intermolecular interactions in UNI-PURE®EX starch-based formulations.

Information about the level of mixture heterogeneity can be obtained from the proton relaxation decay times T_{1H} and $T_{1\rho H}$. The intrinsic relaxation decay times of the pure components will be averaged out towards a single decay time if homogeneous mixing is achieved or if the dimensions of existing molecular domains are smaller than the maximum path length over which proton-proton spin diffusion can occur. Table 3.5 presents the T_{1H} values of pure components and blends with variable sorbitol content. Note that for the mixtures the decay times of the resonances 2, 3 and 4 were determined by using a relative short cross-polarisation contact time (1 ms) and recycling delay (5 s). In this way, the contribution of theophylline to resonance 2 was completely suppressed. In all blends, regardless the sorbitol content, sorbitol and UNI-PURE®EX starch were mixed homogeneously on the T_{1H} scale, since a single decay time is observed for the corresponding resonances 2, 3 and 4. Both components clearly relaxed very efficiently via the short T_{1H} of UNI-PURE®EX starch. It was remarkable that this T_{1H} value goes through a maximum for a blend containing 11.25 % sorbitol. In contrast, theophylline remained phase separated and the extremely long T_{1H} relaxation time of about 50 s indicated the preservation of the crystalline state in the blends.

Table 3.5 also presents the $T_{1\rho H}$ decay times of pure components and of mixtures with variable sorbitol content. The $T_{1\rho H}$ relaxation of pure sorbitol behaved bi-exponentially with both a short and very long decay time. This pointed to a (semi) crystalline state which could also explain the rather long T_{1H} decay time. In the processed blends sorbitol became amorphous with only a short $T_{1\rho H}$ decay time. Additionally, sorbitol in the mixtures also relaxed via the efficient pathway of UNI-PURE®EX starch, which indicated that they are homogeneously mixed on the nm-level. Furthermore, the relaxation occurred more efficiently at higher sorbitol concentration. The $T_{1\rho H}$ decay time of theophylline is so extremely long that it could not be determined experimentally, confirming the crystalline state of theophylline in the blends.

Table 3.5 *T_{1H} and $T_{1\rho H}$ decay times of the pure components and of blends with variable sorbitol concentration as measured by ^{13}C solid-state NMR. Resonances 1 and 5 originate from theophylline, 2 from theophylline and UNI-PURE®EX starch, 3 and 4 from UNI-PURE®EX starch and sorbitol. The extremely long $T_{1\rho H}$ of theophylline can not be determined experimentally (ND: not determined).*

	Resonance					Resonance				
	1	2	3	4	5	1	2	3	4	5
	T_{1H} (s)					$T_{1\rho H}$ (ms)				
UNI-PURE®EX starch		0.76	0.75	0.70			4.6	3.9	4.2	
Sorbitol			10.5	10.5				11 (21%) 172(79%)	11 (21%) 172(79%)	
Theophylline	51.2	45.2			54.0	ND	ND			ND
0 % sorbitol	50.0	0.61	0.61	0.62	51.0	ND	4.5	4.6	5.0	ND
7.5 % sorbitol	48.4	0.68	0.67	0.65	49.6	ND	2.6	2.4	2.5	ND
11.25 % sorbitol	52.2	0.76	0.74	0.72	46.3	ND	2.8	2.4	2.3	ND
20 % sorbitol	48.3	0.67	0.66	0.65	50.7	ND	1.9	1.8	2.1	ND

As a conclusion, it was demonstrated that the T_{1H} relaxation time increased with the amount of sorbitol, reaching a maximum for a blend containing 11.25 % sorbitol. The relaxation is completely determined by UNI-PURE®EX starch. Adding sorbitol to the formulation reduced the molecular mobility of starch with an increase of T_{1H}. However, in blends with higher sorbitol concentration (20%), sorbitol acted as a plasticiser, providing increased starch mobility and reducing the T_{1H} value. Above mentioned observations are in good agreement with MTR measurements: at lower sorbitol levels the mean torque increased due to strong starch-sorbitol interactions and a lower molecular mobility, whereas at the highest sorbitol level (20%, w/w) the mean torque decreased due to the plasticising effect of the higher molecular mobility. Gaudin et al. (1999) already described the plasticising and anti-plasticising effect of sorbitol on starch as a function of sorbitol concentration: at low sorbitol levels brittle starch-sorbitol films were formed, whereas the mechanical strength of these films increased at sorbitol concentrations above 21%. These interactions between starch and sorbitol were also confirmed by NMR, showing that at low sorbitol levels the molecular mobility was lower due to strong hydrogen bonding between sorbitol and starch molecules. When exceeding a critical sorbitol concentration, other interactions were identified, namely starch-sorbitol-sorbitol interactions. This clustering of sorbitol molecules was associated with higher system mobility and therefore sorbitol exhibited a plasticising effect.

3.4.4 Development and optimisation of starch-based pellets containing anhydrous theophylline as model drug

3.4.4.1 Pellet yield

Table 3.6 lists the actual values of pellet yield (fraction of pellet size between 710 and 1400 µm), fines (fraction of pellet size <710 µm), pellet sphericity (aspect ratio, AR and two-dimensional shape factor, e_R) and size (mean Feret diameter), while Table 3.7 presents the results of the ANOVA analysis of the responses modelled in the experimental design.

Table 3.6 *Results of pellet yield, fines, aspect ratio (AR), two-dimensional shape factor (e_R) and Feret diameter (FD).*

Run	Coded values				Results				
	A	B	C	D	Yield (%)	Fines (%)	AR	e_R	FD (µm)
1	-1	0	0	-1	83.6	16.3	1.17	0.51	1013
2	0	0	1	-1	73.4	26.5	1.14	0.55	980
3	-1	0	0	1	77.2	16.5	1.15	0.54	1062
4	0	0	0	0	88.4	8.9	1.14	0.54	1047
5	0	0	0	0	87.4	10.0	1.12	0.57	1066
6	0	-1	-1	0	85.8	1.8	1.15	0.53	1140
7	-1	0	1	0	37.4	62.3	1.13	0.55	996
8	-1	0	-1	0	90.2	7.8	1.20	0.48	1073
9	1	0	0	1	83.7	1.7	1.16	0.52	1155
10	-1	-1	0	0	59.2	35.3	1.13	0.56	1159
11	0	0	-1	-1	94.0	6.0	1.25	0.45	1095
12	-1	0.78	0	0	39.0	60.9	1.15	0.52	1063
13	0	0.78	-1	0	45.9	54.1	1.16	0.52	1020
14	1	0	-1	0	90.1	0.6	1.19	0.48	1168
15	0	0.78	1	0	7.6	92.4	1.16	0.51	952
16	1	0.78	0	0	14.8	85.2	1.14	0.54	918
17	0	-1	0	1	71.1	4.8	1.15	0.53	1134
18	0	0	0	0	87.6	11.0	1.13	0.55	1071
19	0	0	0	0	86.6	11.9	1.11	0.58	1020
20	0	0	-1	1	76.3	0.7	1.15	0.52	1193
21	0	0.78	0	-1	35.5	64.5	1.19	0.48	963
22	0	-1	0	-1	75.8	22.5	1.13	0.54	1062
23	0	0	0	0	88.9	9.3	1.12	0.57	1054
24	1	0	0	-1	91.8	7.9	1.18	0.50	1048
25	1	-1	0	0	79.2	3.0	1.16	0.53	1108
26	0	0	1	1	73.5	21.9	1.17	0.52	1022
27	0	-1	1	0	42.1	47.2	1.14	0.54	1042
28	1	0	1	0	80.9	18.5	1.14	0.54	1029
29	0	0.78	0	1	33.1	46.9	1.15	0.52	1069

The results of the ANOVA analysis of pellet yield (Table 3.7) suggested that a reduced cubic model can be used for data fitting ($P<0.05$) and process optimisation. The lack-of-fit was not significant ($P>0.05$) and the predicted R-squared value was in reasonable agreement with the R-squared value adjusted for the degrees of freedom. All linear and quadratic factors of the model (except the quadratic term of water) were significant ($P<0.05$) as well as some interaction terms. Therefore, when evaluating the influence of a specific factor (variable) on process yield, significant interactions between spheronisation speed and the other variables and between sorbitol and binder level should be considered. The regression equation in terms of the coded values is the following (Eq. 3.7):

$$\begin{aligned}\text{Yield (\%)} = {} & 88.11+3.67*A-32.83*B-5.85*C-3.27*D-4.82*A^2-47.61*B^2- \\ & -8.55*C^2-14.18*A*B+10.91*A*C-2.62*B*C+4.43*C*D- \\ & -9.62*A^2*C-7.83*A*B^2+7.18*A*C^2-18.62*B^2*C \end{aligned} \quad (3.7)$$

Table 3.7 *ANOVA results for yield, aspect ratio and Feret diameter*

Yield		***Aspect ratio***		***Feret diameter***	
Source	*P value*	*Source*	*P value*	*Source*	*P value*
Model	*< 0.0001*	*Model*	*< 0.0001*	*Model*	*< 0.0001*
A: HPMC	*0.0010*	*C: Sph. speed*	*0.0011*	*B: Sorbitol*	*< 0.0001*
B: Sorbitol	*< 0.0001*	*D: Water*	*0.0249*	*C: Sph. speed*	*< 0.0001*
C: Sph. speed	*< 0.0001*	*C^2*	*0.0017*	*D: Water*	*0.0010*
D: Water	*< 0.0001*	*D^2*	*0.0009*	*Lack of Fit*	*0.1008*
A^2	*< 0.0001*	*CD*	*0.0010*	*R^2*	*0.7314*
B^2	*< 0.0001*	*Lack of Fit*	*0.1817*	*Adjusted R^2*	*0.6991*
C^2	*< 0.0001*	*R^2*	*0.7149*		
AB	*< 0.0001*	*Adjusted R^2*	*0.6529*		
AC	*< 0.0001*				
BC	*0.0296*				
CD	*0.0002*				
A^2C	*< 0.0001*				
AB^2	*0.0003*				
AC^2	*< 0.0001*				
B^2C	*< 0.0001*				
Lack of Fit	*0.0724*				
R^2	*0.9977*				
Adjusted R^2	*0.9951*				

Figure 3.7 presents 3D-surface response diagrams of pellet yield as a function of sorbitol and binder level for a fixed (medium) water level at different spheronisation speeds. The

graphs show that a high yield was obtained (>90%). Pellet yield was the highest at medium or low spheronisation speed (Fig. 3.9 a, b). At the highest spheronisation speed, yield increased with increasing binder concentration (Fig. 3.9 c). A higher binder level promoted binding of the wet mass and additionally made the extrudate more resistant to friction forces at higher spheronisation speed. As seen in the previous sections, the lowest yield was obtained for the highest sorbitol level and this was confirmed at each spheronisation speed. When reducing the sorbitol amount to the medium value, the influence of spheronisation speed on pellet yield was less pronounced, being lower only for a lower binder concentration combined with the highest spheronisation speed. A formulation without sorbitol resulted in a lower yield at a higher spheronisation speed and lower binder concentration.

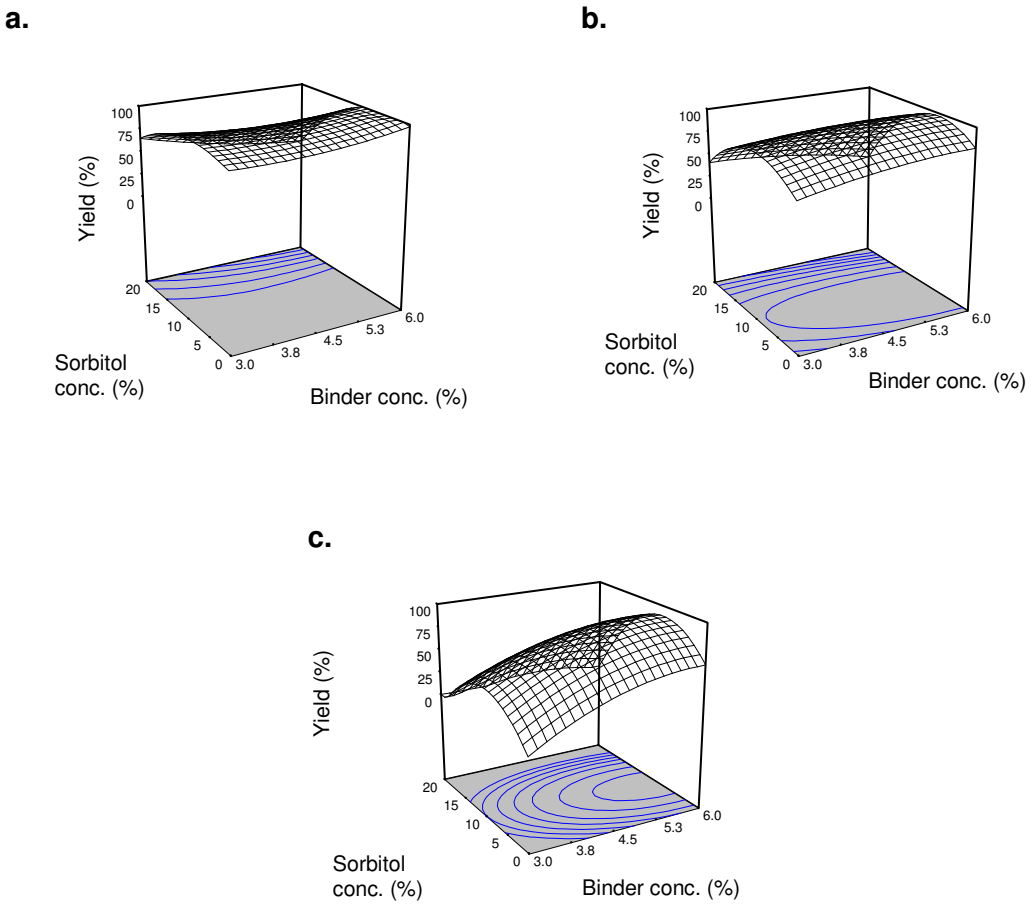


Figure 3.9 *3D surface response diagrams of pellet yield as a function of sorbitol and binder level for fixed amount of water (medium level) and fixed spheronisation speed:* ***a.*** *650 rpm (low),* ***b.*** *850 rpm (medium), and* ***c.*** *1050 rpm (high).*

As already mentioned, the amount of water is a critical parameter for the extrusion/spheronisation process. The optimal water level depended on the sorbitol concentration, being lower if a higher sorbitol level was used. Sorbitol as a water-soluble component dissolved in water during wet massing, reducing the solids amount and inducing pellet agglomeration during spheronisation. Similar relationships between the solubility of drug and filler and the optimal water concentration were reported by Baert et al. (1991), Hileman et al. (1997) and Lustig-Gustafsson et al. (1999).

The response surface graphs (Fig. 3.10) showed that at medium sorbitol level, a high binder level did not increase pellet yield. This is probably due to the low molecular weight of sorbitol, which interacts with the starch molecules and additionally accumulates in the cavities between HPMC molecules and thereby reduces the adhesion of HPMC polymer chains. This effect of low molecular weight materials on self-adhesion of polymers has been described by Millili et al. (1990).

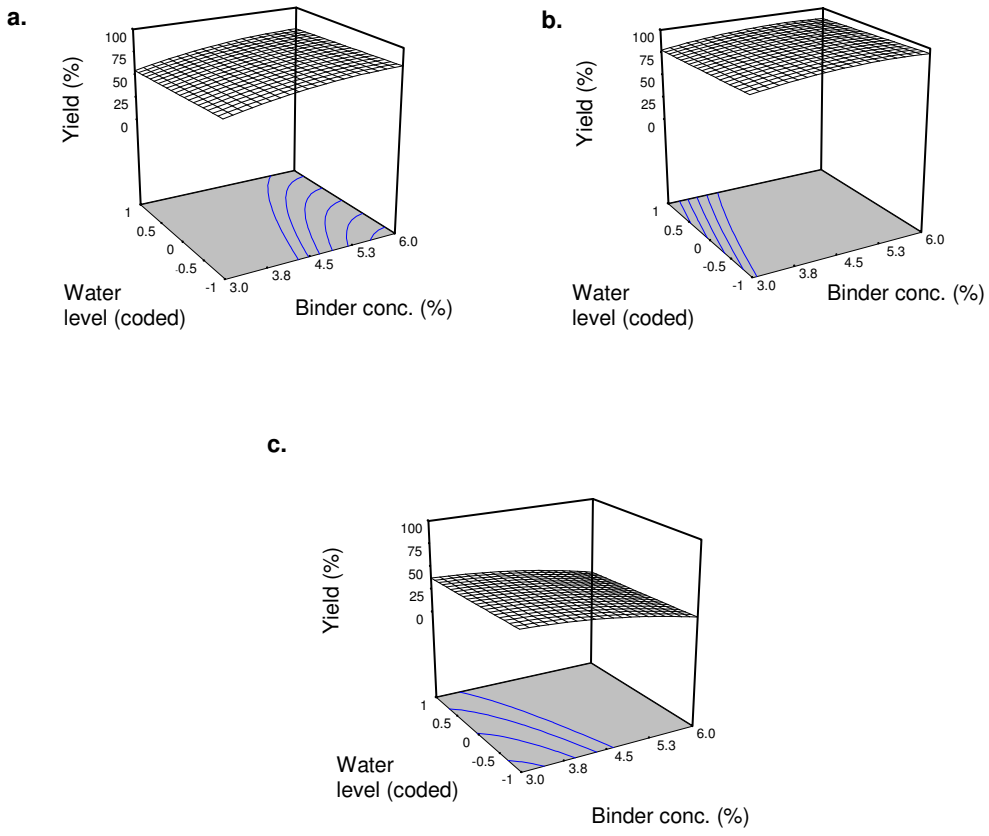


Figure 3.10 *3D surface response diagrams of pellet yield as a function of water and binder level at fixed spheronisation speed (medium) and sorbitol level:* ***a.*** *0% (low),* ***b.*** *11.25% (medium), and* ***c.*** *20% (high).*

3.4.4.2 Pellet sphericity

Table 3.7 lists the results of the ANOVA analysis with the aspect ratio as response value. The quadratic model was significant ($P<0.05$) and the statistics (insignificant lack-of-fit and agreement of predicted and adjusted R-squared values) indicated that the model can be used to describe the data and optimise the process. Spheronisation speed, water level (linear and quadratic functions) as well as their interactions were significant factors ($P<0.05$), while the binder and sorbitol level did not have a significant influence on pellet sphericity. The regression equation in terms of the coded factor values can be presented by the following equation (Eq. 3.8):

$$\text{Aspect ratio} = 1.135 - 0.018*C - 0.012*D + 0.023*C^2 + \\ + 0.024*D^2 + 0.032*C*D \qquad (3.8)$$

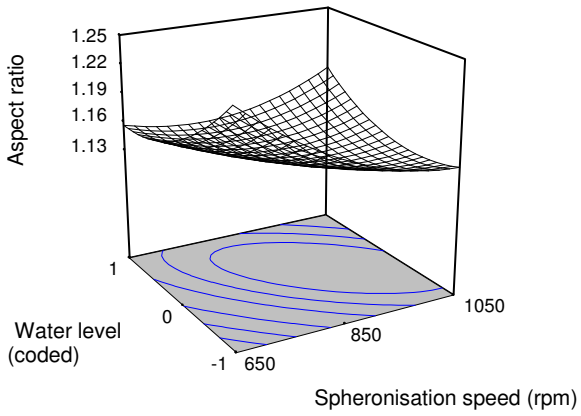


Figure 3.11 *3D surface response diagram of pellet aspect ratio as a function of water level (coded) and spheronisation speed at fixed binder and sorbitol level (medium).*

An increase of spheronisation speed has been already reported as beneficial for pellet sphericity (Baert et al., 1993; Wan et al., 1993; Hellén et al., 1994). For this pellet formulation, we confirmed that increasing spheronisation speed lowered the aspect ratio. Using a suboptimal amount of water resulted in dumbbells, whereas at higher water concentrations agglomeration occurred. Results (Fig. 3.11, Table 3.6) showed that for most formulations the aspect ratio was between 1.12 and 1.20, which complied with the range defined by Chopra et al. (2002) for acceptable pellet sphericity. The significant

interaction between water and spheronisation speed indicated that a low spheronisation speed and water level decreased the sphericity of the end product, confirming the importance of energy input during spheronisation as well as of an adequate water level for successful spheronisation. Sphericity improved at higher spheronisation speeds, but the influence of water was less pronounced, suggesting that a wider water range could be used to obtain acceptable sphericity. The binder concentration and its interactions were not significant, indicating that the concentration range (3-6 %, w/w, dry mass) selected during preliminary study was optimal. Agrawal et al. (2004) employed HPMC as a binder in a higher concentration range (5-10 %, w/w) in pellet formulations containing chitosan and reported significant interactions between binder concentration and spheronisation speed.

ANOVA analysis of shape factor (e_R) resulted in similar significant factors compared to aspect ratio as a response parameter, with sorbitol as an additional significant factor (P=0.0493). The shape factor was lower at high sorbitol level due to an increase of pellet surface roughness, since the shape factor combined by definition pellet geometry and surface roughness (Podczeck et al., 1994). Due to the low mechanical strength of extrudates at this sorbitol concentration, fine particles were sticking to the surface of larger pellets during spheronisation, thus increasing surface roughness (Fig. 3.15 d).

3.4.4.3 Pellet size

The ANOVA analysis of pellet size (mean Feret diameter) is presented in Table 3.7. Significant linear model (P<0.05), insignificant lack-of-fit test (P>0.05) and agreement of predicted and adjusted R-squared values allow data modelling. Only linear functions of spheronisation speed, water and sorbitol concentration were significant (P<0.05). The regression equation (Eq. 3.9) in terms of the coded factor values is:

$$\text{Feret diameter} = 1056.6 - 60.5*B - 55.6*C + 39.4*D \quad (3.9)$$

3D surface response diagrams of pellet size (Fig. 3.12) showed that for all batches the mean pellet diameter was between 900 and 1200 µm. Furthermore, a higher water concentration generated larger pellets due to particle agglomeration during spheronisation. Additionally, at higher spheronisation speed excessive fragmentation of the extrudates occurred at the beginning of the spheronisation phase, yielding smaller pellets. Furthermore, the results revealed that increasing sorbitol concentrations reduced

pellet size. The addition of sorbitol provided a more uniform breaking of the extrudates at the beginning of the spheronisation phase. Formulations with the highest sorbitol concentration had the lowest pellet size due to insufficient mechanical strength of extrudates.

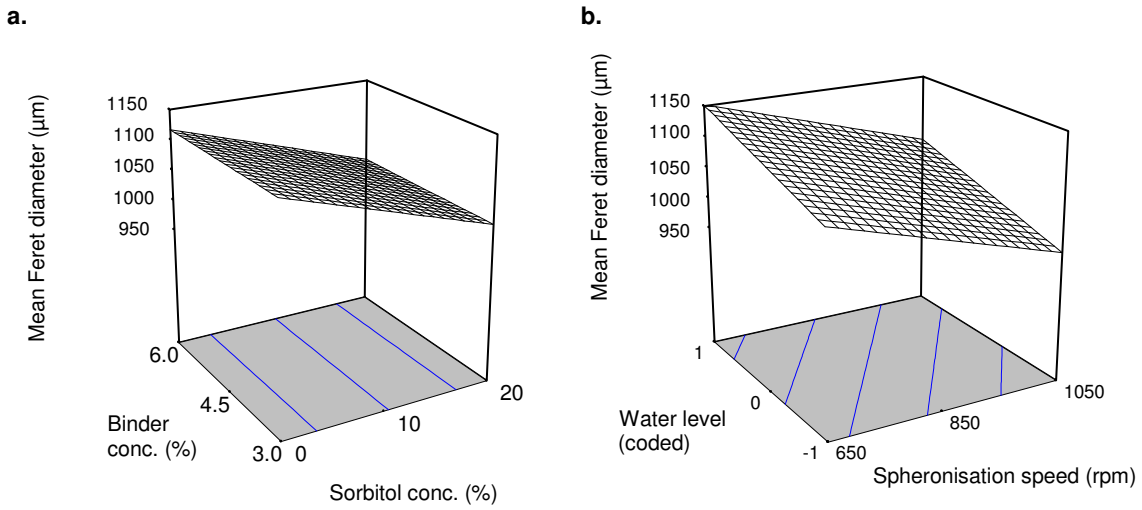


Figure 3.12 *3D surface response diagrams of pellet size (mean Feret diameter) as a function of:* ***a.*** *sorbitol and binder level at fixed spheronisation speed and water level (medium), and* ***b.*** *spheronisation speed and water level at fixed sorbitol and binder level (medium).*

3.4.4.4 Validation of model prediction

The regression equations of pellet yield and aspect ratio were used to determine the levels of all variables resulting in an optimal process in terms of maximum yield and minimal aspect ratio. For example, Figure 3.13 presents the spheronisation speed and sorbitol concentration providing a pellet yield above 85% and an aspect ratio below 1.15 for medium binder and water level. It can be observed that an acceptable yield and sphericity is to be expected at a spheronisation speed between 750 and 950 rpm combined with sorbitol concentrations up to about 10 % (w/w).

To test the validity of the model, one point of the model was selected and the good agreement between the predicted and actual values (Table 3.8) indicated that the proposed statistical models can be used as a tool for successful process optimisation.

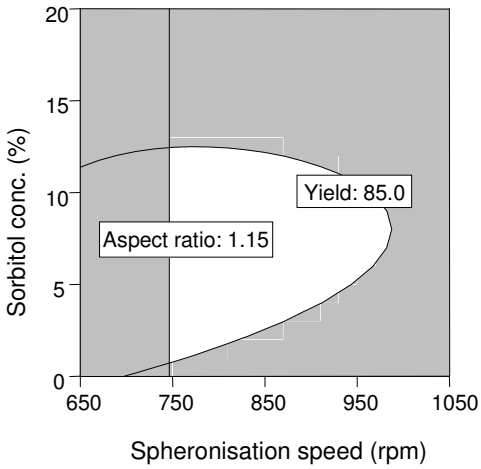


Figure 3.13 *Graphical presentation of process optimisation (yield>85% and AR<1.15) at medium water and binder level.*

Table 3.8 *Predicted and actual response values for a formulation consisting of 25% theophylline, 5.8 % binder, 9.7 % sorbitol and UNIPURE®EX starch (water level: 0.33 (coded value); spheronisation speed: 846 rpm).*

Response	***Prediction value***	***SEM***	***95% CI - low***	***95% CI - high***	***Obtained value***
Yield (%)	*91.36*	*0.97*	*89.27*	*93.45*	*91.80*
Aspect ratio	*1.134*	*0.005*	*1.123*	*1.144*	*1.125*
Shape factor	*0.541*	*0.007*	*0.527*	*0.556*	*0.552*

3.4.4.5 X-ray diffraction

X-ray diffractograms of raw materials and pellets (Fig. 3.14) support the solid state NMR findings that theophylline preserved its crystallinity, while sorbitol became amorphous during processing. Furthermore, no differences between diffraction patterns of formulations with different sorbitol content were observed.

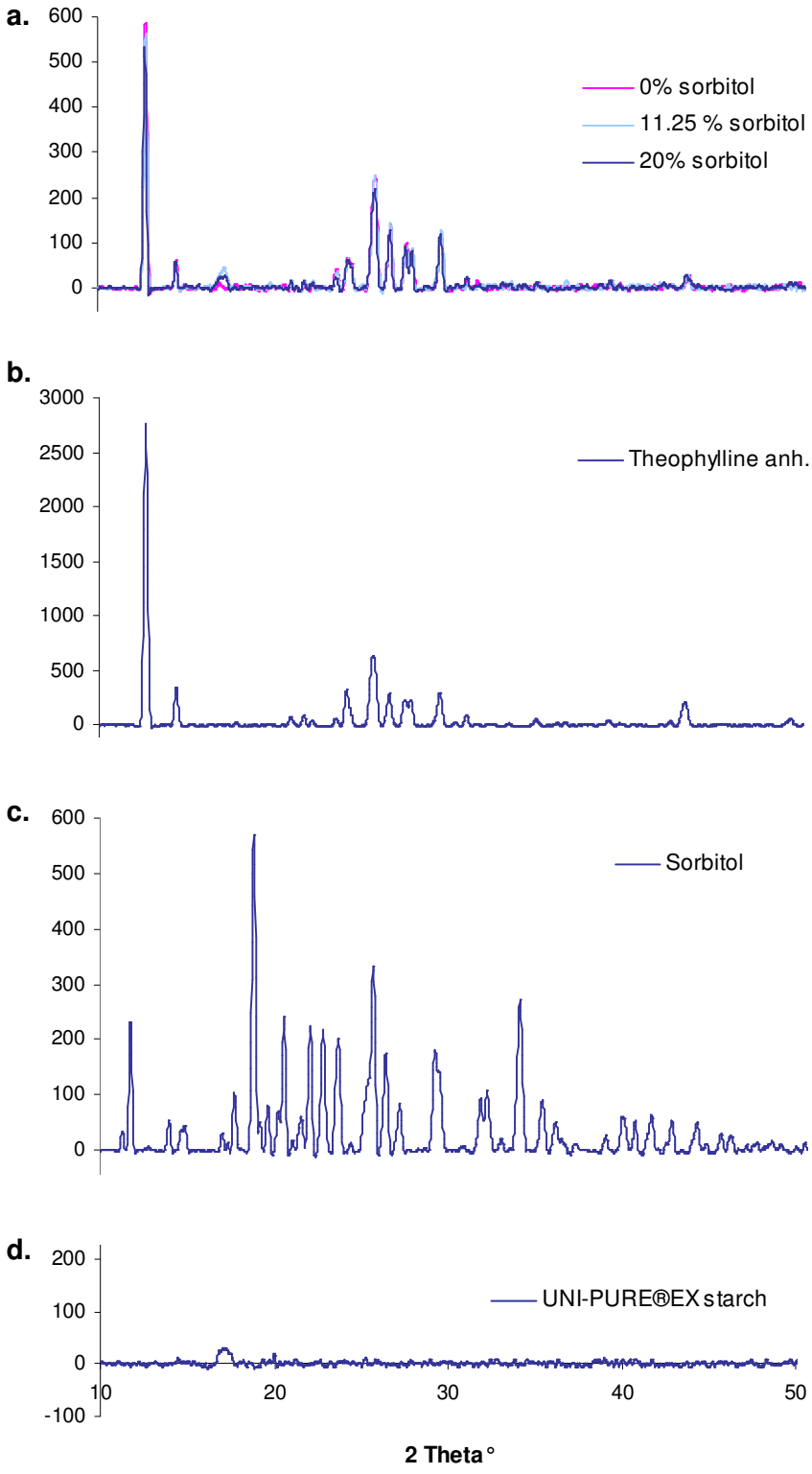


Figure 3.14 *X-ray powder diffraction patterns:* ***a.*** *pellets containing 0, 11.25 and 20 %, w/w of sorbitol,* ***b.*** *anhydrous theophylline,* ***c.*** *sorbitol, and* ***d.*** *UNI-PURE®EX starch.*

3.4.4.6 Pellet surface morphology

SEM pictures showed that sorbitol had a significant effect on surface morphology (Fig. 3.15): pellets without sorbitol had a cracked surface, whereas pellets containing sorbitol had a smoother surface. Since the presence of cracks is not a preferred feature for pellets intended for coating, the addition of sorbitol to the formulation is crucial to obtain a successful sustained-release coat.

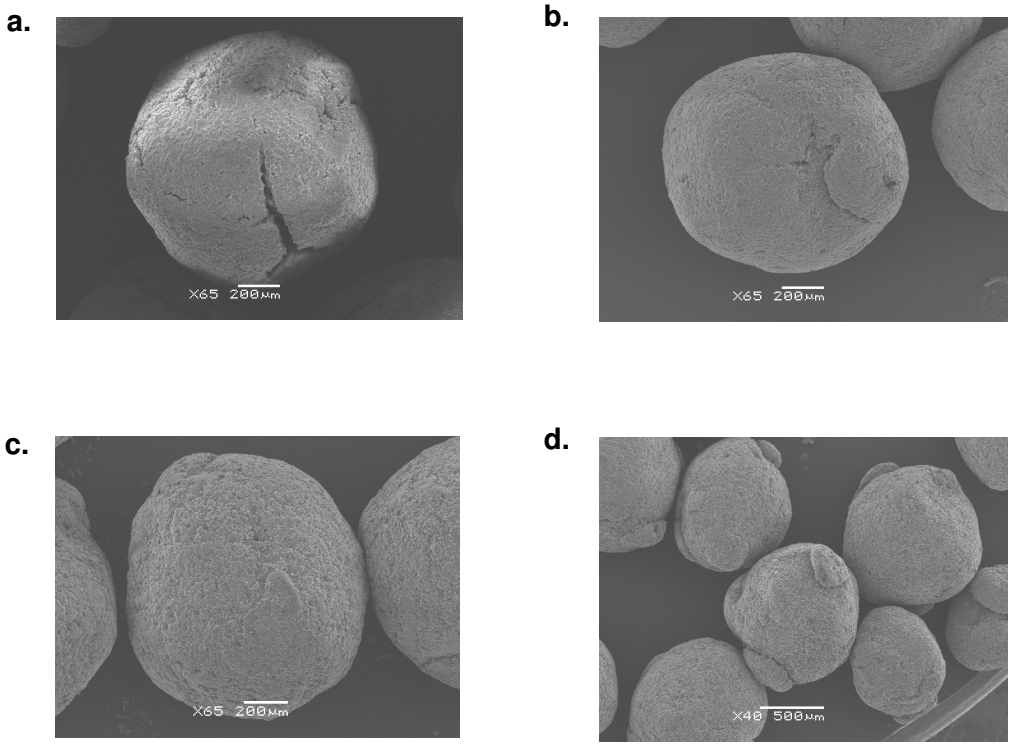


Figure 3.15 *SEM pictures of pellets containing different amounts of sorbitol (w/w, dry mass):* ***a****. 0 %,* ***b.*** *11.25 %,* ***c.*** *20 % and* ***d.*** *20 %*

3.4.4.7 Friability

Starch-based pellets had a high mechanical strength, since friability values of less than 0.01% were obtained for all batches. In general, a low friability indicates the pellets ability to withstand the shear forces during fluid bed coating.

3.4.4.8 Disintegration

Disintegration tests showed that pellets containing starch as the main excipient disintegrated, the disintegration time being determined by the sorbitol level: pellets with 20% sorbitol disintegrated within 2 min, whereas the other batches (irrespective of sorbitol level) had a similar disintegration time (between 5 and 7 min). The other variables of the experimental design did not have a significant influence on disintegration time.

3.4.4.9 In-vitro drug release

Drug release profiles (Fig. 3.16) showed that all starch-based pellets completely released theophylline in less than 20 minutes, which is significantly faster than MCC-based pellets (only 50 % drug was released within the same time interval), mainly due to the ability of starch-based pellets to disintegrate.

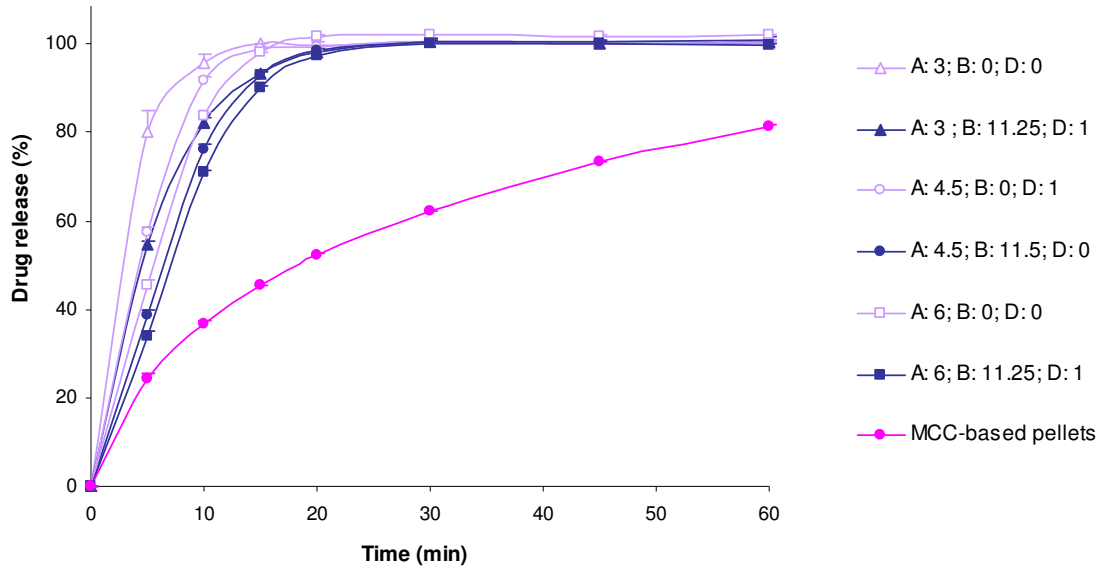


Figure 3.16 *Dissolution profiles of pellets containing different amounts of a binder (A: 3, 4.5 or 6 % w/w, dry mass) and sorbitol (B: 0 or 11.25 % w/w, dry mass). Water level (D) is presented in coded values.*

The binder and sorbitol concentration only determined drug release during the initial 10 minutes of the test: pellets containing a high amount of binder or sorbitol at medium level had a slightly slower drug release. In contrast, formulations without sorbitol had slightly

faster drug release, possibly due to surface irregularities (cracks). Nevertheless, immediate drug release was obtained for all batches, irrespective of formulation and process parameters.

Figure 3.17 shows the dissolution profiles of theophylline from pellets produced using the same variables of the experimental designs (central point was repeated five times). Since the standard deviation (three samples of each batch were analysed) for each sampling point was less than 1%, it can be concluded that the dissolution profiles are reproducible.

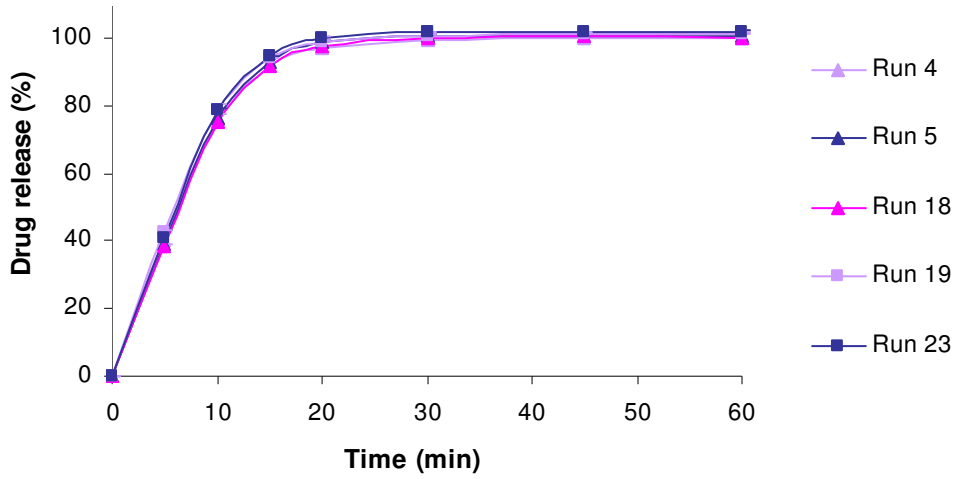


Figure 3.17 *Dissolution profiles of pellet batches produced using the same (central) point of the experimental design (SD values for n=3 are included in the graph).*

3.5 Conclusion

UNI-PURE®EX starch was successfully used as the main excipient in formulations intended for extrusion/spheronisation. However, a binder was necessary to obtain an acceptable yield and the addition of sorbitol improved the surface properties of the pellets. Based on the high process yield (>90%), good pellet sphericity (AR<1.2), low friability (<0.01%), fast disintegration (<10 min) and complete drug release in less than 20 minutes for all formulations, UNI-PURE®EX starch can be proposed as an alternative to microcrystalline cellulose during processing of formulations (containing 25% drug) via extrusion/spheronisation.

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4

IN-VITRO AND IN-VIVO EVALUATION OF IMMEDIATE-RELEASE STARCH-BASED PELLETS CONTAINING POORLY SOLUBLE MODEL DRUGS

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A. Dukić-Ott, J.P. Remon, P. Foreman, C. Vervaet
"Immediate release of poorly soluble drugs from starch-based pellets prepared via extrusion/spheronisation."

4.1 Introduction

An important disadvantage of MCC-based pellets is the lack of disintegration (Chapter 2) and therefore 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 enteric-coated pellets or colon targeted drug delivery, where immediate drug release is required after the functional coating has dissolved in gastrointestinal fluids. The lack of MCC-based pellet disintegration becomes very critical if the active component has poor solubility in water, since the drug release is prolonged. As reviewed in the second chapter, MCC formulations have been modified in order to increase drug release, or alternative excipients as substitutes for MCC have been investigated.

The first part of this study (Chapter 3) showed the potential of UNI-PURE® EX starch to produce spherical pellets with a narrow particle size distribution and high process yield. The most influential formulation and process variables have been identified. The most important feature of UNI-PURE® EX starch-based pellets was found to be their

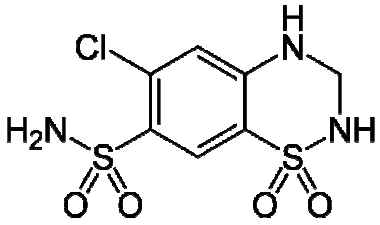
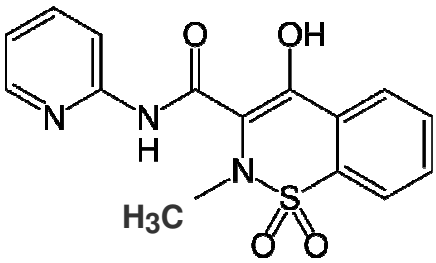
disintegration, which might provide a solution for the slower release of poorly soluble drugs observed in MCC-based pellets.

It was shown that due to pellet disintegration, the release of anhydrous theophylline as a model drug with medium water solubility was complete in less than 20 minutes. This part of the 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.

4.2 Materials

The physicochemical properties of hydrochlorothiazide and piroxicam, used as model drug used in this part of the study, are presented in Table 4.1, while Tables 4.2 and 4.3 list the excipients used for pellet preparation and the materials used for determination of hydrochlorothiazide in dog plasma, respectively.

Table 4.1 *Physicochemical properties of hydrochlorothiazide and piroxicam (Martindale, 2005)*

Properties		***Structural formula***
Name:	*Hydrochlorothiazide*	
Chemical name:	*6-chloro-3,4-dihydro-2H-1,2,4-benzothiazidine-7-sulphonamide-1,1-dioxide*	
Producer:	*Bufa (Uitgeest, The Netherlands)*	
Solubility in water:	*Very slightly soluble* [a]	
Particle size D[v,0.5]:	*102.4 (± 9.6) µm*	
pK_a :	*8.8 ; 9.9*	
Name:	*Piroxicam*	
Chemical name:	*4-hydroxy-2-methyl-N-(2-pyrydil)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide*	
Producer:	*Sagran (Milan, Italy)*	
Solubility in water:	*Practically insoluble* [a]	
Particle size D[v,0.5]:	*9.9 (± 0.6) µm*	
pK_a :	*1.8; 5.1*	

[a] *Source: Ph. Eur. 4*

Table 4.2 *Excipients used during extrusion/spheronisation.*

Excipient name	***Trade name***	***Producer***
Modified (resistant) starch	*UNI-PURE® EX starch*	*National Starch and Chemical Co., Bridgewater, New Jersey, USA*
Hydroxypropylmethylcellulose	*Methocel® E15 LV EP Pharm*	*Colorcon, Dartford, UK*
Sorbitol	*Sorbidex® P 16616*	*Cerestar, Vilvoorde, Belgium*
Microcrystalline cellulose	*Avicel® PH 101*	*FMC, Cork, Ireland*
Demineralised water was used as granulation liquid.		

Table 4.3 *Materials used for determination of hydrochlorothiazide in dog plasma.*

Excipient name	***Producer***
Hydrochlorothiazide	*Sigma Chemical Co., St. Louis, MO, USA*
Hydroflumethiazide	*Sigma Chemical Co., St. Louis, MO, USA*
Methyl tert.-buthylether, HPLC quality	*Sigma Chemical Co., St. Louis, MO, USA*
Acetonitrile, HPLC quality	*Biosolve, Valkenswaard, The Netherlands*
Toluene, HPLC quality	*Biosolve, Valkenswaard, The Netherlands*
Tetrahydrofurane, HPLC quality	*Biosolve, Valkenswaard, The Netherlands*
NaOH, pro analyse	*VWR International, Fontenay sous Bois, France*
KH_2PO_4, pro analyse	*VWR International, Fontenay sous Bois, France*
Distilled water	

4.3 Methods

4.3.1 Pellets containing hydrochlorothiazide as model drug - Experimental design

To identify significant formulation variables a 2^4 - factorial design with a central point for curvature estimation was used. Table 4.4 lists the four factors at both levels used in the design. Hydrochlorothiazide (10 and 50 % w/w, dry mass) and HPMC concentration (4 and 7 % w/w, dry mass) were numerical 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 the following: O (formulation without sorbitol), S (formulation containing sorbitol), L (low water level) and H (high water level). Sorbitol concentration was set to 10 % of the UNI-PURE® EX starch content in the formulation.

Table 4.4 *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]	*L*	*H*

[a] *Sorbitol concentration is expressed as the percentage of UNI-PURE® EX starch amount in the formulation: O=formulation without sorbitol, S=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 4.5 lists all experiments with the coded and actual values of the variables. The total number of experiments was 20 (including 4 experiments using the central point of each numerical variable combined with each level of both categorical variables). Experiments were performed in a randomised order using the same process parameters.

Table 4.5 *2^4- factorial design (randomised order) presented in coded and actual values: A - HCT conc. (% w/w, dry mass), B - HPMC conc. (% w/w, dry mass), C - sorbitol conc. (% w/w, dry mass) and D - water conc. (% w/w, wet mass).*

Run	Coded values				Actual values			
	A	B	C	D	A	B	C	D
1	0	0	O	L	30	5.5	0	42.86
2	1	-1	O	L	50	4	0	37.50
3	-1	-1	O	H	10	4	0	50.00
4	-1	1	S	L	10	7	8.3	42.86
5	1	1	O	H	50	7	0	37.50
6	0	0	S	L	30	5.5	6.5	40.30
7	-1	-1	O	L	10	4	0	49.37
8	-1	-1	S	H	10	4	8.6	45.95
9	0	0	S	H	30	5.5	6.5	41.18
10	1	-1	O	H	50	4	0	39.39
11	1	1	O	L	50	7	0	35.48
12	1	1	S	L	50	7	4.3	33.33
13	1	-1	S	H	50	4	4.6	36.51
14	-1	1	S	H	10	7	8.3	44.44
15	1	1	S	H	50	7	4.3	35.48
16	-1	-1	S	L	10	4	8.6	44.44
17	1	-1	S	L	50	4	4.6	35.48
18	0	0	O	H	30	5.5	0	44.44
19	-1	1	O	L	10	7	0	48.05
20	-1	1	O	H	10	7	0	48.72

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

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

4.3.2 Pellets containing piroxicam as model drug

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.

Reference pellets with the same piroxicam concentration were prepared with MCC as the main excipient, to compare the drug release profiles. Furthermore, the same process parameters and an optimised amount of water as granulation liquid were used for extrusion/spheronisation.

4.3.3 Pellet production

The model drug (hydrochlorothiazide or piroxicam), 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: 1mm). 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).

4.3.4 Pellet and powder characterisation

Methods for determining the pellet yield, size, sphericity, friability and disintegration were described in the previous chapter, Section 3.3.4. Scanning electron microscopy (also described in Section 3.3.4) has been used to visually observe the morphology of

hydrochlorothiazide, piroxicam and UNI-PURE® EX starch powders.

4.3.4.1 Laser diffraction

The particle size of hydrochlorothiazide, piroxicam and UNI-PURE® EX starch powders was determined by laser diffraction (Mastersizer-S long bed, Malvern Instruments, Malvern, UK) with data acquisition time of 2 ms. A small sample dispersion unit (stirrer speed of 1500 rpm) was used to suspend powders in a dispersion medium: Miglyol 812N, capric triglyceride, refraction index 1.4493 (Sasol, Witten, Germany) with 0.2 % of polysorbate 80 (Tween® 80, Alpha Pharma, Nazareth, Belgium). All measurements were performed in triplicate and the particle size was expressed as D [v,0.5].

4.3.4.2 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 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.

4.3.4.3 Dissolution

The dissolution tests were performed using a dissolution apparatus according to the USP paddle apparatus (VK 8000, VanKel, New Jersey, USA) at a rotational speed of 100 rpm and temperature of 37°C. The 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 hydrochlorothiazide and phosphate buffer (pH 6.8) for pellets with piroxicam. 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 hydrochlorothiazide and piroxicam pellets, respectively, by means of a double-beam spectrophotometer (Shimadzu UV-1650PC, Shimadzu Co., Kyoto, Japan). Each batch was analysed in triplicate.

4.3.5 Bioavailability testing

4.3.5.1 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 12h as well as during the experiment (24h), while access to water was unlimited. In a randomised cross-over design, each dog received 50 mg of hydrochlorothiazide 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 hydrochlorothiazide, while the other was formulated with 50% of hydrochlorothiazide (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 24h, collected into heparinised borosilicate test tubes and after centrifugation at 1400g for 10 min, plasma was stored at -20°C until analysed.

4.3.5.2 Analysis of plasma samples

100 µL of internal standard (IS) solution was added to 500 µL of plasma sample, vortexed for 15 s and after adding 5 mL of methyl *tert.*-buthylether vortexed for another 2 min. After 5 min of centrifugation at 2500g, 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 2500g, 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. 200 µL 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.

Hydrochlorothiazide plasma concentrations were determined using a validated high performance liquid chromatography (HPLC) method (Vervaet et al., 1997).

2.1.1.1 Pharmacokinetic and statistical analysis

Individual hydrochlorothiazide plasma concentrations were adjusted for the body weight of the dogs and plotted against the time. $AUC_{0\rightarrow24}$ 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_{0-24h} between a pellet formulation and the immediate release tablets. Data were statistically analysed using SPSS 14 software (SPSS, Chicago, USA). Multiple comparisons of $AUC_{0\rightarrow24h}$ 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).

4.3.6 Validation of an HPLC method for determination of hydrochlorothiazide in dog plasma

Method was validated based on the ICH-guidelines (1995). The following validation criteria have been taken into account: specificity, linearity, precision, accuracy, recovery, detection limit and quantification limit.

4.3.6.1 HPLC system

The HPLC-system consisted of an isocratic pump (L-7110, Merck Hitachi, Tokyo, Japan), automatic injection system (234 Autoinjector, Gilson, Middleton, WI, USA) with a 100 µL loop, a precolumn (LiChrospher® 100 RP-18, 4 x 4 mm, 5 µm, Merck, Darmstadt, Germany) followed by a reversed-phase C-18 column (LiChrospher® 100 RP-18, 250 x 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 (50 mL 0.2 M KH_2PO_4 + 29.1 mL 0.2 M NaOH + H_2O ad 200 mL; 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 the detector was set to 272 nm.

4.3.6.2 Sample preparation

The stock solution of hydrochlorothiazide (50 µg/mL) was prepared by dissolving 50 mg of hydrochlorothiazide in 10 mL of methanol and adding distilled water up to 1000 mL. The IS solution was prepared by dissolving hydroflumethiazide in 10 mL of methanol and dilution with distilled water to obtain a concentration of 1.25 µg/mL. The stock solution of hydrochlorothiazide was used to prepare the standard solutions (concentrations: 0.050, 0.125, 0.250, 0.500, 1.0, 1.5, 5 and 10 µg/mL) for the method validation and the IS was always added in the same concentration (1.25 µg/mL).

For the determination of calibration curves, 100 µL of IS solution (1.25 µg/mL) was added to 500 µL of blank plasma (zero point) and to 400 µL of blank plasma together with 100 µL of standard HCT solutions to obtain serum concentrations of 10, 25, 50, 100, 200, 500, 1000 and 2000 ng/mL. This mixture was vortexed for 15 s and after adding 5 mL of methyl *tert.*-buthylether for another 2 min. After 5 min of centrifugation at 4000 rpm (2524g), 4.5 mL of organic phase was removed into new test tube and dried under a N_2-stream at 40°C until the evaporation of the organic solvent. The residue was further dissolved in 200 µL of distilled water, followed by addition of 3 mL toluene for the extraction of lipofilic serum components. The mixture was further vortexed for 2 min and after 10 min of centrifugation at 4000 rpm, the toluene layer was removed. 3 mL of toluene was added once again, and the extraction procedure was repeated. After the organic phase was removed, the mixture was dried under a N_2-stream at 40°C. 200 µL 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.

4.3.6.3 Specificity

ICH definition: Specificity is the ability to asses unequivocally the analyte in the presence of components that may be expected to be present (Chan et al., 2004).

Comparing the chromatogram of blank dog plasma (Fig. 4.1 a) spiked with the one of blank dog plasma spiked with hydrochlorothiazide (HCT, conc. 200 ng/mL) and internal standard (IS, hydroflumethiazide conc. 1.25 µg/mL) (Fig. 4.1 b) it is clear that no interference exists between hydrochlorothiazide, internal standard, endogenous components of plasma and/or components used for extraction. When observing the chromatogram of dog plasma after intake of hydrochlorothiazide reference tablets

(Esidrex® 50 mg) (Fig. 4.1 c), similar retention times of hydrochlorothiazide and internal standard were obtained as with spiked plasma: 5.5 and 9.8 s for hydrochlorothiazide and internal standard, respectively (Fig. 4.1 b). Since no interfering peaks have been observed, the method is specific for the determination of hydrochlorothiazide and hydroflumethiazide (internal standard) in dog plasma.

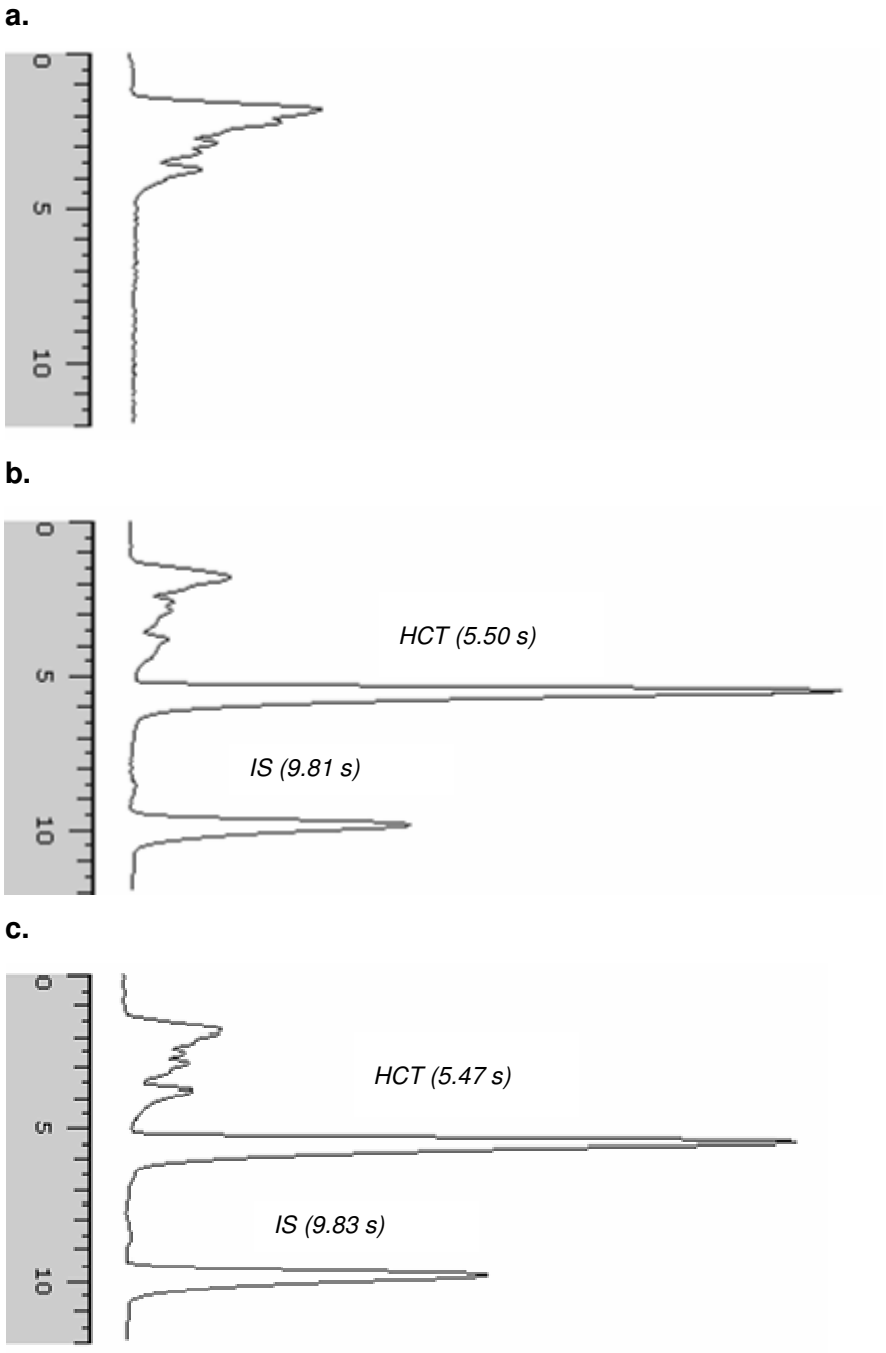


Figure 4.1 *Chromatograms of HCT analysis in plasma:* ***a.*** *blank dog plasma,* ***b.*** *blank dog plasma spiked with hydrochlorothiazide (HCT, conc. 200 ng/mL) and internal standard (IS, hydroflumethiazide, conc. 1.25 µg/mL) and* ***c.*** *dog plasma after intake of HCT tablets (Esidrex® 50 mg).*

4.3.6.4 Linearity

ICH definition: The linearity of an analytical procedure is its ability (within a given range) to obtain results that are directly proportional to the concentration of analyte in the sample (Chan et al., 2004).

It is recommended to use five to eight concentrations (excluding blank value) to define a standard curve (Shah et al., 1992). The linearity of a standard curve is expressed by the correlation coefficient, the intercept and the slope of each individual calibration curve and of the average response curve. Eight concentrations in the range from 0 to 2000 ng/mL were used for determination of the standard curve. A correlation coefficient (R^2) > 0.9999 demonstrates linearity of the standard curve in the entire concentration range.

Table 4.6 *Linearity: Within-day variation (n=5).*

HCT conc. (ng/mL)	***Peak area (HCT / Internal standard)***							
	1	***2***	***3***	***4***	***5***	***Average***	***SD***	***CV***
0	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*--*
10	*0.042*	*0.046*	*0.052*	*0.049*	*0.044*	*0.046*	*0.004*	*8.63*
25	*0.102*	*0.097*	*0.105*	*0.119*	*0.098*	*0.104*	*0.009*	*8.32*
50	*0.222*	*0.244*	*0.217*	*0.238*	*0.229*	*0.230*	*0.011*	*4.73*
100	*0.457*	*0.417*	*0.488*	*0.486*	*0.454*	*0.460*	*0.029*	*6.28*
200	*0.909*	*0.889*	*0.915*	*0.910*	*0.874*	*0.899*	*0.017*	*1.92*
500	*2.265*	*2.236*	*2.305*	*2.267*	*2.216*	*2.258*	*0.034*	*1.49*
1000	*4.514*	*4.451*	*4.548*	*4.442*	*4.469*	*4.485*	*0.045*	*1.01*
2000	*9.161*	*8.834*	*9.154*	*8.960*	*8.836*	*8.989*	*0.162*	*1.81*
Slope	*0.00457*	*0.00442*	*0.00457*	*0.00447*	*0.00443*	*0.00449*	*0.00008*	*1.67*
Intercept	*-0.01027*	*0.00308*	*0.00246*	*0.01254*	*0.00263*	*0.00209*	*0.00812*	*-*
Corr. coef. (R^2)	*0.99995*	*0.99996*	*0.99997*	*0.99996*	*0.99996*	*0.99996*	*0.00001*	*-*

The variation within one day is presented as the mean value of the slopes obtained after five injections of eight standard solutions during one day (Table 4.6), while the variation between days was assessed from the mean value of the slopes obtained after five injections of eight standard solutions during one month (Table 4.7). The mean slope and its coefficient of variation calculated within one day (0.00449; 1.67 %) and between days

(0.00453; 2.78 %) calibration curves only show small differences.

Table 4.7 *Linearity: Between-day variation (n=5)*

HCT conc. (ng/mL)	***Peak area (HCT / Internal standard)***							
	1	***2***	***3***	***4***	***5***	***Average***	***SD***	***CV***
0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*-*
10	*0.051*	*0.048*	*0.045*	*0.042*	*0.043*	*0.046*	*0.004*	*8.19*
25	*0.107*	*0.107*	*0.104*	*0.102*	*0.126*	*0.109*	*0.010*	*8.97*
50	*0.227*	*0.223*	*0.207*	*0.222*	*0.221*	*0.220*	*0.008*	*3.50*
100	*0.446*	*0.452*	*0.396*	*0.457*	*0.472*	*0.445*	*0.029*	*6.46*
200	*0.915*	*0.860*	*0.882*	*0.909*	*0.901*	*0.894*	*0.022*	*2.50*
500	*2.288*	*2.193*	*2.224*	*2.265*	*2.359*	*2.266*	*0.064*	*2.82*
1000	*4.493*	*4.440*	*4.677*	*4.514*	*4.784*	*4.581*	*0.144*	*3.14*
2000	*9.064*	*8.716*	*8.824*	*9.161*	*9.374*	*9.028*	*0.264*	*2.92*
Slope	*0.00453*	*0.00437*	*0.00446*	*0.00457*	*0.00471*	*0.00453*	*0.00013*	*2.78*
Intercept	*0.00072*	*0.00703*	*0.00354*	*-0.01027*	*-0.00001*	*0.00020*	*0.00648*	*-*
Corr. coef. (R^2)	*0.99997*	*0.99992*	*0.99918*	*0.99995*	*0.99988*	*0.99978*	*0.00034*	*-*

4.3.6.5 Precision

The precision of a method is a measure of data spreading around the mean value during repeated determinations. It can be defined at three levels (*ICH definition*): repeatability (intra-assay precision), which is the precision under the same operating conditions over a short interval of time; intermediate precision (within-laboratory precision), which expresses within-laboratory variations like different days, analysts, equipment, etc.; reproducibility, which is the precision between laboratories. Precision is expressed as the coefficient of variation (CV, %) of series of measurements for each standard concentration of the calibration curve (Lee et al., 2004).

CV values for repeatability (Table 4.6) are between 1.01 and 8.63 % for the whole concentration range of the standard curve, while the values for intermediate precision (Table 4.7) are in the range between 2.50 and 8.97 % for the same concentration range. As recommended by Shah et al. (1992), the precision around the mean should not exceed

15 % CV, except for the lowest concentration, where it should not exceed 20 % CV. It is clear that the method is precise to determine hydrochlorothiazide levels in plasma.

4.3.6.6 Accuracy

ICH definition: The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the determined value (Chan et al., 2004). It is expressed as the percent agreement between the mean determined and the true concentration.

It is determined from blank plasma samples spiked with known amounts of hydrochlorothiazide (standard concentrations) and then analysed by HPLC. Obtained values of hydrochlorothiazide concentration are compared with the theoretical concentration of hydrochlorothiazide. Each concentration was determined ten times. Table 4.8 lists the mean accuracies and their coefficients of variation (CV) for both within-day and between-day accuracy. Since all CV values are below the acceptance limit of 15 % CV, as suggested by Shah et al. (1992), we can conclude that the method is accurate.

Table 4.8 *Mean accuracies (CV) (%) within-day (n=5) and between-day (n=5).*

HCT conc. (ng/mL)	***Mean accuracy (CV), %***	
	Within-day (n=5)	***Between-day (n=5)***
10	*103.1 (14.0)*	*100.4 (10.9)*
25	*110.6 (6.3)*	*104.5 (7.1)*
50	*98.8 (5.2)*	*103.1 (4.6)*
100	*98.3 (5.2)*	*102.2 (6.4)*
200	*100.1 (0.9)*	*101.4 (2.2)*
500	*99.6 (0.6)*	*99.9 (0.6)*
1000	*100.2 (0.9)*	*98.8 (2.3)*
2000	*100.0 (0.2)*	*100.3 (0.5)*

4.3.6.7 Recovery

The amount of analyte in biological samples can be reduced during sample treatment like extraction with organic solvents, etc. The closeness of agreement (in %) between the peak surface area of an analyte in non-extracted and extracted medium for the same

analyte concentration is represented by the recovery (Lee et al., 2004).

The recovery was determined for both hydrochlorothiazide and internal standard. Blank plasma was spiked with known concentrations of hydrochlorothiazide and internal standard and the sample was treated according to the HPLC method. The obtained peak areas were compared with the peak areas obtained after HPLC analysis of aqueous hydrochlorothiazide and internal standard solutions of the same concentration. Recovery was presented as the mean value of 10 determinations for standard hydrochlorothiazide concentrations of 10, 50, 200, 500 and 1000 ng/mL, as well as for internal standard (Table 4.9).

Table 4.9 *Mean recoveries (%) (± SD) and CV (%) of HCT and internal standard from plasma (n=10).*

Concentration	***Mean recovery (%) ± SD***	***CV (%)***
HCT 10 ng/mL	*96.3 ± 9.4*	*9.7*
HCT 50 ng/mL	*82.8 ± 3.8*	*4.6*
HCT 200 ng/mL	*77.1 ± 10.1*	*13.1*
HCT 500 ng/mL	*78.3 ± 2.6*	*3.3*
HCT 1000 ng/mL	*78.5 ± 3.0*	*3.8*
IS 1.25 µg/mL	*84.8 ± 6.5*	*7.7*

4.3.6.8 Detection and quantification limits

Detection limit (DL) and quantification limit (DQ) were calculated from the mean calibration curve (n=10), according to the following equations (Eq. 4.1 and 4.2):

$$DL = (3.3 * \sigma) / S \quad (4.1)$$

$$DQ = (10 * \sigma) / S \quad (4.2)$$

where σ represents the standard deviation of the Y-intercept of the mean calibration curve and S the slope of the mean calibration curve (Lee et al., 2004).

The detection limit for HCT determination in dog plasma was 4.45 ng/mL and the quantification limit was calculated as 13.49 ng/mL.

4.4 Results and discussion

4.4.1 In-vitro evaluation of hydrochlorothiazide pellets

In the previous chapter (Chapter 3) it was shown 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 for the 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 results (Chapter 3).

Table 4.10 *2^4-Factorial design (randomised order) presented in coded terms (A: HCT level, B: HPMC level, C: sorbitol level, D: water level) and the corresponding results of pellet yield, aspect ratio (AR), two-dimensional shape factor (e_R) and Feret diameter (FD).*

Run	Coded values				Responses			
	A	B	C	D	Yield (%)	AR	e_R	FD (µm)
1	0	0	O	L	82.7	1.12	0.57	1100
2	1	-1	O	L	65.3	1.12	0.55	1133
3	-1	-1	O	H	46.1	1.12	0.58	1162
4	-1	1	S	L	90.9	1.12	0.56	1038
5	1	1	O	H	51.5	1.16	0.53	1246
6	0	0	S	L	86.9	1.26	0.45	1300
7	-1	-1	O	L	50.8	1.15	0.53	1096
8	-1	-1	S	H	53.9	1.15	0.55	1117
9	0	0	S	H	76.7	1.15	0.54	1210
10	1	-1	O	H	50.0	1.11	0.59	1147
11	1	1	O	L	85.1	1.12	0.56	1095
12	1	1	S	L	81.8	1.16	0.54	1155
13	1	-1	S	H	52.4	1.19	0.47	1246
14	-1	1	S	H	92.3	1.16	0.52	1038
15	1	1	S	H	37.1	1.16	0.51	1170
16	-1	-1	S	L	53.6	1.13	0.57	1071
17	1	-1	S	L	77.7	1.12	0.55	1093
18	0	0	O	H	80.7	1.14	0.55	1151
19	-1	1	O	L	89.0	1.14	0.55	1142
20	-1	1	O	H	83.5	1.15	0.52	1155

Table 4.11 *ANOVA results for yield and Feret diameter.*

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

4.4.1.1 Pellet yield

Figure 4.2 presents the water concentrations which have been used in the experimental design in order to maximize pellet yield. Water concentration was directly related to the sorbitol and hydrochlorothiazide concentration: it was lower in formulations containing sorbitol and when the hydrochlorothiazide 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 a water-soluble filler or drug has been reported by Baert et al. (1991), Hileman et al. (1997), Lustig-Gustafsson et al. (1999) and Sousa et al. (2002). The effect of sorbitol on water concentration was higher for batches with a lower hydrochlorothiazide 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 hydrochlorothiazide 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 hydrochlorothiazide concentration in the powder mixture reduced the total powder surface area as hydrochlorothiazide particles (D [v,0.5]=104.4 µm) are larger compared to UNI-PURE® EX starch particles (D [v,0.5]=43 µm), thus liquid saturation was obtained at lower water levels (Holm, 1997). Similar relationships between the amount of granulation liquid and the particle size were observed by Kristensen et al. (1985) (granule growth by coalescence was achieved at lower liquid saturation values when increasing the mean particle size of dicalcium phosphate powder) and Bains et al.

(1991) (substituting part of the MCC-fraction by the smaller sized barium sulphate increased the amount of liquid required for successful extrusion/spheronisation).

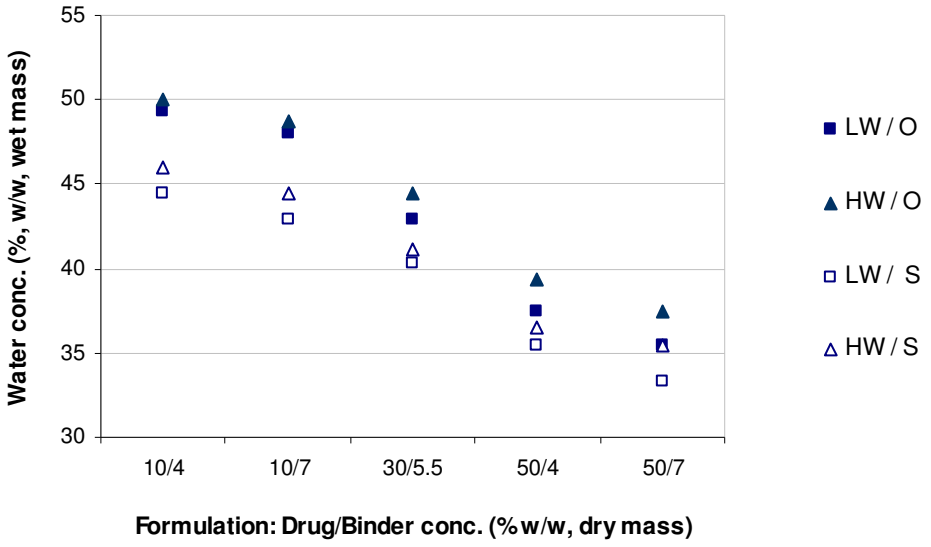


Figure 4.2 *Water levels (% w/w, wet mass; LW-low and HW-high) used in formulations without (O) and with sorbitol (S) for different drug (HCT) and binder (HPMC) concentrations (% w/w, dry mass).*

Table 4.11 presents the results of ANOVA analysis 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 (Eq. 4.3):

$$\text{Yield (\%)} = 66.32 - 3.70*A + 10.09*B - 6.97*D - 8.83*A*B - 6.90*A*D \qquad (4.3)$$

3D diagrams of pellet yield in function of binder level and drug load are presented in Figure 4.3. The weight distribution of the different pellet formulations are presented in Figure 4.4, indicating that a pellet yield (710-1400 µm fraction) higher than 90% could be obtained.

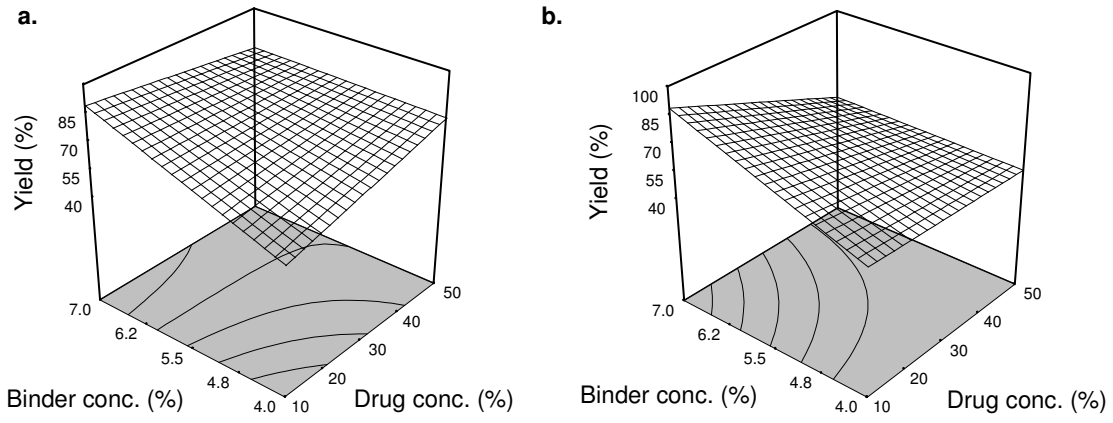


Figure 4.3 *3D diagrams of pellet yield as a function of binder and drug level for formulations without sorbitol and different water levels:* ***a.*** *low water level and* ***b.*** *high water level.*

For formulations with a low hydrochlorothiazide 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 obtained in the first part of the study (Chapter 3) and were reported by Agrawal et al. (1994), who used HPMC as a binder in chitosan-based pellets. In addition, 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 hydrochlorothiazide load and low water level, pellet yield was high, irrespective of the binder level. Furthermore, at the same (low) binder concentration, higher hydrochlorothiazide amounts significantly increased pellet yield due to the difference in morphology between hydrochlorothiazide and starch particles: spherical UNI-PURE® EX starch particles (Fig. 4.5 a) 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 hydrochlorothiazide particles (Fig. 4.5 b) can significantly improve the mechanical strength of the wet extrudates via mechanical interlocking of particles (Holm, 1997). At high water levels (Fig. 4.3 b) pellet yield was lower for a higher HCT load due to pellet agglomeration.

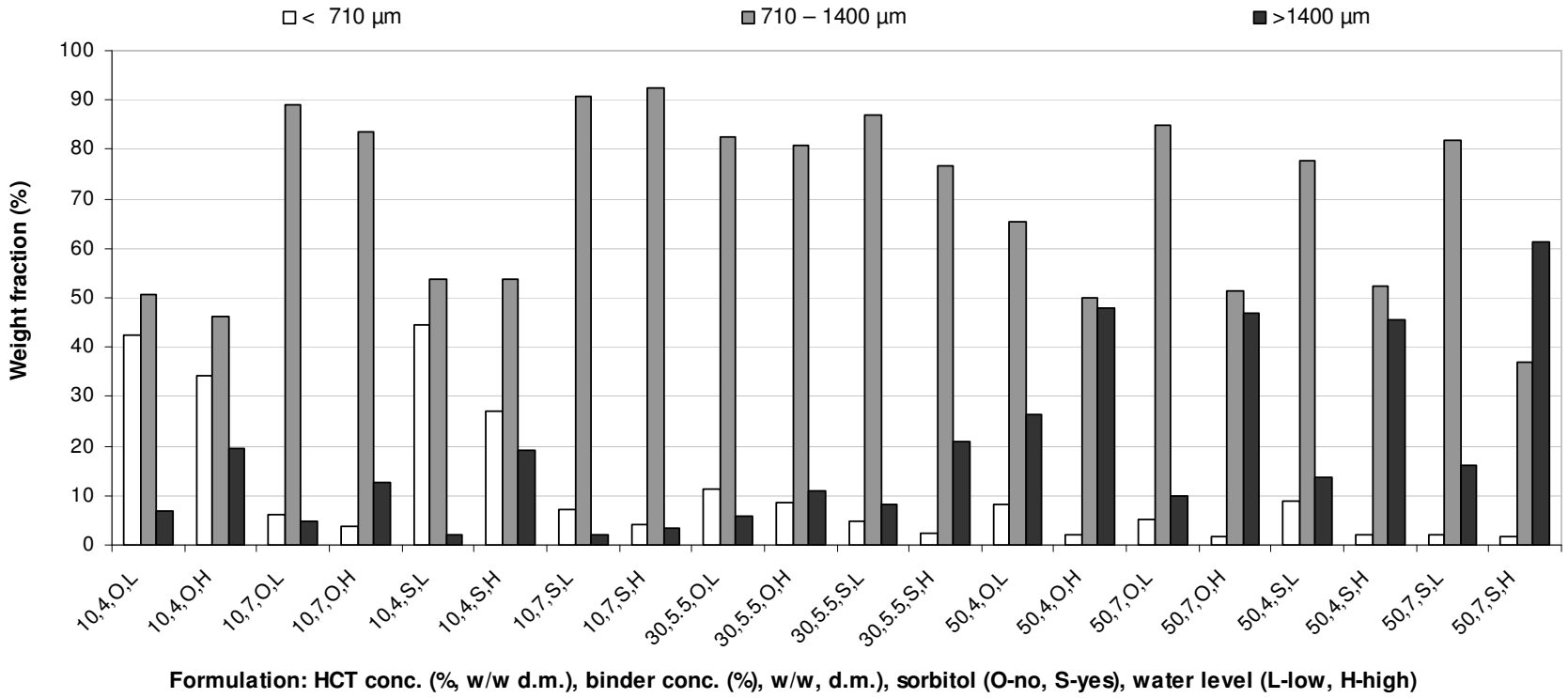


Figure 4.4. *Weight distribution (%) of pellet formulations containing different HCT (10, 30 and 50 % w/w, dry mass), binder (4, 5.5 and 7 %, w/w, dry mass), sorbitol (yes or no) and water (low or high) levels.*

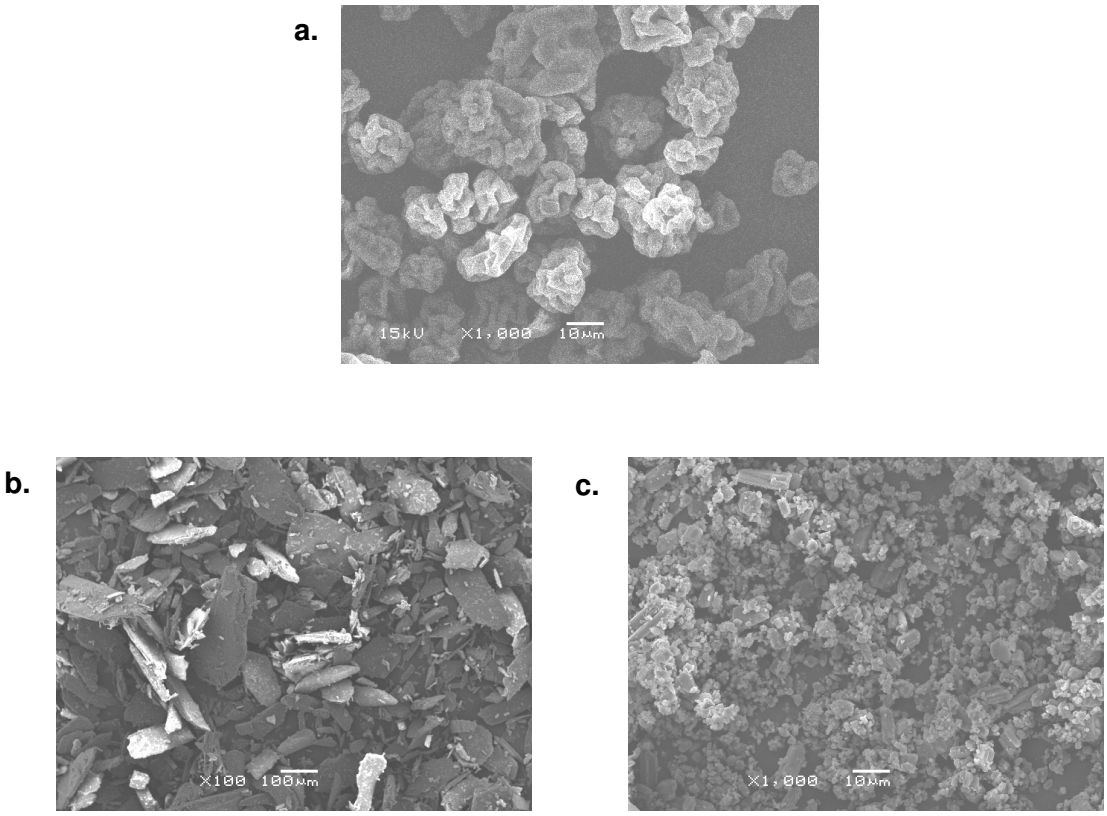


Figure 4.5 *Scanning electron micrographs of:* ***a.*** *UNI-PURE® EX starch,* ***b.*** *hydrochlorothiazide and* ***c.*** *piroxicam powder.*

In the previous chapter it was revealed 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).

4.4.1.2 Pellet sphericity

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 (Baert et al., 1993). Since a constant spheronisation speed was used (selected based on the previous results) the formulation variables in this experimental design had a limited effect on pellet sphericity. Majority of pellets had an aspect ratio between 1.11 and 1.19 (except of one formulation with AR of 1.25) and a two-dimensional shape factor >0.50 (except of two formulations with e_R of 0.45 and 0.47), both complying with the ranges for acceptable

pellet sphericity defined by Chopra et al. (2002).

4.4.1.3 Pellet size

The results of ANOVA analysis for pellet size are presented in Table 4.11. 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 (Eq. 4.4):

$$\text{Pellet size } (\mu m) = 1137.24 + 42.51*A + 30.02*D + 26.42*A*D \qquad (4.4)$$

The mean pellet diameter ranged from 1000 to 1300 µm for all batches. Interaction diagrams of pellet size (Fig. 4.6) showed that a higher water concentration generated larger pellets for a higher hydrochlorothiazide load due to particle agglomeration during spheronisation.

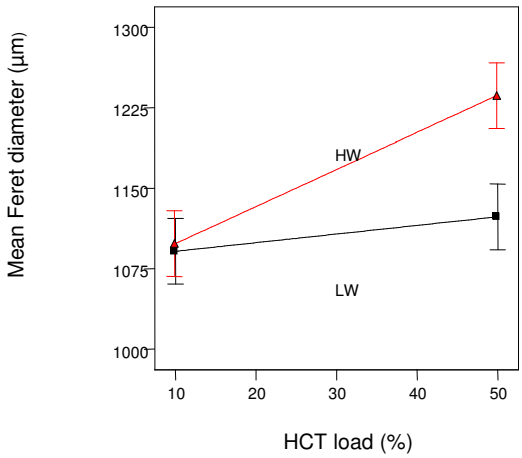


Figure 4.6 *Interaction diagram of pellet size (mean Feret diameter, µm) as a function of HCT load (%) and water level (LW- low water level; HW- high water level).*

4.4.1.4 Water content

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

hydrochlorothiazide load had a higher residual moisture content (6.2 to 7.8 %) than pellets with a higher hydrochlorothiazide concentration (3.6 to 4.4 %), irrespective of binder, sorbitol or water level. This was due to a higher starch content in formulations with low hydrochlorothiazide load since the hydrophilic starch polymer has a higher residual moisture content (9.3 % vs. 0.8 % for hydrochlorothiazide).

4.4.1.5 Friability

Similarly as in the previous study, the friability of all pellet batches was less than 0.01% since the solid HPMC bridges formed during drying yielded pellets with high mechanical strength (Augsburger and Vuppala, 1997).

4.4.1.6 Disintegration

Due to disintegrating properties of UNI-PURE®EX starch as the main excipient, disintegration time of all batches was between 5 and 10 minutes.

4.4.1.7 *In-vitro* drug release

The release profiles of starch-based pellets containing 10 and 50 % of hydrochlorothiazide are presented in Figures 4.7 and 4.8. The *in-vitro* drug release from starch-based pellets was compared to the release of hydrochlorothiazide from MCC-based pellets. It can be observed that more than 80 % hydrochlorothiazide was released in 30 minutes for all starch-based pellet formulations, while MCC-based pellets released less than 40 % hydrochlorothiazide after 75 minutes. 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.

Hydrochlorothiazide 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 hydrochlorothiazide concentration. Nevertheless, immediate release of the poorly soluble drug was obtained, irrespective of the composition.

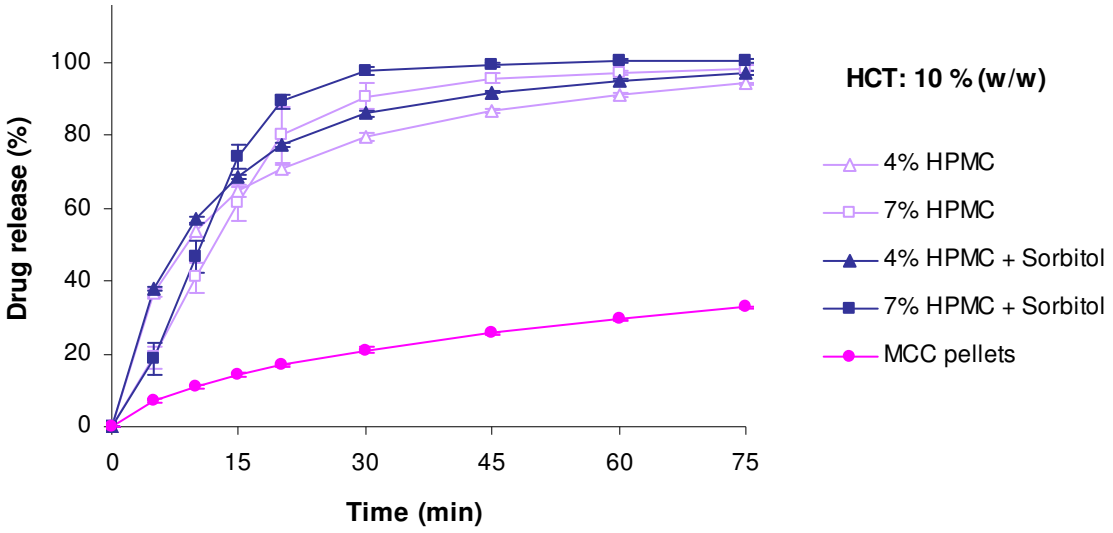


Figure 4.7 *Hydrochlorothiazide (HCT, 10 %) release profiles of pellets containing different amounts of binder (HPMC) and sorbitol, compared to reference pellets (MCC-based).*

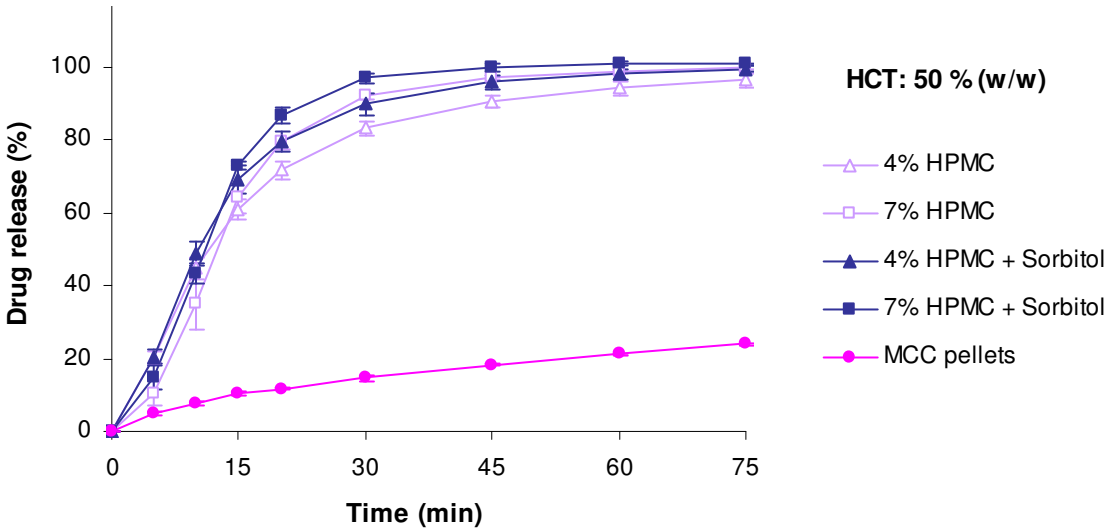


Figure 4.8 *Hydrochlorothiazide (HCT, 50 %) release profiles of pellets containing different amounts of binder (HPMC) and sorbitol, compared to reference pellets (MCC-based).*

4.4.2 In-vitro evaluation of piroxicam pellets

4.4.2.1 Pellet characterisation (pellet yield, size, sphericity and friability)

Piroxicam was used as a second poorly soluble model drug in this study. 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.

4.4.2.2 Pellet disintegration and *in-vitro* drug release

Since piroxicam 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 a non-disintegrating MCC matrix). Furthermore, based on its hydrophobic nature, dissolution of piroxicam was prolonged due to its poor wetting by the dissolution medium.

As expected, starch-based pellets containing piroxicam also disintegrated in less than 15 minutes, which consequently increased the drug release rate: when comparing the dissolution profiles of piroxicam from starch- and MCC-based pellets (Fig. 4.9), it can be observed that more than 90 % piroxicam was released within 45 minutes from starch-based pellets, while only 30 % piroxicam was released from MCC-based pellets during the same period of time.

From Figure 4.9 it can be also observed that addition of sorbitol increased piroxicam release: more than 90 % of piroxicam was released in only 30 minutes, 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 (D [v,0.5]= 9.9 µm) and cohesive powder (Fig. 4.5 c), 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 the immediate release properties of piroxicam (Hoener and Benet, 1996; Schreiner et al, 2005).

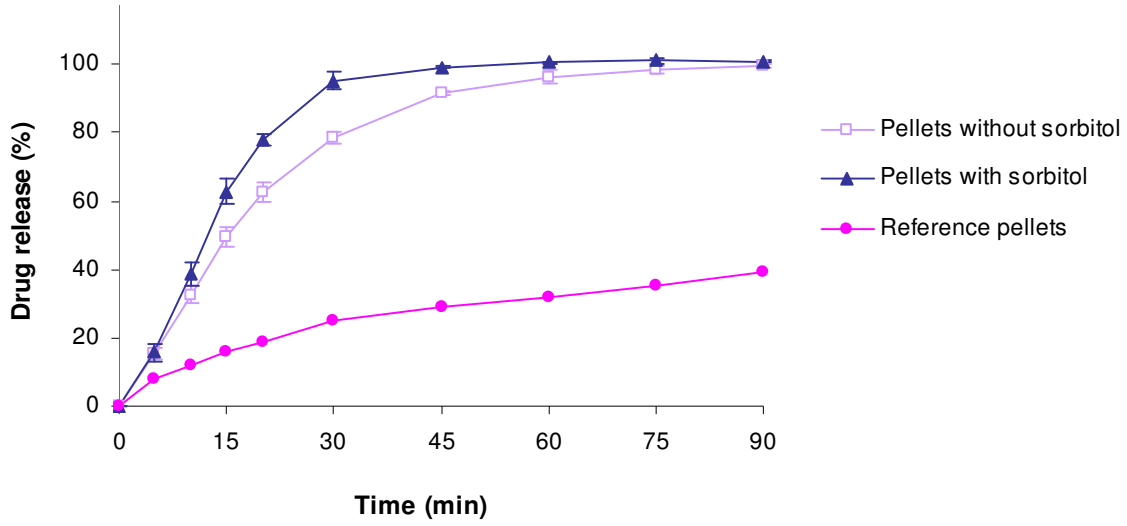


Figure 4.9 *Dissolution profiles of piroxicam from starch-based pellets with and without sorbitol compared with MCC-based pellets.*

4.4.3 In-vivo evaluation of hydrochlorothiazide pellets

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) (Beermann et al., 1976), which combined with its poor solubility in water indicates possible bioavailability problems (Dalton and Meyer, 2002). Therefore, fast dissolution of hydrochlorothiazide is essential for obtaining its maximal concentration at the absorption site.

Fig. 4.10 presents *in-vitro* dissolution profiles of the formulations used in the *in-vivo* study, while Fig. 4.11 presents the mean (n=6) plasma hydrochlorothiazide concentration versus time profiles of both pellet formulations and the immediate-release tablet, while Fig. 4.12 presents individual plasma profiles for all tested formulations. Table 4.12 summarises the pharmacokinetic parameters. No statistically significant differences of $AUC_{0\rightarrow 24h}$, 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.

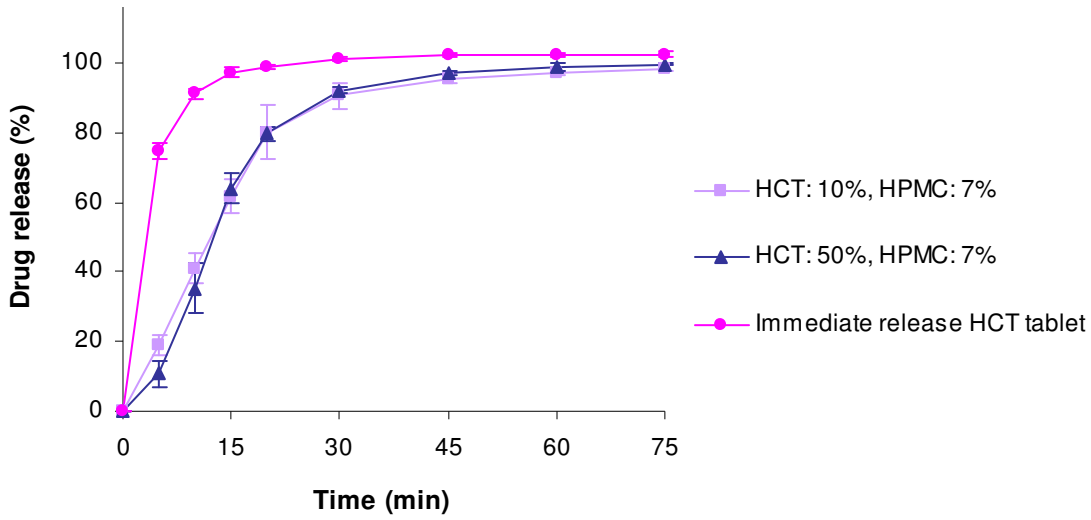


Figure 4.10 *In-vitro drug release profiles for pellets containing 10% and 50% HCT and immediate release HCT tablets.*

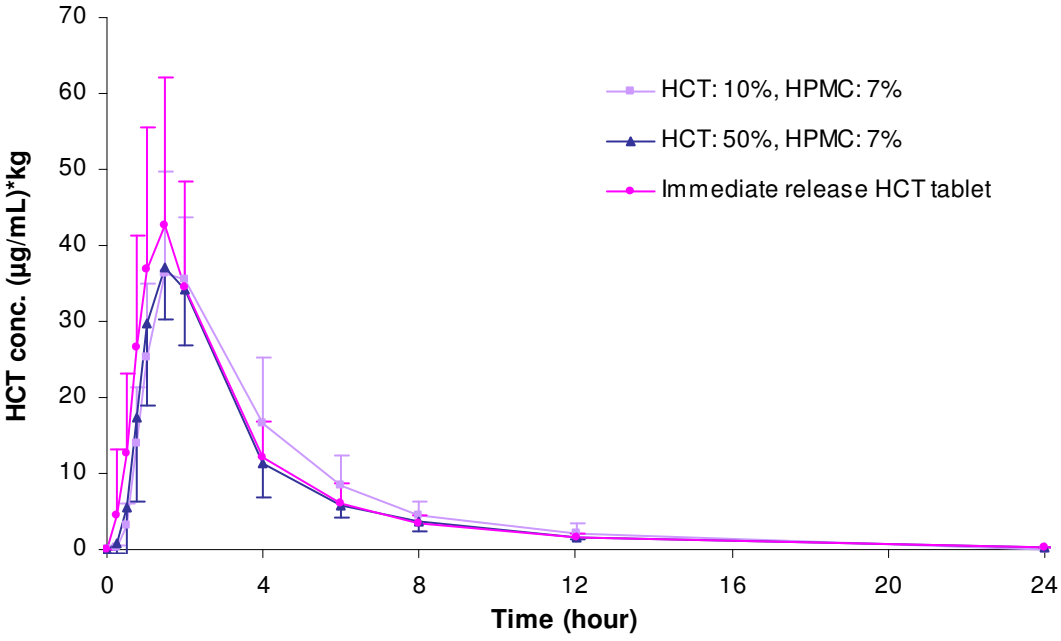


Figure 4.11 *Mean HCT plasma concentration-time profiles (±SD; n=6) obtained after administration of an oral dose of 50 mg HCT for pellets containing 10% and 50% HCT and immediate release HCT tablets*

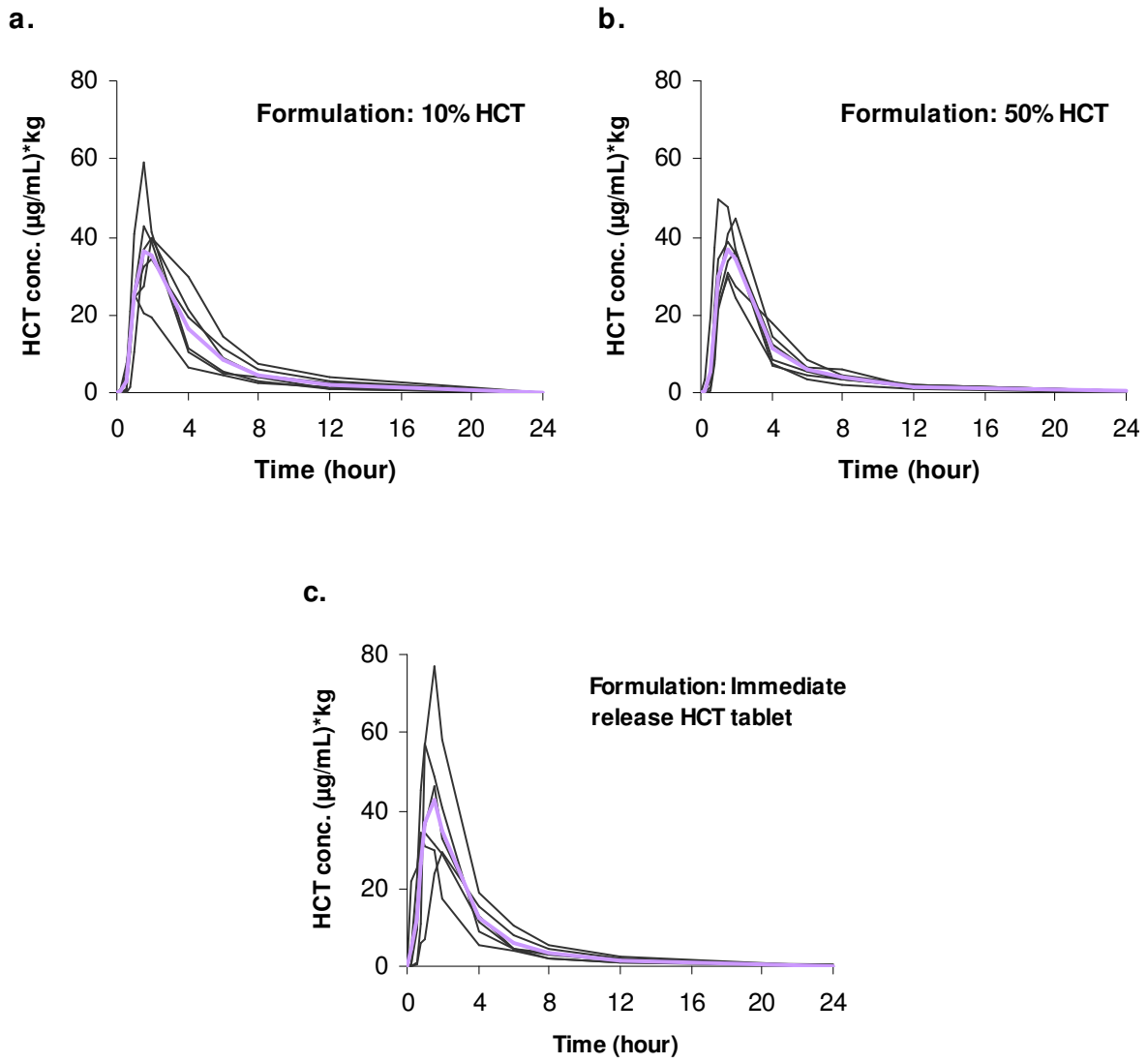


Figure 4.12 *Individual and mean (n=6) HCT plasma concentration-time profiles obtained after administration of an oral dose of 50 mg HCT for pellets containing:* ***a.*** *10% and* ***b.*** *50% HCT and* ***c.*** *immediate release HCT tablets.*

Table 4.12 *Mean $AUC_{0\rightarrow 24h}$, C_{max}, t_{max} and F_{Rel} values (±SD) after oral administration of 50 mg HCT to dogs (n=6).*

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

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

4.5 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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5

IN-VITRO AND IN-VIVO EVALUATION OF ENTERIC-COATED STARCH-BASED PELLET FORMULATIONS

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A. Dukić-Ott, T. De Beer, J.P. Remon, W. Baeyens, P. Foreman, C. Vervaet
"In-vitro and in-vivo evaluation of enteric-coated starch-based pellets prepared via extrusion/spheronisation."

5.1 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 (enteric-coated or colon-targeted drug delivery). Enteric-coated pellets as dosage forms are especially suited for administration of drugs which are not stable in gastric fluids, can cause irritation of gastric mucosa and/or are absorbed in the duodenum or upper intestine (Erkoboni, 2003). After the acid-resistant coating has dissolved in the basic environment of intestine, immediate drug release is essential for complete absorption in the duodenum or upper intestine. However, when producing pellets with MCC as the main excipient immediate release of a poorly water soluble drug can be difficult to obtain due to the lack of disintegration of these pellets (O'Connor and Schwartz, 1985).

The aim of this study was to produce enteric-coated pellet formulations with modified starch as the main excipient. In order to evaluate the influence of model drug solubility on the drug release profiles from coated pellets, two model drugs were used: piroxicam (poor water solubility) and theophylline anhydrous (medium water solubility). The influence of pellet core composition (drug load, drug particle size and sorbitol level) was also evaluated. Moreover, a uniform coating thickness is essential for obtaining modified drug

release profiles, since coating defects could lead to faster drug release or dose dumping. Next to the factors related to the coating process, a smooth surface morphology of the pellet core is very important, since surface roughness results in uneven coating thickness and hence a variable drug release (Porter and Ghebre-Sellassie, 1994). Since the previous experiments (Chapter 3) revealed the influence of sorbitol on surface morphology and several authors reported on the influence of drying method on pellet size, porosity and drug release (Chapter 1), the drying method was included as process variable in this part of the study. 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.

5.2 Materials

The solubility and particle size of anhydrous theophylline and piroxicam, which were used as model drugs in this part of the study, are listed in Table 5.1.

Table 5.1 *Solubility and particle size[a] of model drugs used in this study.*

Model drug:	***Anhydrous theophylline***		***Piroxicam***
	Coarse grade (TC)	*Micronised powder (TM)*	*Micronised powder*
Particle size D [v, 0.5]:	*157.9 (± 3.7) µm*	*19.2 (± 0.5) µm*	*9.9 (± 0.6) µm*
Solubility:	*8.3 g/L at 25°C (in water)*		*Practically insoluble in water [b]*
Producer:	*Roig Farma (Terrassa, Spain)*	*Bufa (Uitgeest, The Netherlands)*	*Sagran (Milan, Italy)*

[a] *Particle size was determined by laser diffraction as described in Chapter 4.*
[b] *Source: Ph. Eur. 4*

Table 5.2 *Excipients used for preparation of coating dispersion.*

Excipient name	***Function***	***Producer***
Eudragit® L30 D-55	*acid-resistant film-forming polymer*	*Röhm, Darmstadt, Germany*
Triethylcitrate	*plasticizer*	*Sigma-Aldrich Chemie, Steinheim, Germany*
Polysorbate 80	*wetting agent*	*Alpha Pharma, Nazareth, Belgium*
Glycerol monostearate	*glidant*	*Federa, Braine-l'Alleud, Belgium*
Demineralised water	*dispersion medium*	

The materials used for pellet coating and for determination of piroxicam in dog plasma are listed in Tables 5.2 and 5.3, respectively, while the same excipients as presented in Table 4.1 (Chapter 4) were used for pellet preparation.

Table 5.3 *Materials used for determination of piroxicam in dog plasma.*

Excipient name*	***Producer***
Meloxicam	*Boehringer Ingelheim, Ingelheim, Germany*
Acetonitrile	*Biosolve, Valkenswaard, The Netherlands*
Methanol	*Biosolve, Valkenswaard, The Netherlands*
Triethylamine	*Sigma-Aldrich Chemie, Steinheim, Germany*
Acetic acid	*Sigma-Aldrich Chemie, Steinheim, Germany*
Diethylether	*VWR International, Leuven, Belgium*
Hydrochloric acid 37%	*VWR International, Leuven, Belgium*

** All solvents and reagents were of HPLC-grade.*

5.3 Methods

5.3.1 Experimental set-up

Table 5.4 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 and 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 (determined by preliminary experiments) was used in the wet massing step, followed by extrusion/spheronisation. Pellets were dried either in an oven or using a fluid-bed drier. Other process parameters were the same for all batches and were selected based on previous experiments with UNI-PURE® EX starch (Chapters 3 and 4).

The drug release of enteric-coated starch-based pellets was compared to the release from coated pellets containing microcrystalline cellulose as the main excipient.

Table 5.4 *Overview of the formulation variables used in the experimental set-up.*

Formulation	***Model drug***	***Drug conc.[1]***	***Sorbitol conc.[1]***	***HPMC conc.[1]***	***Water content[2,3]***
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)**	*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*

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

5.3.2 Pellet production

A uniform dry powder mixture (batch size: 250 g) containing a 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 seconds 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 scrapped 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 extrusion screen (thickness: 1.2 mm, perforation diameter: 1mm). The extrudates were spheronised at 850 rpm during 3 minutes in a spheroniser with a cross-hatched friction plate (Caleva Model 15, Caleva, Sturminster Newton, Dorset, UK). Wet pellets were finally dried for 20 min at 50°C in a fluid-bed (GPCG1, Glatt, Binzen, Germany), except of theophylline pellets which were dried for 20 min at 60°C in a fluid bed or for 24h at 40°C in an oven .

5.3.3 Coating of pellets

400 g of pellet cores (900-1400 µm fraction) were coated using a bottom-spray fluid-bed coating technique with Würster 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 (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 minutes 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 % dry polymer weight gain was obtained.

5.3.4 Pellet and powder characterisation

Pellet cores were characterised by process yield (900-1400 µm fraction), pellet size (mean Feret diameter), sphericity (aspect ratio, AR and two-dimensional shape factor, e_R) as described in Chapter 3 (Section 3.3.4). The scanning electron micrographs of theophylline, piroxicam, MCC and UNI-PURE® EX starch powders were taken as described in Section 3.3.4.

5.3.4.1 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 minimize 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 50 mm Hg, 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, Schröder and Kleinebudde, 1995), using the same maximal intrusion volume and equilibration time. Measurements were performed in duplicate for each sample.

5.3.4.2 Raman spectroscopy

Pellets containing 25 % (w/w) theophylline (with and without sorbitol, dried in an oven and fluid-bed) were evaluated by Raman microscopic measurements 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 10x 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 seconds per spectrum. Before data analysis, spectra were baseline corrected and normalized. Data collection and analysis was done using the HoloGRAMS™ data collection software package, the HoloMAP™ data analysis software package and the Matlab® software package (ver. 6.5).

5.3.4.3 Dynamic vapour sorption

Water sorption isotherms of powders were gravimetrically obtained at 25°C using a DVS Advantage 1 with a Cahn D200 microbalance (Surface Measurement Systems, London, UK). A powder sample (around 10 mg) was dried until constant weight, followed by sorption (increasing the relative humidity up to 90 % with an incremental steps of 10%) and desorption (reducing the relative humidity until 0 % in the steps of 10%) phase. Prior to changing the relative humidity during sorption or desorption, each sample reached an equilibrium (the mass of sample changed less than 0.002% during 10 min). The sample weight was recorded every minute during the experiment.

5.3.4.4 Dissolution

Depending on the model drug used, the dissolution tests were performed using the USP apparatus (VK 8000, VanKel, New Jersey, USA) with paddles (piroxicam pellets) or baskets (theophylline pellets) at a rotational speed of 100 rpm, in 900 mL dissolution medium at 37°C. For enteric-coated pellets, acidic dissolution medium (0.1N HCl) was used during the first 2h, followed by 1h in pH 6.8 phosphate buffer (PB). The pellet amount used for analysis was adjusted to obtain sink conditions. 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 0.1N 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 0.1N 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 XXVII, an enteric coat was successfully applied if less than 10 % of drug is released after 2 h of dissolution in acid dissolution medium (0.1N HCl).

5.3.4.5 Stability study

Pellets containing piroxicam as model drug were used to test the stability of starch-based pellet formulations. Coated and uncoated pellets (with and without sorbitol) were stored for 9 months under controlled relative humidity (RH, %) and temperature (T, C°): 60% RH/25°C and 75% RH/40°C. Samples were taken after 0, 1, 3, 6 and 9 months of storage.

Piroxicam release profiles from coated pellets were determined via dissolution testing for 2 h in 0.1N HCl (to test the quality of enteric coating after storage) and in phosphate buffer (pH 6.8) (to test the immediate release of the drug). The immediate drug release from uncoated pellets in phosphate buffer was also determined. The residual water content of samples was determined by means of Karl-Fischer titration as described in Section 4.3.4.2.

5.3.5 Bioavailability testing

5.3.5.1 Oral administration

Two enteric-coated (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 6 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 for 12h before dosage form administration and until the 12h sample was taken. Water was always available. A minimum wash-out period of 1-week was respected between each 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 1400g for 10 min and stored at -20°C until analysed.

5.3.5.2 Analysis of plasma samples

500 µL of plasma sample was added to the residue after drying of 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 1M 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 1420g. 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.

A validated high performance liquid chromatography (HPLC) method was used to determine piroxicam plasma concentrations (adapted from Debunne et al., 2004).

5.3.5.3 Pharmacokinetic and statistical analysis

Piroxicam plasma concentrations were plotted against 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 $AUC_{0\rightarrow 24h}$ and C_{max} were performed by means of repeated measures multivariate ANOVA analysis within-subjects with the formulation as factor (P-value<0.05).

5.3.6 Validation of an HPLC method for determination of piroxicam in dog plasma

The method was validated based on the ICH-guidelines (1995). Specificity, linearity, precision, accuracy, recovery, detection limit and quantification limit (described in Chapter 4) have been determined as the validation criteria.

5.3.6.1 HPLC system

The HPLC-system consisted of an isocratic pump (L-7110, Merck Hitachi, Tokyo, Japan), automatic injection system (234 Autoinjector, Gilson, Middleton, WI, USA) with a 50 µL loop, a precolumn (LiChrospher® 100 RP-18, 4 x 4 mm, 5 µm, Merck, Darmstadt, Germany) followed by a reversed-phase C-18 column (LiChrospher® 100 RP-18 e, 125 x 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 detector wavelength was 357 nm.

5.3.6.2 Sample preparation

The stock solution of piroxicam (500 µg/mL) was prepared by dissolving 50 mg of piroxicam in 100 mL of methanol. The internal standard solution was prepared by dissolving meloxicam in methanol to obtain a concentration of 20 µg/mL. The stock solution of piroxicam was used to prepare standard solutions of the following concentrations: 5, 10, 15, 25, 40, 50 and 60 µg/mL. The internal standard solution was always added in the same concentration.

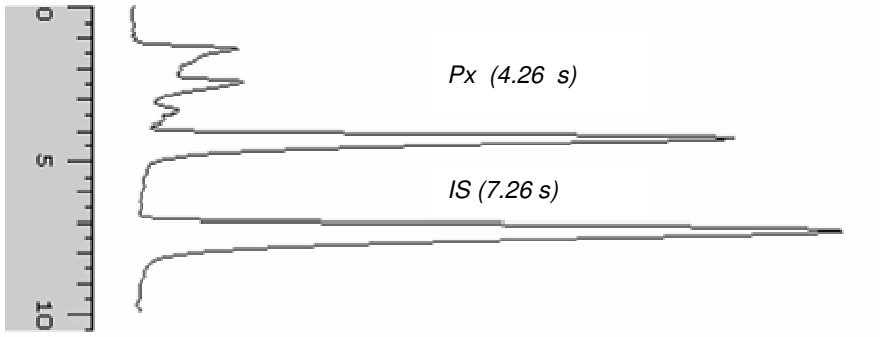
For the determination of calibration curves for method validation, a mixture of 20 µL internal standard solution (20 µg/mL) and 20 µL of standard piroxicam solution was dried under N_2-stream at 40°C. 500 µL of blank plasma was added to the residue in order to obtain the following serum concentrations: 0.2, 0.4, 0.6, 1.0, 1.6, 2.0 and 2.4 µg/mL. The mixture was sonicated for 10 s and vortexed for another 10 s. After adding 250 µL of 1M HCl, the sample was vortexed for another 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 3000 rpm (1420g). The organic layer was then transferred into a new test tube and dried under N_2-stream at 40°C. The residue was dissolved in 200 µL of a mobile phase, vortexed for 10 s and 50 µL of this solution was injected into HPLC-system.

5.3.6.3 Specificity

The absence of interference between piroxicam, internal standard, endogenous plasma components and materials used for the extraction, was confirmed after comparing the chromatogram of blank serum (Fig. 5.1 a) with the one of blank serum spiked with piroxicam (Px, conc. 1.0 µg/mL) and internal standard (IS, meloxicam, conc. 0.8 µg/mL) (Fig. 5.1 b). Furthermore, similar retention times of piroxicam (4.3 s) and internal standard (7.3 s) were obtained from chromatograms of blank plasma spiked with piroxicam and internal standard (Fig. 5.1 b) and of dog plasma after intake of piroxicam reference capsules (Feldene®) (Fig. 5.1 c). Since no interfering peaks have been observed, the method is specific for the determination of piroxicam and meloxicam (internal standard) in dog plasma.

a.

b.



c.

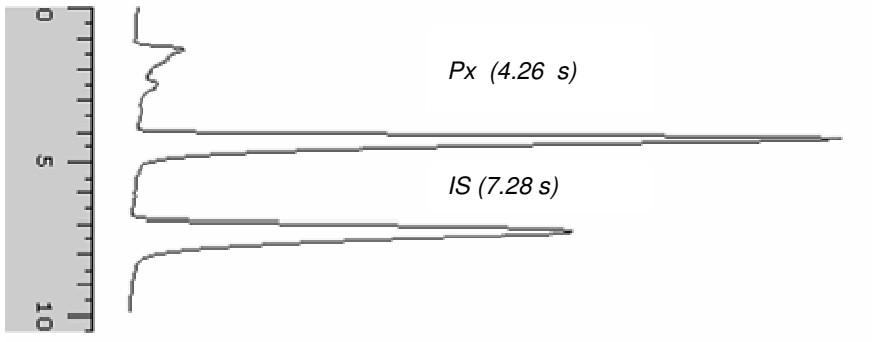


Figure 5.1 *Chromatograms of piroxicam analysis in dog plasma:* ***a.*** *blank plasma,* ***b.*** *blank plasma spiked with piroxicam (Px, conc. 1.0 µg/mL) and internal standard (IS, meloxicam, conc.0.8 µg/mL) and* ***c.*** *dog plasma after intake of piroxicam capsules (Feldene®).*

5.3.6.4 Linearity

Table 5.5 *Linearity: Within-day variation (n=6)*

Px conc. (µg/mL)	***Peak area (Px / Internal standard)***								
	1	***2***	***3***	***4***	***5***	***6***	***Average***	***SD***	***CV***
0.0	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.0*
0.2	*0.17*	*0.18*	*0.17*	*0.20*	*0.16*	*0.18*	*0.18*	*0.01*	*7.2*
0.4	*0.38*	*0.36*	*0.38*	*0.35*	*0.36*	*0.42*	*0.38*	*0.02*	*6.5*
0.6	*0.56*	*0.55*	*0.63*	*0.70*	*0.61*	*0.62*	*0.61*	*0.05*	*8.9*
1.0	*0.95*	*1.01*	*1.20*	*1.03*	*1.00*	*0.96*	*1.02*	*0.09*	*8.8*
1.6	*1.50*	*1.67*	*1.72*	*1.96*	*1.56*	*1.58*	*1.66*	*0.16*	*9.9*
2.0	*1.90*	*2.03*	*2.30*	*2.35*	*2.00*	*2.15*	*2.12*	*0.18*	*8.4*
2.4	*2.22*	*2.30*	*2.65*	*2.59*	*2.51*	*2.67*	*2.49*	*0.19*	*7.6*
Slope	*0.9369*	*0.9986*	*1.1316*	*1.1469*	*1.0341*	*1.0920*	*1.0567*	*0.0815*	*7.7*
Intercept	*-0.0007*	*-0.0101*	*-0.0286*	*-0.0277*	*-0.0357*	*-0.0467*	*-0.0249*	*0.0168*	*-*
Corr. coef. (R^2)	*0.9997*	*0.9964*	*0.9970*	*0.9912*	*0.9981*	*0.9945*	*0.9961*	*0.0030*	*-*

Table 5.6 *Linearity: Between-day variation (n=5)*

PX conc. (µg/mL)	***Peak area (Px / Internal standard)***							
	1	***2***	***3***	***4***	***5***	***Average***	***SD***	***CV***
0.0	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.0*
0.2	*0.18*	*0.18*	*0.21*	*0.23*	*0.23*	*0.21*	*0.02*	*12.0*
0.4	*0.39*	*0.42*	*0.48*	*0.50*	*0.45*	*0.45*	*0.04*	*9.7*
0.6	*0.61*	*0.62*	*0.63*	*0.62*	*0.58*	*0.61*	*0.02*	*2.8*
1.0	*1.01*	*0.96*	*1.08*	*0.97*	*1.04*	*1.01*	*0.05*	*4.9*
1.6	*1.76*	*1.58*	*1.86*	*1.56*	*1.66*	*1.68*	*0.13*	*7.5*
2.0	*2.09*	*2.15*	*2.27*	*2.09*	*2.24*	*2.17*	*0.08*	*3.9*
2.4	*2.63*	*2.67*	*2.68*	*2.59*	*2.71*	*2.66*	*0.05*	*1.8*
Slope	*1.0955*	*1.0920*	*1.1316*	*1.0427*	*1.1181*	*1.0960*	*0.0340*	*3.1*
Intercept	*-0.0383*	*-0.0467*	*-0.0085*	*0.0005*	*-0.0310*	*-0.0248*	*0.0201*	*-*
Corr. coef. (R^2)	*0.9983*	*0.9945*	*0.9986*	*0.9949*	*0.9968*	*0.9966*	*0.0019*	*-*

The variation within one day was calculated as the mean value of the slopes obtained after six injections of seven standard solutions during one day (Table 5.5). The variation

between days was presented as the mean value of the slopes obtained after five injections of seven standard solutions during a period of one month (Table 5.6). The mean slope and its coefficient of variation of the calibration curves calculated for within-day (1.0567; 7.7 %) and between-day (1.0960; 3.1 %) injections only showed small differences.

5.3.6.5 Precision

CV values for repeatability (Table 5.5) and intermediate precision (Table 5.6) for the whole concentration range of the standard curve, are between 7.2 and 9.9 % and 1.8 and 12.0 %, respectively. Based on the recommendations of Shah et al. (1992) mentioned in the Chapter 4, it can be concluded that the method is precise for piroxicam determination in dog plasma.

5.3.6.6 Accuracy

Table 5.7 lists the mean accuracies and their coefficients of variation (CV) for both within-day and between-day accuracy. Since all CV values are below the acceptance limit of 15 % CV, as suggested by Shah et al. (1992), we can conclude that the method is accurate.

Table 5.7 *Mean accuracies (CV) (%) within-day (n=6) and between-day (n=5).*

Px conc. (µg/mL)	***Mean accuracy (CV), %***	
	Within-day (n=6)	***Between-day (n=5)***
0.2	*107.1 (5.5)*	*97.7 (4.4)*
0.4	*108.4 (8.7)*	*93.0 (8.0)*
0.6	*100.1 (4.5)*	*102.0 (3.3)*
1.0	*99.6 (6.5)*	*106.0 (2.1)*
1.6	*98.9 (5.8)*	*102.2 (5.8)*
2.0	*98.4 (1.9)*	*100.3 (1.7)*
2.4	*101.7 (3.3)*	*98.2 (2.2)*

5.3.6.7 Recovery

Recovery was presented as the mean value of 11 determinations for standard piroxicam concentrations of 0.2, 0.4, 0.6, 1.0, 1.6, 2.0, 2.4 µg/mL, as well as for internal standard (Table 5.8). The recovery of piroxicam after extraction varied between 77.3 and 82.4 % depending on the concentration, while 81.6 % of internal standard was recovered.

Table 5.8 *Mean recoveries (%) (± SD) and CV (%) of piroxicam and internal standard from plasma (n=11).*

Concentration	***Mean recovery (%) ± SD***	***CV (%)***
Px 0.2 µg/mL	*77.3 ± 6.3*	*8.1*
Px 0.4 µg/mL	*79.0 ± 5.4*	*6.9*
Px 0.6 µg/mL	*80.6 ± 9.0*	*11.2*
Px 1.0 µg/mL	*82.4 ± 9.9*	*12.0*
Px 1.6 µg/mL	*82.0 ± 10.5*	*12.8*
Px 2.0 µg/mL	*78.2 ± 8.0*	*10.3*
Px 2.4 µg/mL	*81.8 ± 9.1*	*11.1*
IS 0.8 µg/mL	*81.6 ± 9.3*	*11.4*

5.3.6.8 Detection and quantification limits

Detection and quantification limits were calculated from the mean calibration curve (n=11) based on the equations presented in the previous chapter (Section 4.3.6.8). The detection limit for piroxicam determination in dog plasma was 0.05 µg/mL and the quantification limit was calculated as 0.16 µg/mL.

5.4 Results and discussion

5.4.1 In-vitro evaluation of pellets

Based on the previous experiments (Chapters 3 and 4), HPMC was included as a binder in the starch-based pellet formulations. Furthermore, it was shown in Chapter 3 that including sorbitol into starch-based pellet formulation had a twofold effect on pellet properties: firstly, a higher mechanical strength of extrudates (increased 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 (Chapter 3), hydrochlorothiazide (Chapter 4) and piroxicam (Chapter 4) was immediate, irrespective of drug solubility, pellet formulation and process parameters due to the 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 maximum yield and acceptable sphericity. Extrusion and spheronisation parameters have been selected based on the experiments presented in Chapters 3 and 4. Furthermore, the influence of drying method on pellet properties has been studied by several authors (Chapter 1). In this study, 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.

5.4.1.1 Pellet characterisation (pellet yield, size, sphericity and friability)

Table 5.9 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 (Wesdyk et al., 1990; Ragnarsson and Johansson, 1988). As discussed in Chapter 4, optimal water level for successful extrusion/spheronisation was lower when introducing sorbitol as water-soluble excipient and when increasing theophylline concentration in the pellet formulation (Table 5.4).

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. (2002), sphericity can be described as acceptable. In addition, the data presented at Table 5.9 showed that pellets were slightly more spherical (lower AR and higher e_R) when sorbitol was added and pellets were dried in a fluid-bed.

Table 5.9 *Pellet yield (%), sphericity (aspect ratio, AR; two-dimensional shape factor, e_R) and size (mean Feret diameter, FD) for pellets died in an oven (O) and fluid-bed (FB).*

Drying method:	***O***	***FB***	***O***	***FB***	***O***	***FB***	***O***	***FB***
Formulation	***Yield (%)***		***AR***		***e_R***		***Mean FD (µm)***	
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*

Mean pellet core 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. 5.2). As reported by Kleinebudde (1994), pellets made from water-absorbing excipients tend to shrink during drying and the extent of shrinking depends on the drying method. Bashaiwoldu et al. (2004) 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 of time 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.

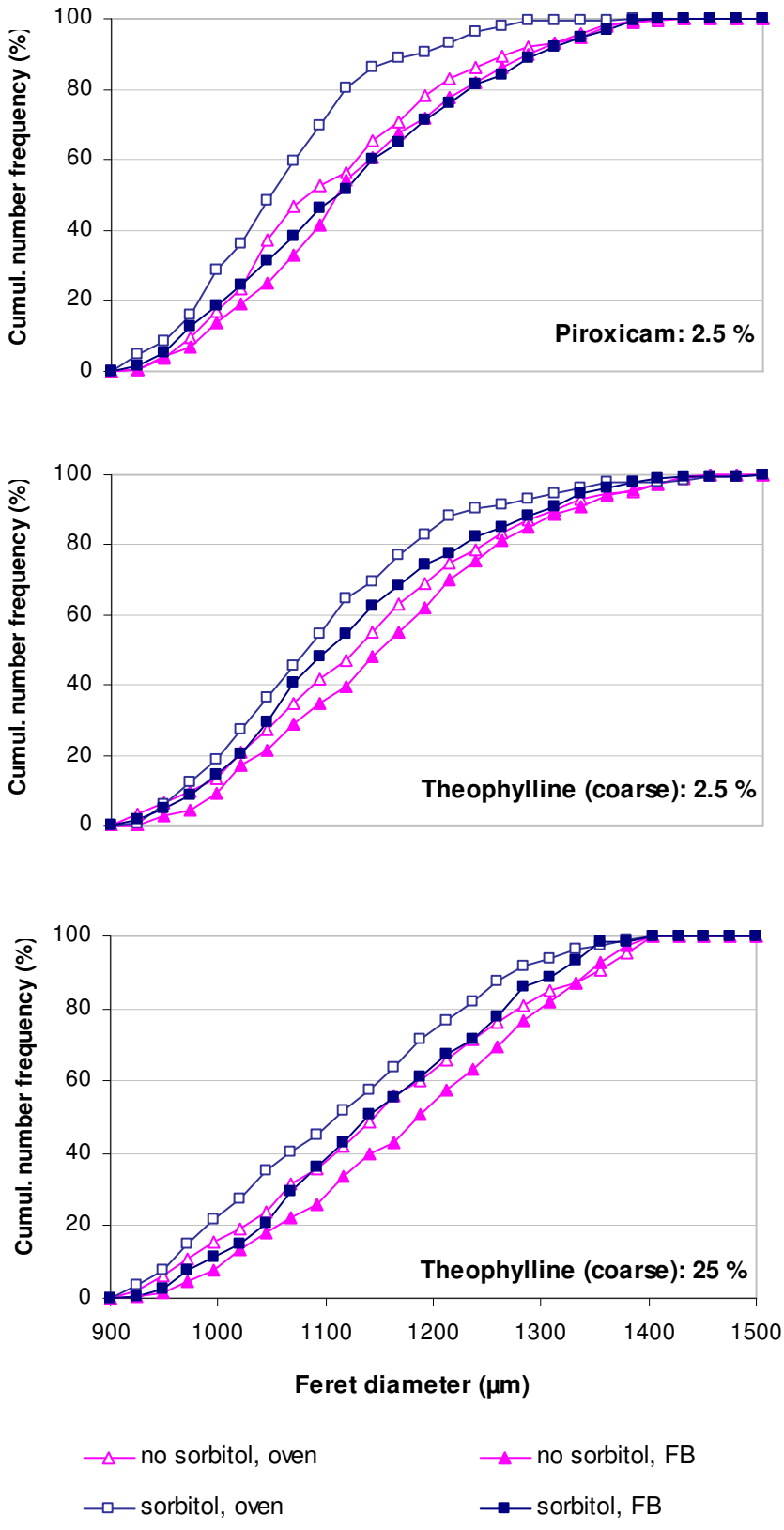


Figure 5.2 *Cumulative number frequency distributions of pellets containing piroxicam (2.5 %) and coarse theophylline (2.5 and 25 %).*

5.4.1.2 Mercury intrusion porosimetry

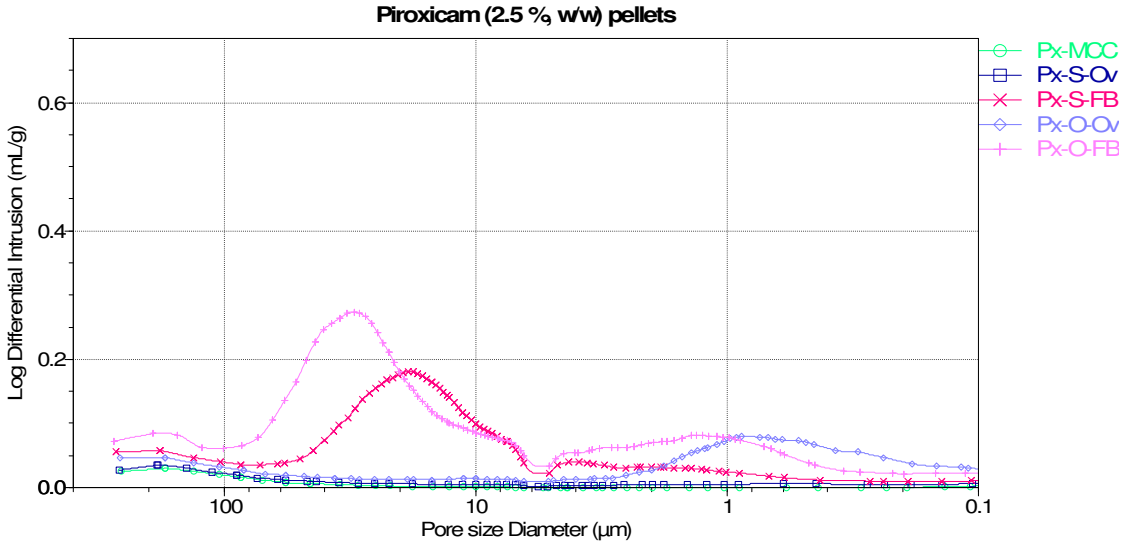
Mercury intrusion porosimetry was used to determine pore size distribution of pellet cores. Since a smooth pellet surface structure is important for a successful coating process, the evaluation of cracks and large pores on the pellet surface was of major interest. Mercury intrusion volumes in the low-pressure 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 Figure 5.3. Due to the logarithmic transformation ($\Delta V/\Delta \log R$), the large pores fraction is overemphasised, which is in this case useful for comparison of the 6-100 µm pore range of the different pellet cores (Juppo, 1996; Meyer and Klobes, 1999).

Figure 5.3a 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 pores 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 6-80 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.

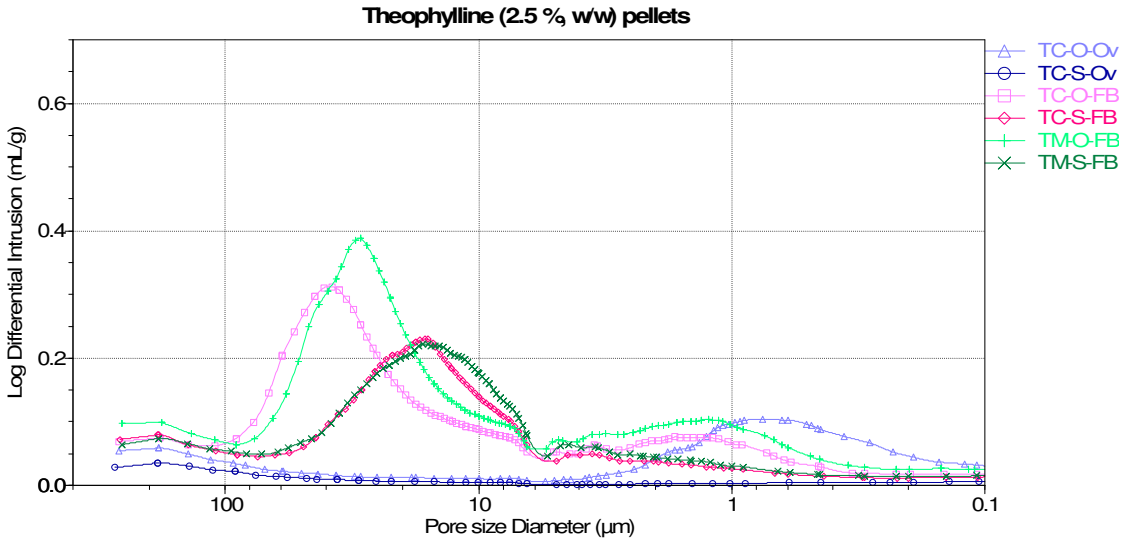
Above mentioned observations were supported by SEM photos of the pellets: cracks on the surface of fluid-bed dried starch-based pellets and a smooth surface in case of oven-dried starch-based pellets and MCC-based pellets (Fig. 5.4).

A similar influence of drying method and sorbitol addition on pellet surface morphology was observed for pellets containing anhydrous theophylline as model drug (Fig. 5.3 b,c).

a.



b.



c.

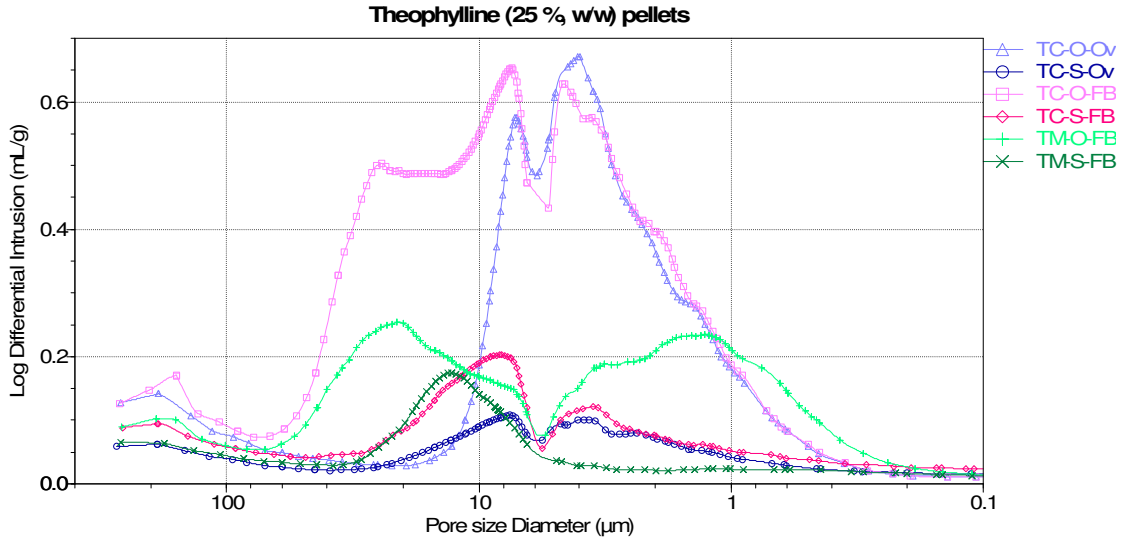


Figure 5.3 *Mercury intrusion volumes vs. pore size distribution (n=2) of pellets containing:* ***a.*** *piroxicam, and theophylline (coarse (T) and micronised (TM)) at* ***b.*** *2.5 % and* ***c.*** *25 % load.*

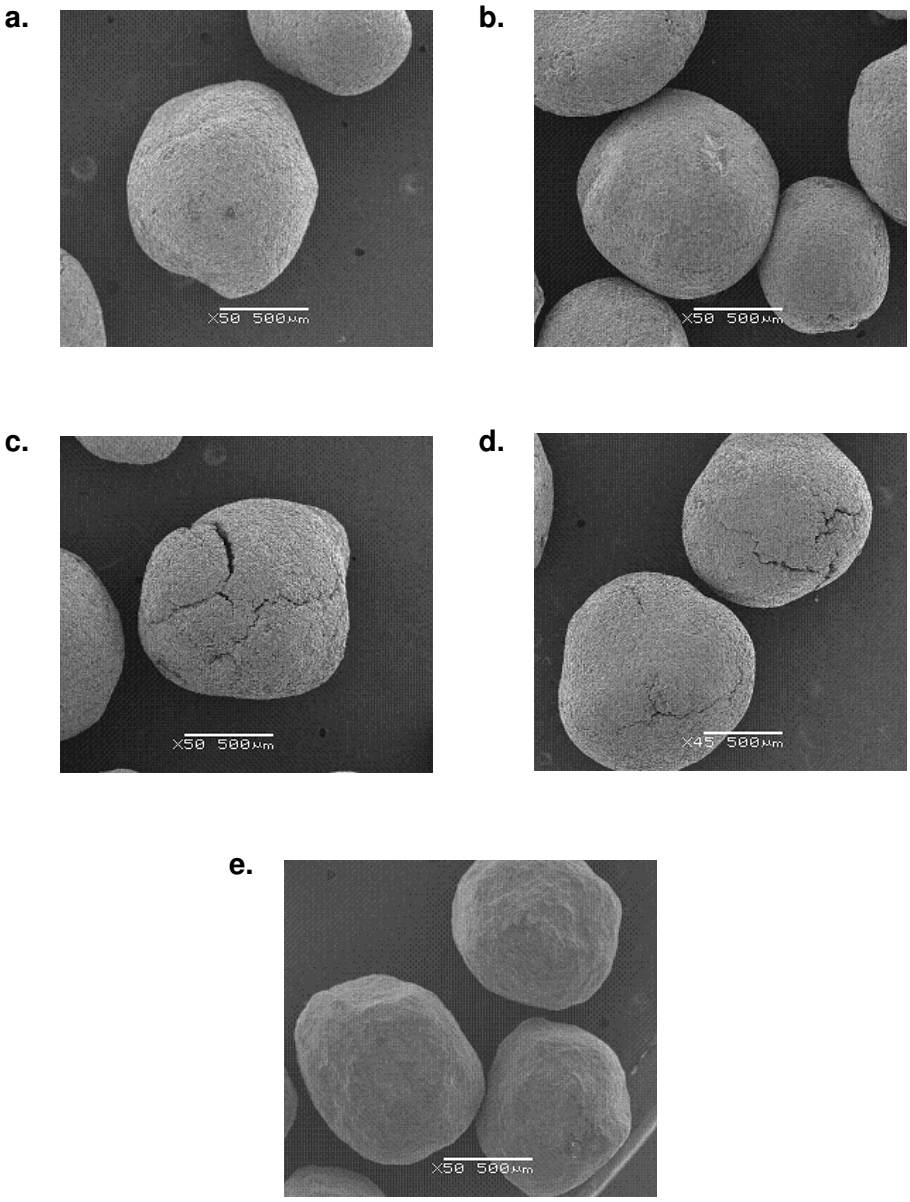


Figure 5.4 *Scanning electron micrographs of starch-based piroxicam pellets dried in oven without (**a**) and with (**b**) sorbitol, of starch-based piroxicam pellets dried in fluid bed without (**c**) or with sorbitol (**d**) and of microcrystalline cellulose-based piroxicam pellets dried in fluid-bed (**e**).*

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 (Scherer, 1990). 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 (Bataille et al., 1993).

In contrast, fluid-bed drying is 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 (Lieberman and Rankell, 1970). This faster drying rate and therefore higher pressure gradient of evaporating liquid might be a driving force for pellet contraction and can lead to crack formation (Scherer, 1990; Hasatani et al., 1993; Berggren et al., 2001).

The probability of crack formation can be reduced by increasing the network strength (Scherer, 1990). It was shown in Chapter 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. 5.5 a) which - in contrast to globular starch particles - provides a higher mechanical strength (Fig. 5.5 b). 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 case of sorbitol addition may be a consequence of improved mechanical strength of starch-based wet extrudates, as shown in Chapter 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. 5.3 b,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 (higher network strength) and therefore lower extent of crack formation: addition of a higher concentration of needle-like (coarse) theophylline powder (Fig. 5.5 c) and a lower level of globular starch particles (Fig. 5.5 b) increased the wet mass consistency due to mechanical interlocking of particles. Moreover, increasing the concentration of micronised theophylline powder (Fig. 5.5 d) increased the wet mass consistency due to a larger particle surface area (Holm, 1997). 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 (1992) who reported that reducing the particle size of theophylline powder in pellet formulations decreased the fraction of large pores.

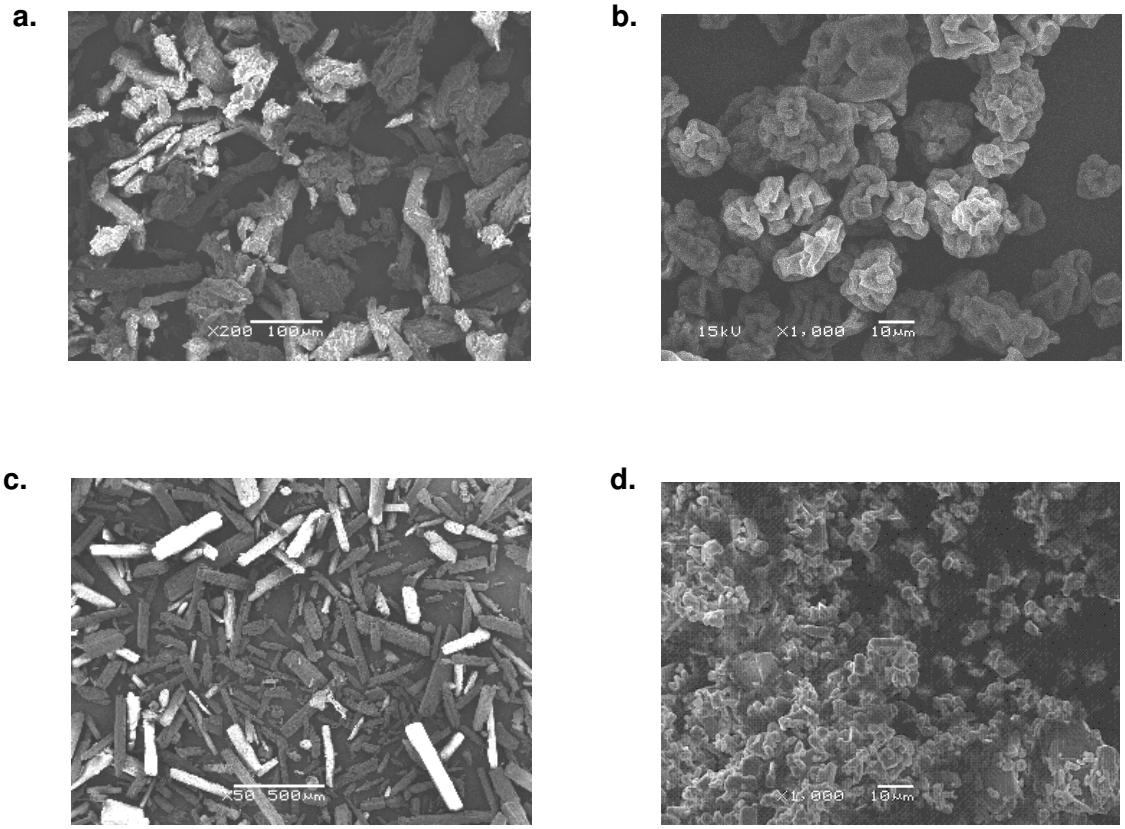


Figure 5.5 *Scanning electron micrographs of (**a**) Avicel® PH101, (**b**) UNI-PURE® EX starch, (**c**) coarse and (**d**) micronised anhydrous theophylline powder.*

5.4.1.3 Raman spectroscopy

The Raman spectra at the 1650 – 1730 cm^{-1} range of different theophylline forms (anhydrous, monohydrate and metastable), as well as the spectra of excipients used in pellet formulations are presented in Figure 5.6 a. It can be observed that all theophylline forms are easily distinguished and there is no spectral overlap originating from other excipients. Figure 5.6 b shows the Raman spectra of pellet formulations containing different sorbitol levels and dried in an oven and fluid-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 forms have a significant influence on drug release. Herman et al. (1988) reported that the transition of anhydrous theophylline into a monohydrate polymorph

occured during wet granulation. If theophylline dehydration during drying is not complete, the dissolution might be prolonged due to theophylline monohydrate which has a lower aqueous solubility (Shefter and Higuchi, 1963). Furthermore, depending on the drying conditions, theophylline monohydrate dehydration into a stabile anhydrous form can occur via a metastable form with lower dissolution rate compared to the stabile anhydrous polymorph (Phadnis and Suryanarayanan, 1997; Airaksinen et al., 2004).

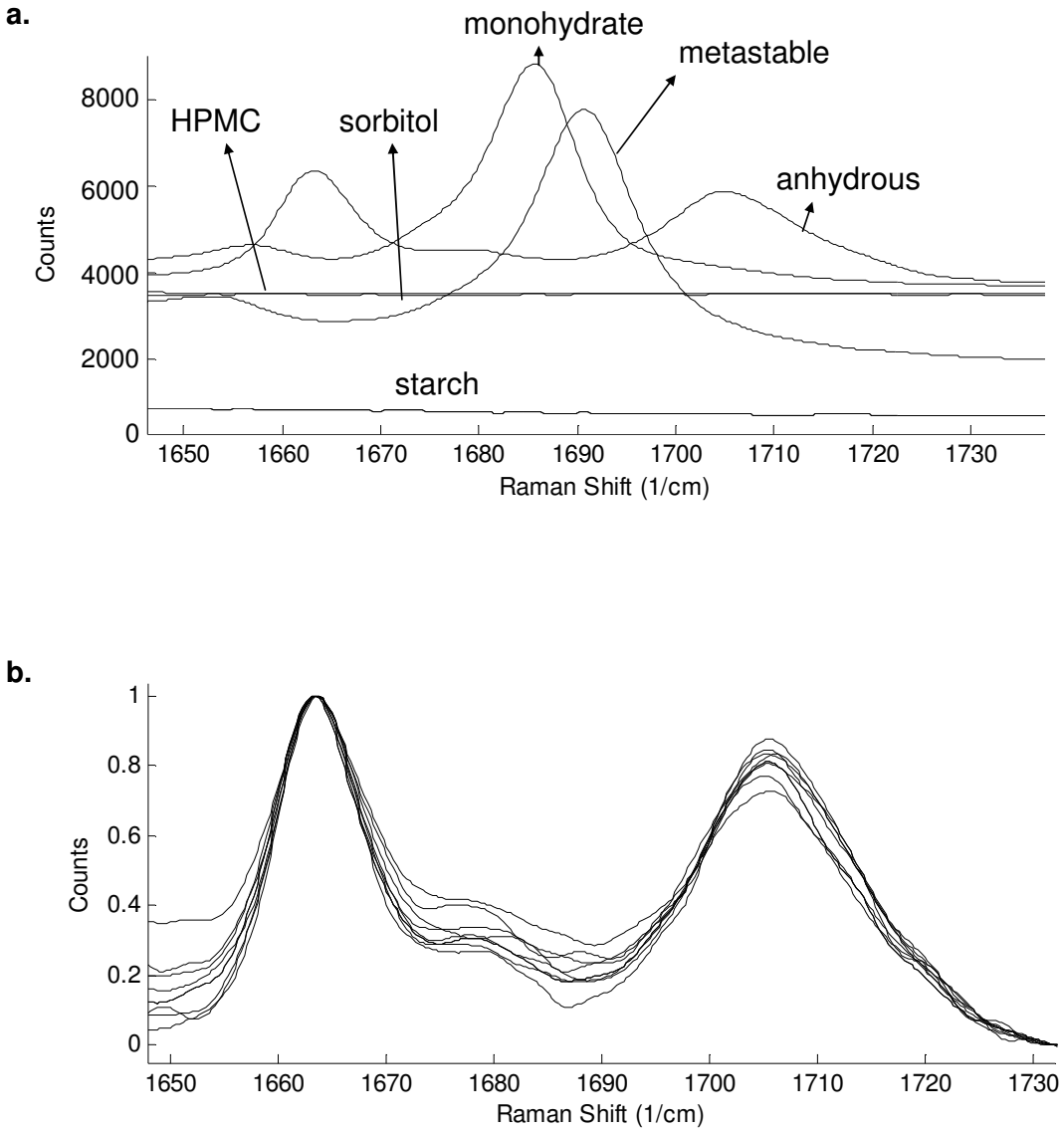


Figure 5.6 *Raman spectra: (**a**) raw materials including theophylline monohydrate and metastable form; (**b**) pellets dried in oven and fluid-bed, with and without sorbitol (25 % theophylline) and sampled at the surface and inside of the pellets .*

5.4.1.4 *In-vitro* drug release

Figures 5.7 and 5.8 show theophylline release profiles from enteric-coated pellets (with 15 and 30 % polymer weight gain, respectively) during 2 hours in acidic dissolution medium (0.1N HCl).

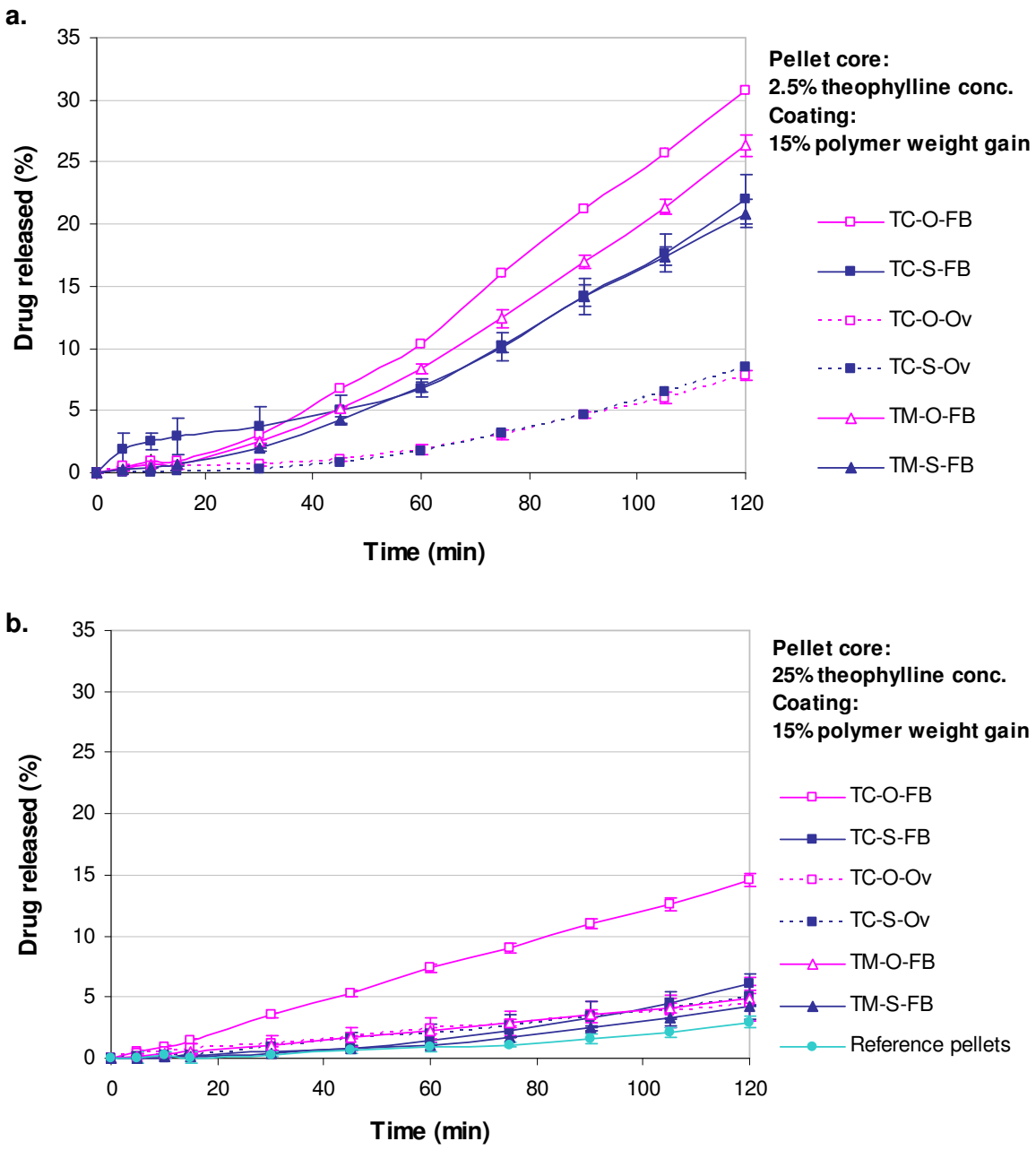


Figure 5.7 *In-vitro drug release in 0.1N HCl during 2h from enteric-coated pellets (15 % polymer weight gain) containing (**a**) 2.5 % (w/w, dry mass) and (**b**) 25 % (w/w, dry mass) of theophylline anhydrous. Legend: TC and TM – coarse and micronised theophylline; S and O – formulation with and without sorbitol; FB and Ov – pellet cores dried in fluid-bed and oven.*

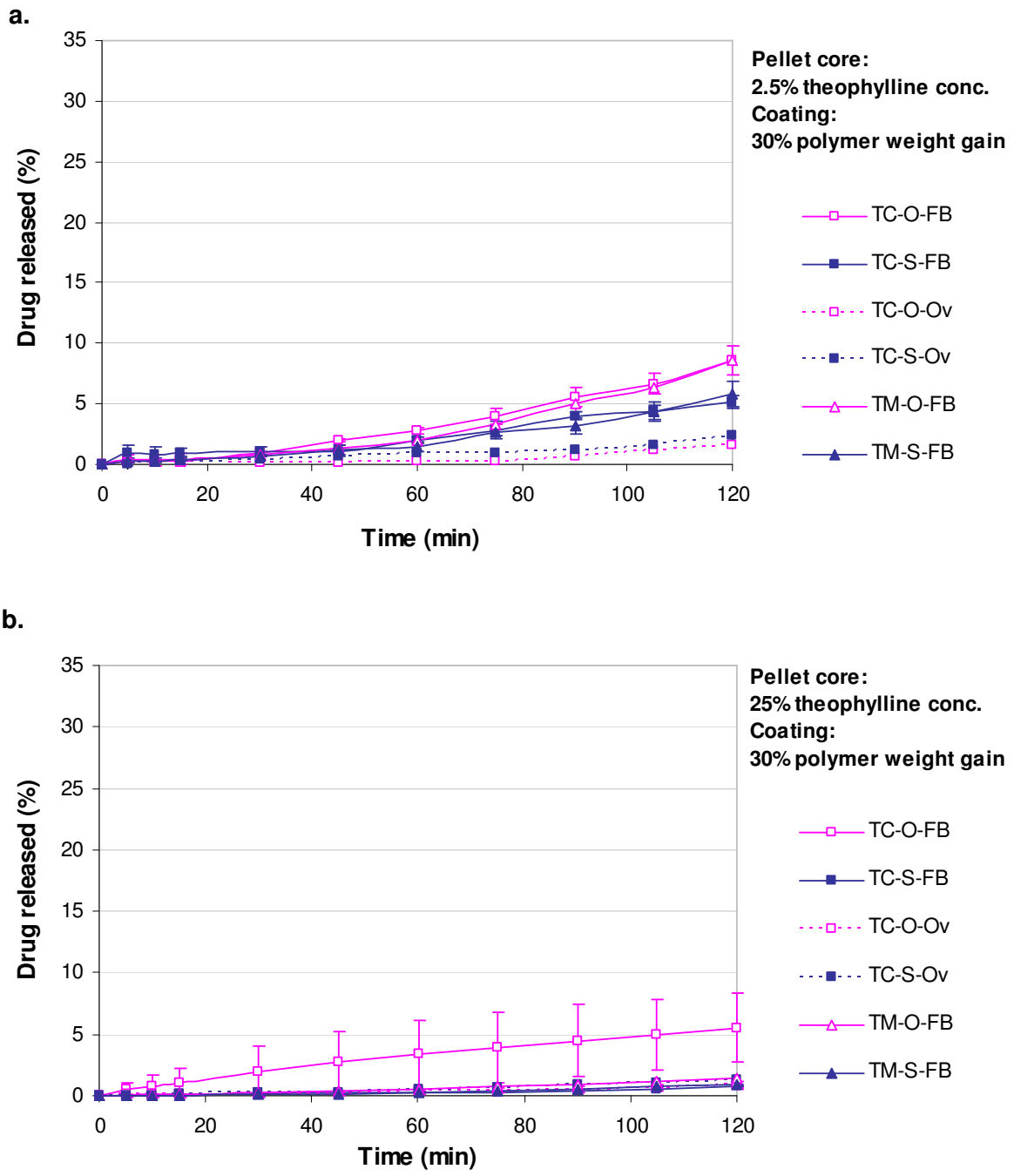


Figure 5.8 *In-vitro drug release in 0.1N HCl during 2h from enteric-coated pellets (30 % polymer weight gain) containing (**a**) 2.5 % (w/w, dry mass) and (**b**) 25 % (w/w, dry mass) of theophylline anhydrous. Legend: TC and TM – coarse and micronised theophylline; S and O – formulation with and without sorbitol; FB and Ov – pellet cores dried in fluid-bed and oven.*

For pellets coated with 15 % 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).

Furthermore, since the reference pellets (MCC-based) containing 25% theophylline (coarse), fluid-bed dried and coated until 15% polymer weight gain released only 3% of the drug after 2h in acidic medium (Fig. 5.7), 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 promoted the formation of an uneven coating thickness (especially in the case of larger pores or cracks) and resulted in a faster drug release (Porter and Ghebre-Sellassie, 1994).

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. 5.7 a). This correlated with the worst surface defects as identified via mercury intrusion. The slightly lower drug release for micronised theophylline is also linked with 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. 5.7 b) 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 2h in acid medium was less then 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.

Figures 5.9 and 5.10 present drug release from enteric coated pellets (containing 2.5 and 25 % theophylline, respectively) during 45 minutes of dissolution test in phosphate buffer (pH 6.8). As expected, the theophylline release was faster completed in case of lower coating thickness (in 20 and 30 minutes for formulations coated until 15 and 30 % polymer weight gain, respectively) due to a shorter lag time period required for dissolution of polymer film. In addition, it can be observed that for the same coating thickness, the

release profiles were similar for all formulations containing a lower theophylline level (Figure 5.9 a,b). In contrast, for pellets with a higher drug level (Figure 5.10 a,b), theophylline release was initially faster for formulations without sorbitol, which might be related to the pellet surface structure (more cracks). Nevertheless, due to pellet disintegration the release of theophylline in phosphate buffer was complete in less than 30 minutes for all formulations, irrespective of the theophylline level.

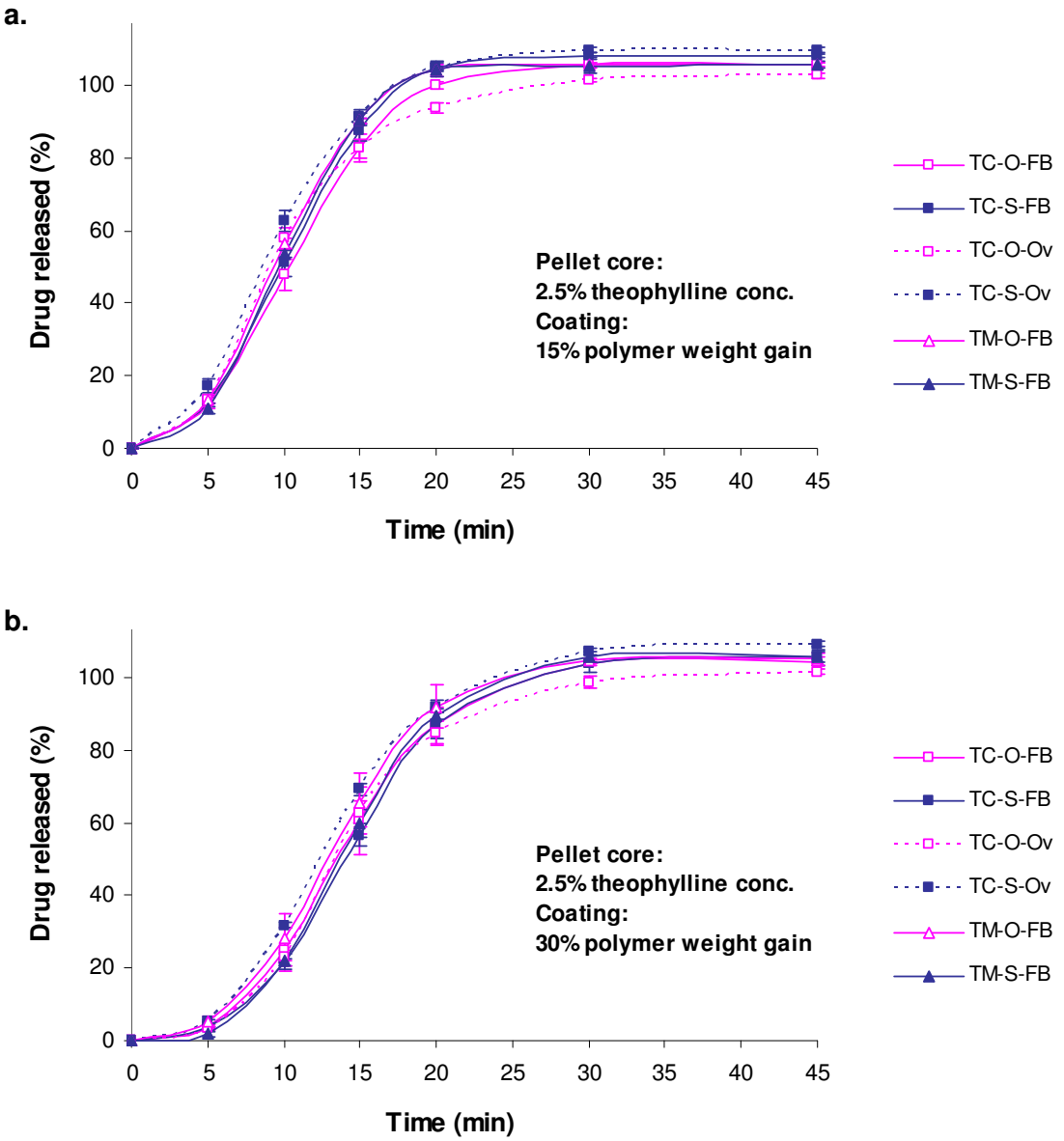


Figure 5.9 *In-vitro drug release in phosphate buffer (pH 6.8) during 45 min from pellets containing anhydrous theophylline (2.5 %) and enteric-coated until (**a**) 15 % polymer weight gain and (**b**) 30 % polymer weight gain. Legend: TC and TM – coarse and micronised theophylline; S and O – formulation with and without sorbitol; FB and Ov – pellet cores dried in fluid-bed and oven.*

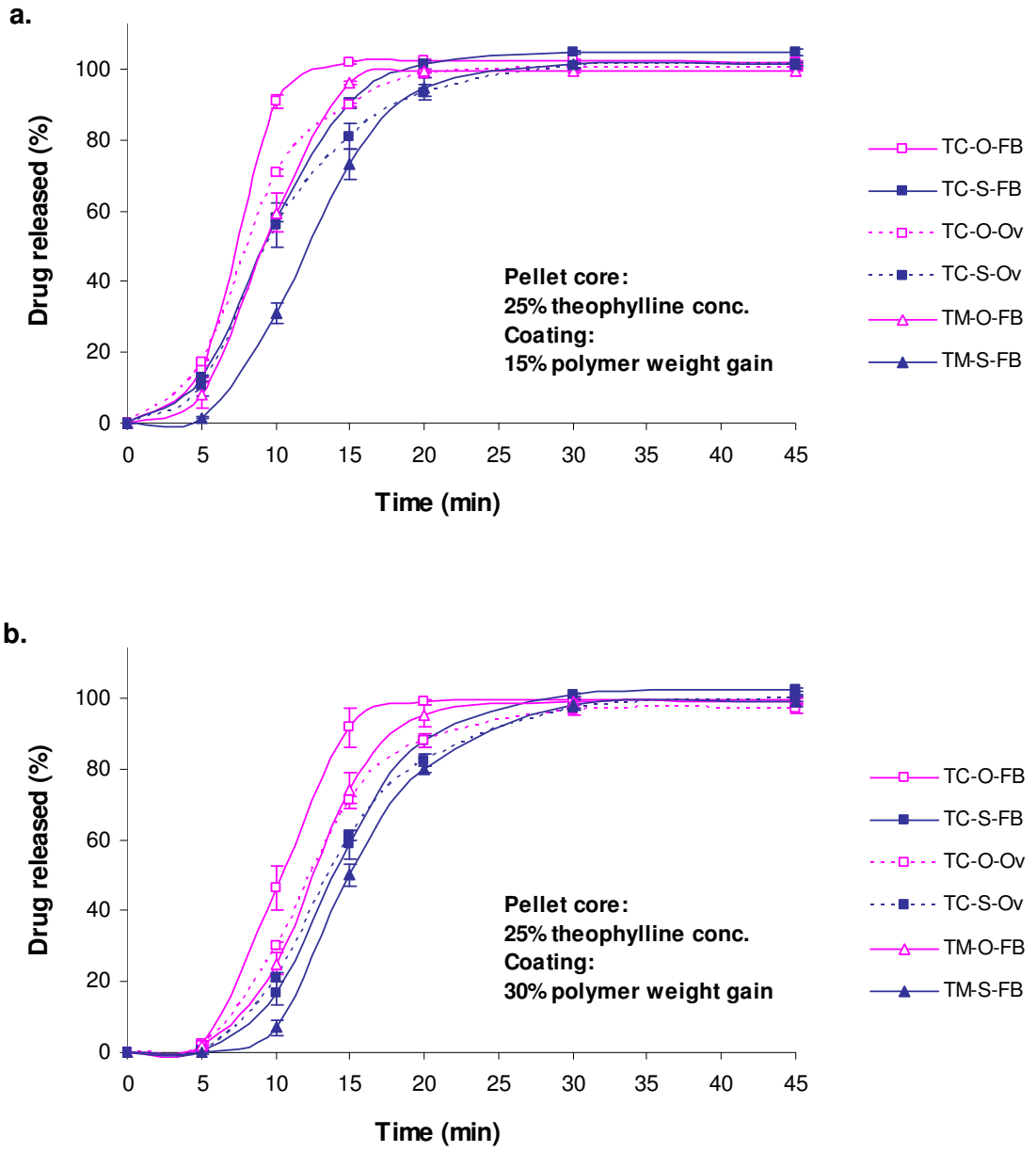


Figure 5.10 *In-vitro drug release in phosphate buffer (pH 6.8) during 45 min from pellets containing anhydrous theophylline (25 %) and enteric-coated until (**a**) 15 % polymer weight gain and (**b**) 30 % polymer weight gain. Legend: TC and TM – coarse and micronised theophylline; S and O – formulation with and without sorbitol; FB and Ov – pellet cores dried in fluid-bed and oven.*

Initial piroxicam release from coated pellets in phosphate buffer (pH 6.8) was faster from pellets with lower coating thickness (Fig. 5.11). In addition, pellet formulations containing sorbitol showed faster release due to wetting effect of sorbitol, as explained in Chapter 4.

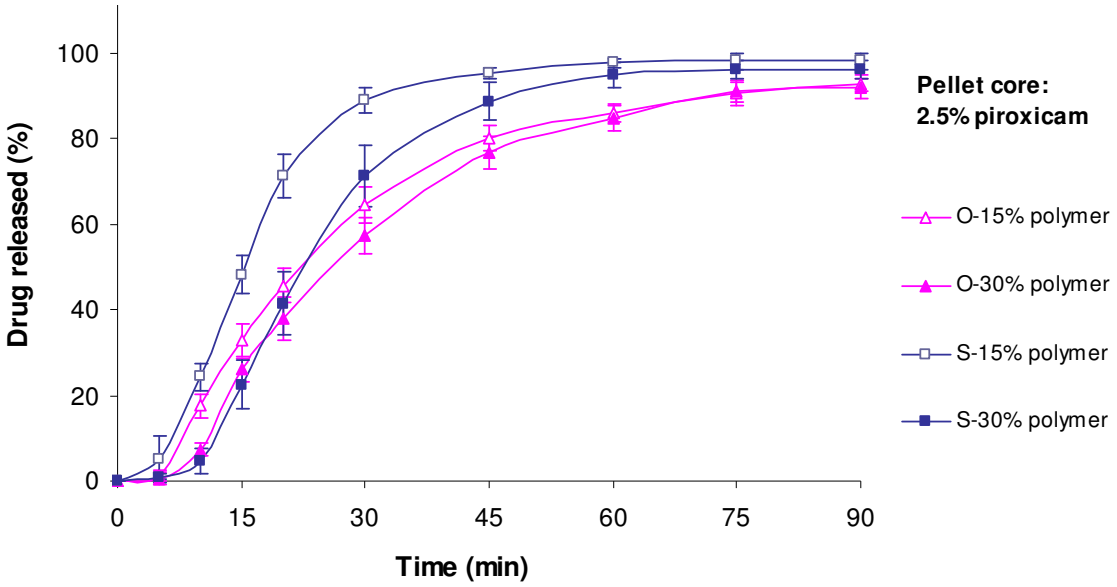


Figure 5.11 *In-vitro drug release in phosphate buffer (pH 6.8) during 90 min from enteric-coated piroxicam pellets (2.5 %) dried in fluid-bed. Legend: S and O – formulation with and without sorbitol; 15 % and 30 % refers to polymer weight gain after coating.*

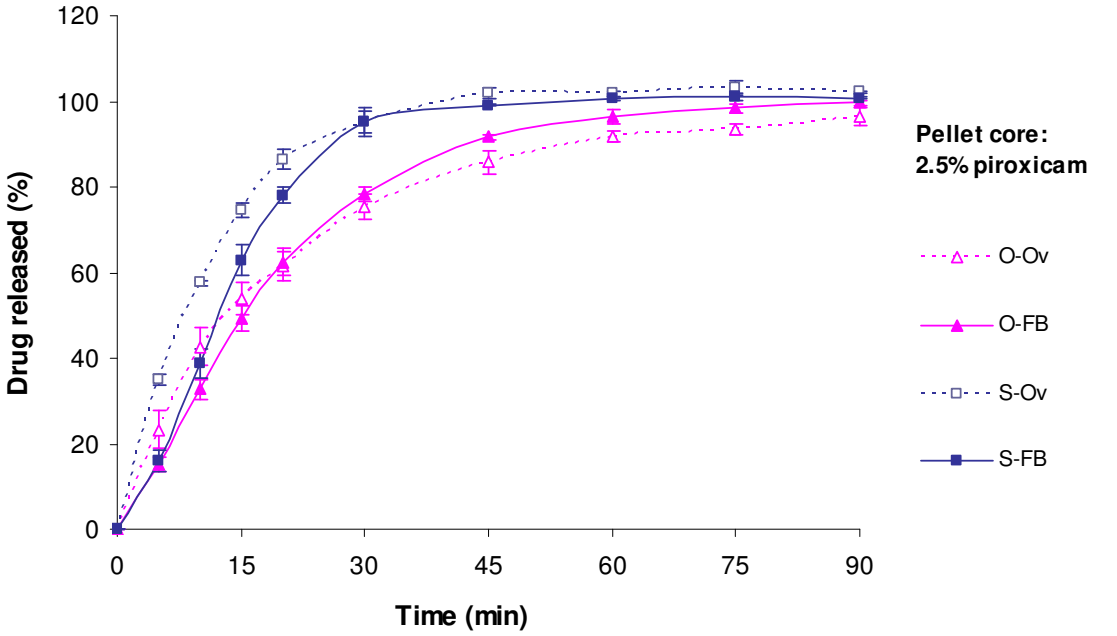


Figure 5.12 *In-vitro drug release in phosphate buffer (pH 6.8) during 90 min from uncoated pellets containing piroxicam (2.5 %) Legend: S and O – formulation with and without sorbitol; FB and Ov – pellets dried in fluid-bed and oven.*

Figure 5.12 presents the drug release profiles in phosphate buffer (pH 6.8) from piroxicam pellet cores dried in an oven and fluidised bed. It can be observed that piroxicam release is initially faster from oven-dried pellets, irrespective of sorbitol level. This might be explained by the difference in pellet size distribution between oven and fluid-bed dried pellets (Fig. 5.2): oven dried pellets were slightly smaller and, due to a higher surface area, more exposed to dissolution medium (Pinto et al., 1997). This effect of pellet fraction size is more pronounced if a drug has poor water solubility.

5.4.2 Stability study

Coated and uncoated starch-based pellets containing piroxicam as model drug were stored for 9 months under controlled relative humidity and temperature (60% RH/25°C and 75% RH / 40°C).

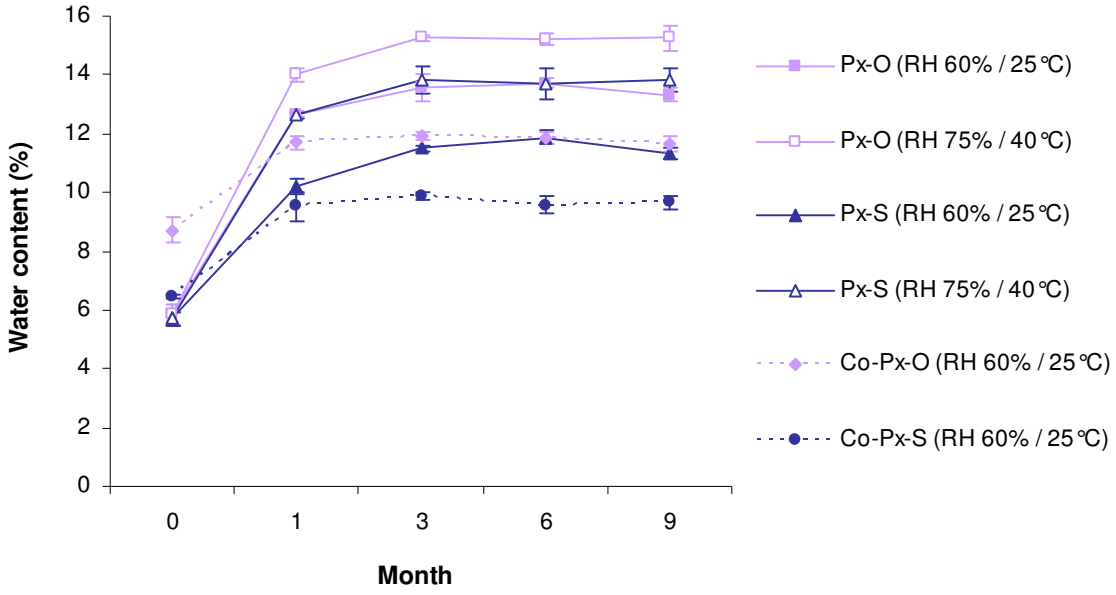


Figure 5.14 *Mean water content (±SD, n=3) of pellets (Px- piroxicam, Co- coated pellets, O- pellets without sorbitol, S- pellets with sorbitol) after 0, 1, 3, 6 and 9 months storage under controlled relative humidity and temperature (60% RH/25°C and 75% RH/40°C).*

After one month of storage at the higher relative humidity and temperature, coated pellets agglomerated due to sticking of the polymer film. Similar behaviour of piroxicam pellets coated with a mixture of Eudragit® polymers (L 30 D-55/ FS 30 D in a 60/40 ratio) and stored for one month under similar storage conditions has been reported by Debunne

(2004). This effect was attributed to the plasticising effect of water following water sorption at higher temperature: the T_g of film forming polymer is reduced, the molecular mobility of polymer chains increased and consequently sticking of coated pellets occured.

Figure 5.14 shows the results of residual water content of pellets as determined by Karl-Fischer titration. It can be observed that equilibrium water content is reached after 3 months storage for all formulations. Moreover, pellets containing sorbitol and coated pellets had a lower residual water content compared with the pellet formulations without sorbitol and uncoated pellets. This may be explained by the higher moisture sorption of starch (Fig. 5.15), which is present in a higher concentration in uncoated pellets and in formulations without sorbitol.

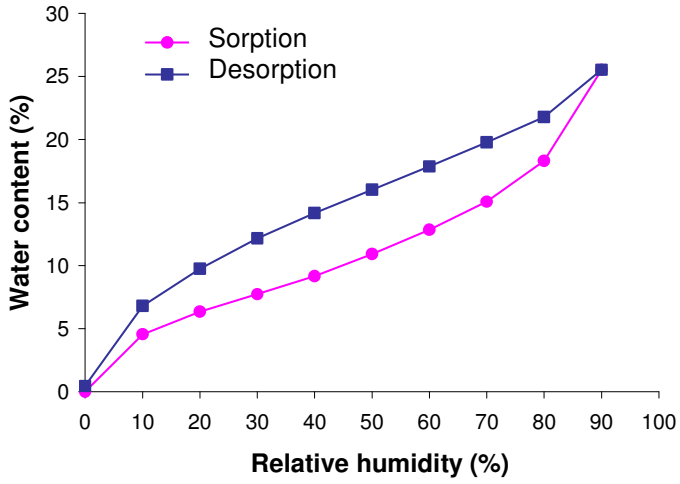


Figure 5.15 *Water sorption and desorption curves of UNI-PURE® EX starch.*

Piroxicam release profiles from uncoated pellets in phosphate buffer showed no change in the release after storage of the pellets at 60% RH/25°C and at 75% RH/40°C during the 9 month period (Fig. 5.16). The same was observed for coated pellets stored at 60% RH / 25°C (Fig 5.17). All drug release profiles were normalised, due to the large difference in residual water content. Furthermore, coated pellets stored at 60% RH / 25°C did not show any change of piroxicam release in acid medium (less than 1% of piroxicam was released after 2 h in 0.1N HCl).

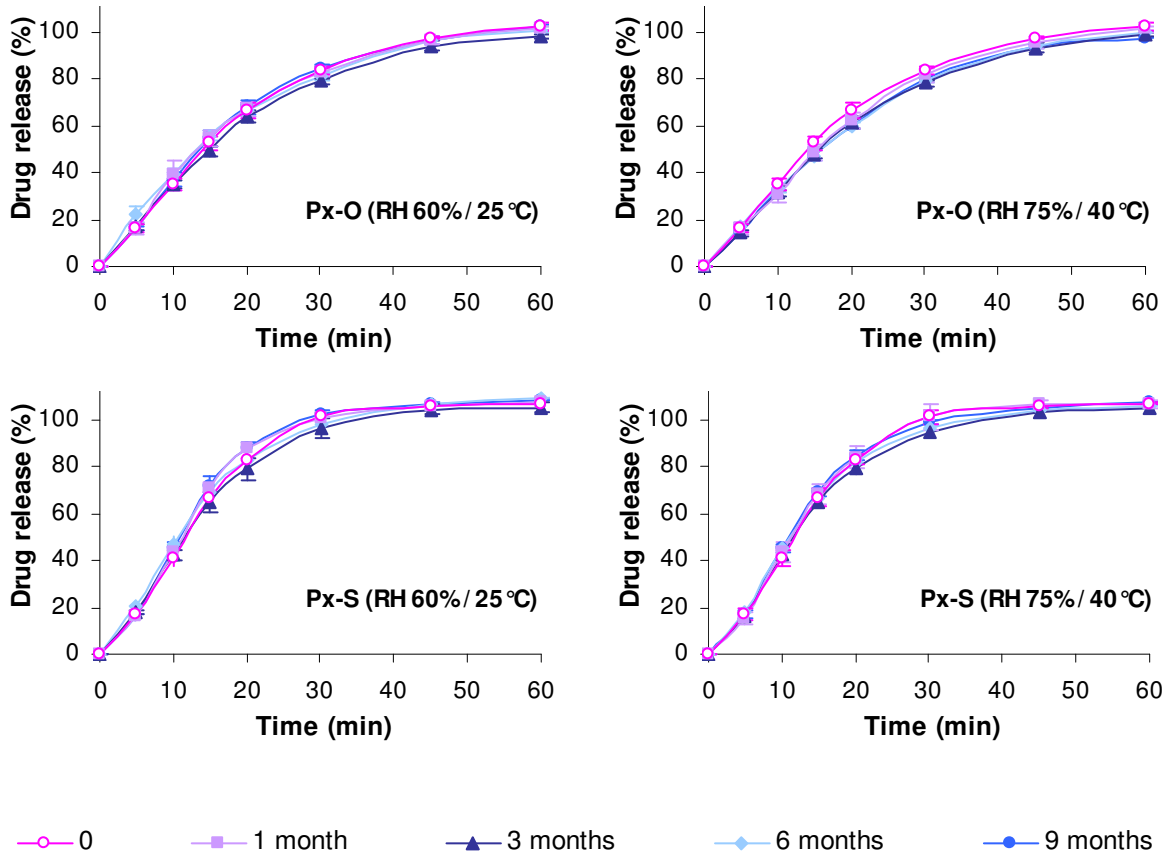


Figure 5.16 *In-vitro drug release in phosphate buffer (pH 6.8) from uncoated fluid-bed dried pellets containing piroxicam (Px, 2.5 %) without (O) and with sorbitol (S), after 0, 1, 3, 6 and 9 months storage under controlled relative humidity and temperature (60% RH/25 °C and 75% RH/40 °C).*

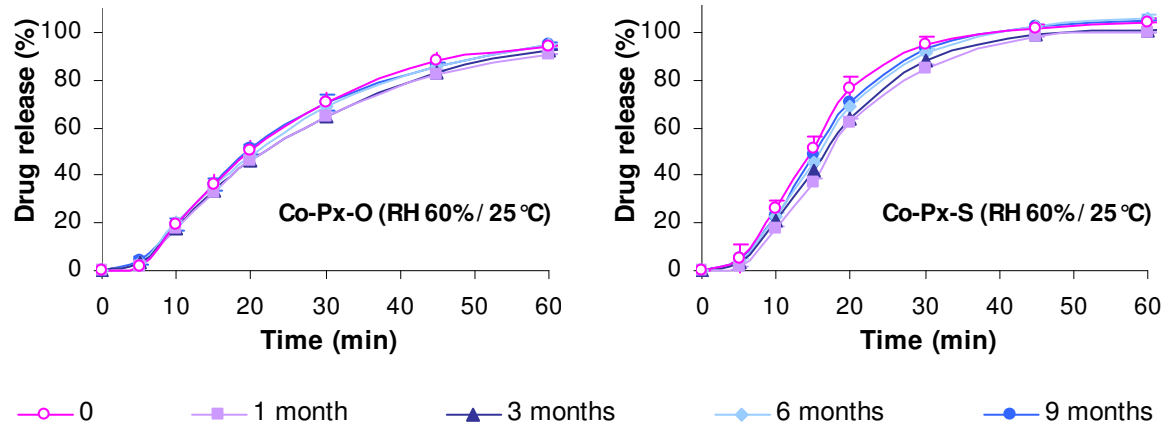


Figure 5.17 *In-vitro drug release in phosphate buffer (pH 6.8) from coated fluid-bed dried pellets containing piroxicam (Px, 2.5 %) without (O) and with sorbitol (S), after 0, 1, 3, 6 and 9 months storage under controlled relative humidity and temperature (60% RH/25 °C).*

5.4.3 *In-vivo evaluation of piroxicam pellets*

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.

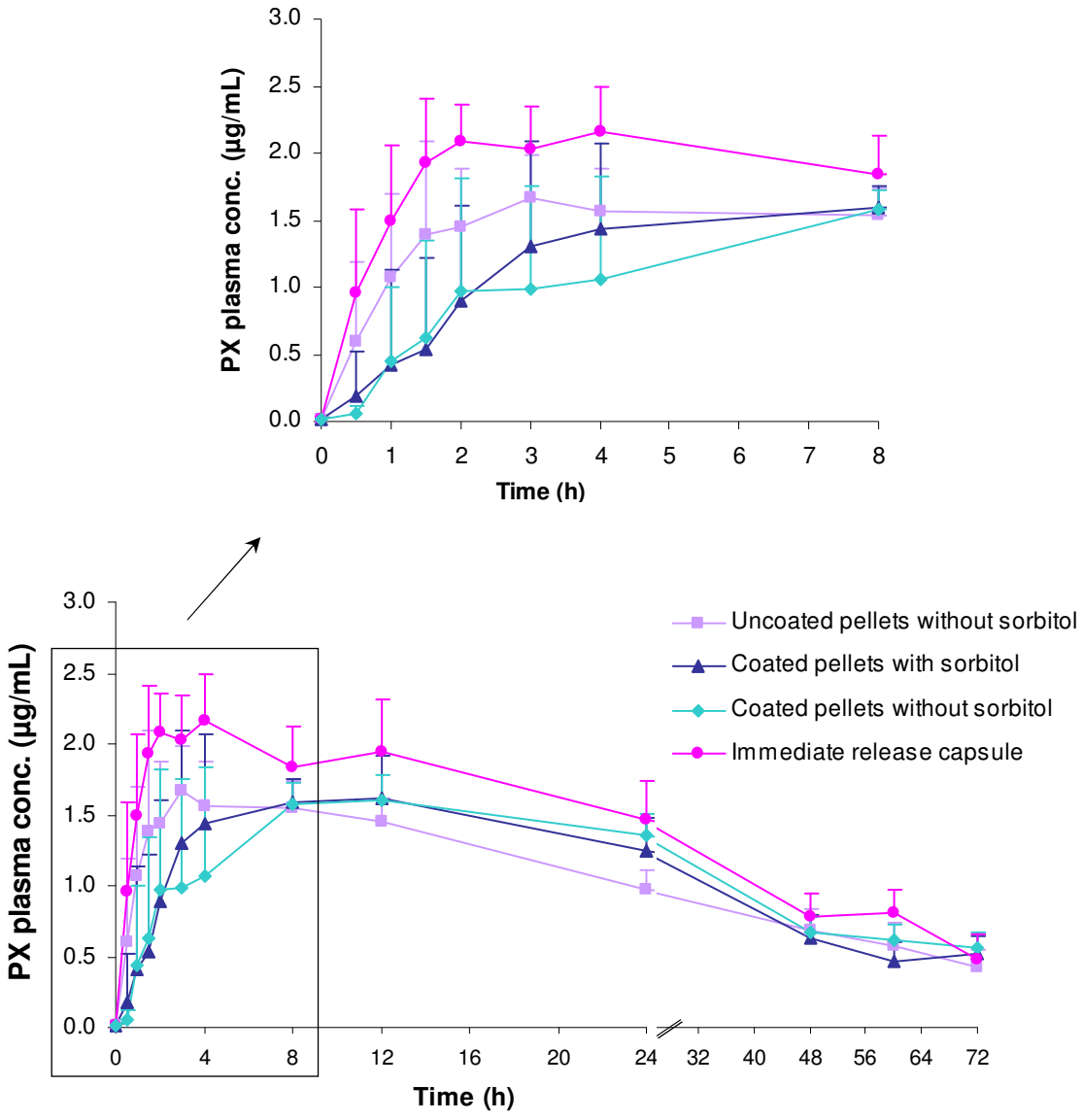


Figure 5.13 *Mean piroxicam plasma concentration-time profiles (±SD; n=6) obtained after administration of an oral dose of 0.3 mg piroxicam / kg body weight, formulated as uncoated and coated pellets as well as immediate release capsules.*

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 fast disintegrating formulation (Feldene®-capsules used as a reference).

Fig. 5.13 presents the mean (n=6) piroxicam plasma concentration versus time profiles of the pellet formulations and the immediate-release capsule, while Fig. 5.14 shows individual plasma concentration-time profiles. The pharmacokinetic parameters are summarised in Table 5.10.

Table 5.10 shows that there are no statistically significant differences of $AUC_{0\rightarrow 72h}$ and C_{max} between th 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. 5.13 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® L 30 D-55), which dissolves only at pH>6 (i.e., when the coated pellets are emptied from the stomach). In addition, one and four hours 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 (Debunne et al., 2004; Galbraith and McKellar, 1991, Polli et al., 1996).

Table 5.10 *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).*

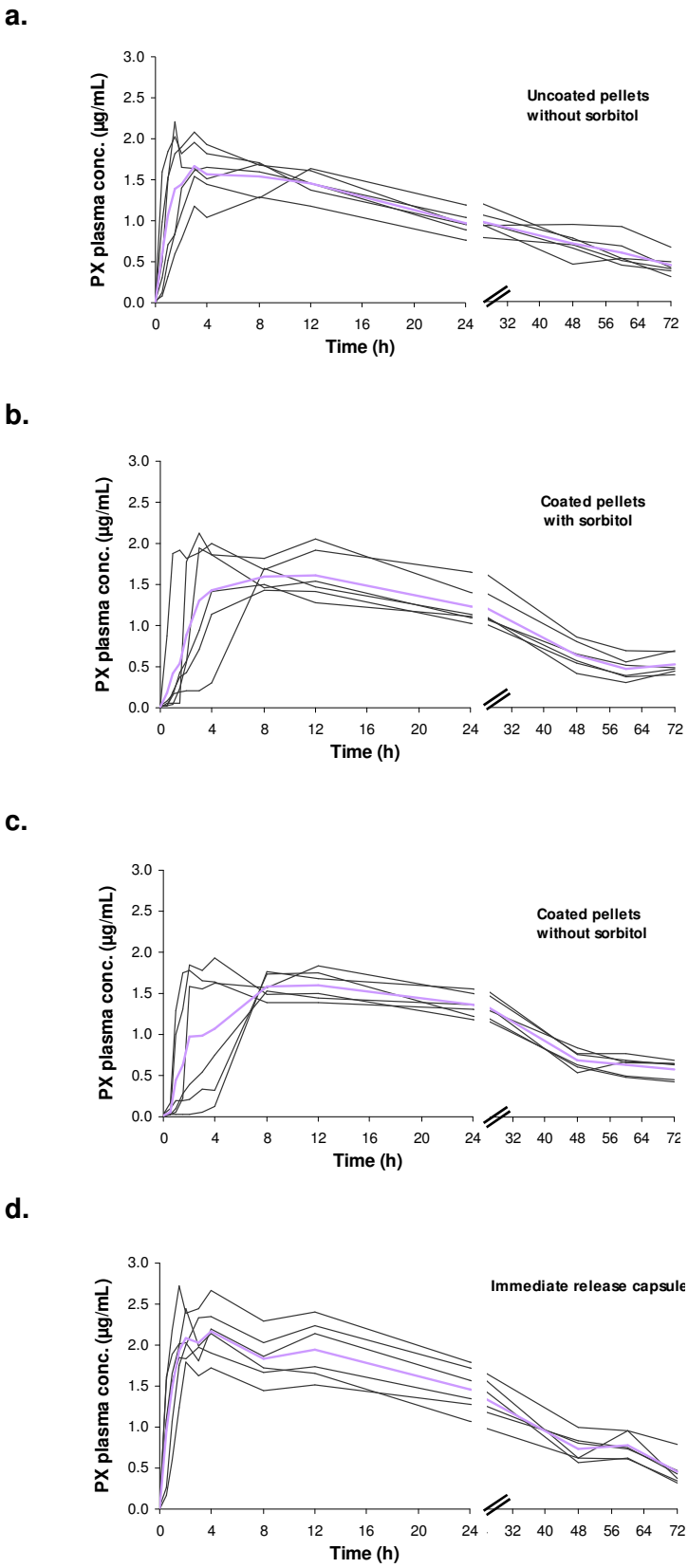


Figure 5.14 *Individual and mean (n=6) Px plasma concentration-time profiles obtained after oral administration of piroxicam formulated as:* ***a.*** *uncoated pellets without sorbitol,* ***b.*** *coated pellets with and* ***c.*** *without sorbitol and* ***d.*** *immediate release capsule.*

5.5 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 two hours 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.

5.6 References

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GENERAL CONCLUSION AND FUTURE PERSPECTIVES

This study demonstrated the performance of UNI-PURE® EX starch as the main excipient during production of pellets via extrusion and spheronisation. It was possible to obtain pellets with a high process yield (>90 %), acceptable sphericity (aspect ratio < 1.2) and low friability (<0.01 %). In contrast to microcrystalline-based pellet formulations, the main feature of starch-based pellets is their fast disintegration (< 10 min), which promotes faster release of drugs with poor water solubility. Therefore, irrespective of the model drug solubility, all starch-based pellet formulations in this study showed immediate drug release profiles. In addition, two *in-vivo s*tudies in dogs showed no significant differences in bioavailability (AUC and C_{max}) of two poorly soluble model drugs (hydrochlorothiazide and piroxicam) compared to immediate release reference formulations (P>0.05).

Concerning the formulation properties, pellets with UNI-PURE® EX starch as the main excipient required a binder to obtain acceptable pellet yield and sphericity. Furthermore, addition of sorbitol increased the mechanical strength of the wet mass and consequently improved pellet yield and surface properties. Water content was another important formulation variable. Compared to MCC-based pellet formulations, the optimal water content range was narrower in case of starch-based pellets. Furthermore, spheronisation time was limited to 3 min and the optimal spheronisation speed was at moderate intensity (around 850 rpm). Moreover, a smooth pellet surface morphology is an important pellet feature, especially when the application of a functional coating is needed. The results of the study showed that the quality of enteric coating (drug release after 2 h in acid dissolution medium) depended on the surface irregularities of the pellet cores and on the drug solubility. Cracks in the pellet surface were reduced if sorbitol was added to the pellet formulation or when using oven drying. Nevertheless, applying a higher coating thickness (up to 30 % of the polymer weight gain) yielded satisfactory drug release profiles from

enteric-coated pellets.

Future challenges for the application of UNI-PURE® EX starch during extrusion/spheronisation are linked to several issues:

- All experiments have been performed with a single batch of UNI-PURE® EX starch.

Since this research project is the first reported study on use of this excipient in extrusion/spheronisation process, batch-to-batch variability of UNI-PURE® EX starch could represent the problem when assessing reproducibility of pellet quality. Therefore, the future work might involve optimisation of excipient specification in order to reduce batch-to-batch variability. In addition, it will be important to identify those starch specification parameters which determine its extrusion/spheronisation behaviour (e.g. degree of crystallinity, amylose/amylopectin ratio, etc.)

- All formulations used in this study were produced on laboratory scale using a single type of equipment. It is known that the change of equipment type and the batch size (due to a difference in energy input and generation of heat due to longer processing time) can affect the material behaviour, thus influencing pellet quality. Therefore, scale-up would be another challenging step when evaluating the performance of UNI-PURE® EX starch as excipient in extrusion/spheronisation.
- Finally, the success of using UNI-PURE® EX starch in extrusion/spheronisation, depends to a great extent on the amount and properties of the drug which is being formulated.

SUMMARY

1 Pellets are spherical free-flowing granules with a narrow size distribution, typically varying between 500 and 1500 µm for pharmaceutical applications. Being multiparticulate solid dosage forms, pellets offer several important advantages when compared single-unit dosage forms such as tablets. A number of methods are used to produce pellets: extrusion/spheronisation, layering, direct pelletisation and high-shear pelletisation. Extrusion/spheronisation is a multiphase process which comprises several distinct steps: a uniform powder mixture of drug and excipient(s) is initially wet massed by addition of a liquid binder, followed by pressing of the moistened mass through an extrusion screen (extrusion) to form cylindrical extrudates, which are subsequently broken into smaller cylindrical rods and rounded into spherical granules by means of a fast-rotating friction plate (spheronisation) and finally dried. Each production step is a distinct process and involves control over a number of process parameters in order to obtain pellets of required quality.

2 Next to the variables relating to the process of extrusion/spheronisation, formulation variables (e.g. granulation liquid type and concentration) as well as the API and excipients properties (e.g. concentration, solubility and particle size distribution) significantly influence the pellet properties. In Chapter 2, an overview of the commonly used excipients and their influence on pellet properties (sphericity, size distribution, mechanical strength and drug release) was presented. Microcrystalline cellulose (MCC) is commonly used as excipient in extrusion/spheronisation due to its favorable rheological properties when wetted with granulation liquid, thus assuring the production of high quality pellets . However, in several cases microcrystalline cellulose is not the excipient of choice, the most important disadvantage being lack of disintegration of MCC-based pellets, which leads to prolonged drug release from pellets formulated with poorly water soluble drugs. In order to circumvent the disadvantages of MCC, different

research approaches have been reviewed in this chapter: modification of MCC-based pellet formulations by addition of other excipients and partial or complete substitution of MCC by an alternative excipient. The aim of this study was to evaluate UNI-PURE® EX starch as an alternative to MCC in the production of pellets via extrusion/spheronisation. This modified starch was obtained by an enzymatic debranching of amylose-rich starch. After debranching, the starch was retrograded and further isolated by extrusion or drying, yielding a crystalline, high-amylose material consisting of D-anhydroglucose units linked by α-1,4-D-glycosidic bonds and organised into double-helical crystalline chains. Due to its double-helical structure, the α-1,4-D-glycosidic linkages are inaccessible to α-amylase in small intestine and therefore it belongs to the group of resistant starches.

3 In the first part of Chapter 3, preliminary experiments were performed in order to identify the formulation and process variables, as well as their optimal ranges for the preparation of pellets with high process yield and acceptable sphericity. It was revealed that a binder was necessary to obtain an acceptable yield and that the addition of sorbitol (when used in a specific concentration range) improved the mechanical strength of the wet mass as well as the pellet surface properties. Mixer torque rheometry revealed the influence of formulation variables on wet mass consistency and indirectly on pellet yield, while solid state NMR confirmed the starch-sorbitol interaction at a molecular level. In the second part of this chapter, the process optimisation of a pellet formulation (containing theophylline anhydrous (25%, w/w) as model drug) was performed by means of surface response methodology. The Box-Behnken surface response design included four variables at three levels: binder concentration (HPMC; 3, 4.6 and 6 %, w/w), sorbitol concentration (0, 11.25 and 22.5 %, w/w), spheronisation speed (650, 850 and 1050 rpm) and water level (which depended on the sorbitol level in the formulation). Pellet yield, sphericity (aspect ratio and two-dimensional shape factor, e_R) and size (mean Feret diameter) were modelled as responses. Pellet friability, disintegration properties and drug release profiles were also determined. It was possible to obtain a high pellet yield (>90%) and all variables of the design as well as their interactions were significant for pellet yield ($P<0.05$). Pellet sphericity was acceptable ($AR<1.2$), while spheronisation speed and water level, as well as their interactions were significant variables. The mean pellet size was between 900 and 1200 µm, with spheronisation speed, water and sorbitol level as significant variables. All pellet formulations had a low friability (<0.01%), fast disintegration (<10 min) and complete drug release in less than 20 minutes.

4 In Chapter 4, UNI-PURE® EX starch was evaluated as the main excipient for immediate-release pellets containing poorly soluble drugs (hydrochlorothiazide and piroxicam). 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 (mean Feret diameter) and sphericity (aspect ratio and two-dimensional shape factor, e_R). The optimal granulation liquid content depended on the 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 of pellets (containing 50 mg hydrochlorothiazide) was determined after oral administration to 6 dogs. An HPLC method, validated according to the ICH-guidelines, was used for determination of drug levels in dog plasma. No interference with endogenous components was detected. Calibration curves were linear in the whole concentration range (r^2 = 0.99986 ± 0.00026; n=10). The recovery of hydrochlorothiazide (10-2000 ng/mL range) after extraction varied between 77.1 and 96.3 %, while 84.8 % of internal standard (hydroflumethiazide) 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. The bioavailability ($AUC_{0\rightarrow 24h}$ and C_{max}) of hydrochlorothiazide pellets in dogs was not significantly different from fast-disintegrating immediate-release hydrochlorothiazide tablets ($P>0.05$).

5 In Chapter 5, pellet cores containing UNI-PURE® EX 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 and two-dimensional shape factor, e_R), friability, surface morphology and drug release were evaluated. Binder (HPMC) and granulation liquid (water) level were optimised in order 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 6-80µm 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 6 dogs. An HPLC method, validated according to the ICH-guidelines, was used for determination of drug levels in dog plasma. Interference of piroxicam and meloxicam (internal standard) with endogenous components was not detected. The calibration curves were linear (R^2 = 0.99655 ± 0.00247; n=11) in the 0.2 to 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 internal standard 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. Values of $AUC_{0\rightarrow 72h}$ and C_{max} after oral administration of piroxicam pellets to dogs were not significantly different from the values obtained from immediate release capsules (P>0.05).

SAMENVATTING

1 De focus van dit onderzoeksproject zijn pellets als farmaceutische doseringsvorm. Pellets worden gedefiniëerd als sferische partikels met goede vloei-eigenschappen en met een nauwe deeltjesgroottedistributie (voor farmaceutische toepassingen variërend tussen 500 en 1500 µm). Als multiparticulaire doseringsvorm bieden pellets een aantal belangrijke voordelen ten opzichte van single-unit vormen zoals tabletten. Pellets kunnen via een aantal technieken worden geproduceerd: extrusie/sferonisatie, layering, directe pelletisatie en high-shear pelletisatie. Extrusie/sferonisatie, de techniek aangewend tijdens dit onderzoeksproject, bestaat uit een aantal verschillende stappen: in een eerste fase wordt een poedermengsel van het geneesmiddel en de hulpstoffen bevochtigd door toevoeging van een granulatievloeistof. Vervolgens wordt deze vochtige massa doorheen een extrusiescherm geperst (extrusie) waardoor cylindrische extrudaten worden gevormd. Deze worden daarna in kleinere cylindrische staafjes opgebroken en afgerond tot sferische granules (pellets) door middel van een roterende frictieplaat (sferonisatie). De laatste stap in het extrusie/sferonisatie-proces is een droogfase. Op basis van literatuurgegevens is het duidelijk dat het controleren van de procesparameters tijdens de verschillende stappen van het proces essentieel is om de kwaliteit van de pellets te waarborgen.

2 De eigenschappen van de pellets worden echter niet alleen duidelijk beïnvloed door de procesvariabelen gedurende het extrusie-/sferonisatieproces, maar ook door de formulatieparameters (vb. type en concentratie van de granulatievloeistof) en door de eigenschappen van het actief bestanddeel en de hulpstoffen (vb. concentratie, oplosbaarheid en deeltjesgroottedistributie). In Hoofdstuk 2 wordt een overzicht gegeven van de belangrijkste hulpstoffen aangewend tijdens extrusie/sferonisatie en hun invloed op de eigenschappen van de pellets (sfericiteit,

deeltjesgroottedistributie, hardheid en geneesmiddelvrijstelling). Microkristallijne cellulose (MCC) wordt het frequentst aangewend als hulpstof tijdens extrusie/sferonisatie aangezien de rheologische eigenschappen van dit product – na bevochtigen met water – optimaal zijn voor extrusie en sferonisatie zodat de kwaliteit van de pellets verzekerd wordt. In bepaalde gevallen is het gebruik van MCC echter niet aangewezen omwille van een aantal specifieke nadelen, waarvan het niet desintegreren van MCC-pellets het belangrijkste is. Dit heeft een verlengde geneesmiddelvrijstelling tot gevolg indien de pellets geformuleerd zijn met een slecht wateroplosbaar geneesmiddel. Om de nadelen van MCC tijdens extrusie/sferonisatie te vermijden kunnen verschillende strategieën worden aangewend die besproken worden in dit hoofdstuk: het aanpassen van formulaties op basis van MCC door toevoeging van specifieke hulpstoffen (vulmiddel, bindmiddel, lubrifieermiddel, desintegrator, ...) en het (gedeeltelijk of volledig) vervangen van de MCC-fractie door een alternatieve hulpstof die eveneens optimale eigenschappen voor extrusie/sferonisatie bezit. Deze laatste strategie wordt aangewend tijdens dit onderzoeksproject waarbij de extrusie/sferonisatie-eigenschappen van een specifiek zetmeelderivaat (UNI-PURE® EX zetmeel) worden geëvalueerd. Dit zetmeelderivaat is een kristallijn product met een hoog amylose-gehalte dat wordt bekomen na enzymatische behandeling en retrogradatie van zetmeel en dat is opgebouwd uit D-anhydroglucose eenheden verbonden via α-1,4-D-glycosidische bindingen en georganiseerd in een kristallijne dubbele helix. Door deze dubbele helix-structuur zijn de α-1,4-D-glycosidische bindingen ontoegankelijk voor α-amylase in de dunne darm en behoort UNI-PURE® EX zetmeel tot de groep van resistente zetmelen.

3 Via preliminaire testen uitgevoerd tijdens het eerste deel van Hoofdstuk 3 werden de formulatie- en procesparameters geïdentificeerd die van belang zijn voor het produceren van pellets met een hoge opbrengst en een goede sferiiciteit. Hieruit bleek dat een bindmiddel noodzakelijk was om een aanvaardbare opbrengst te garanderen en dat het toevoegen van sorbitol (binnen een specifieke concentratiegebied) de mechanische sterkte van de vochtige massa en ook de oppervlakte-eigenschappen van de pellets verbeterden. Met behulp van een mixer torque rheometer werd de invloed van de formulatieparameters op de consistentie van de vochtige massa (en onrechtstreeks op het rendement van het extrusie/sferonisatie proces) aangetoond, terwijl vaste stof NMR de interactie tussen zetmeel en sorbitol op een moleculair niveau bevestigde. In het tweede deel van dit hoofdstuk werd via de oppervlakterespons methodologie de optimalisatie beschreven van een extrusie/sferonisatie-proces voor een formulatie op basis van anhydrische theophylline

(25%, w/w, aangewend als modelgeneesmiddel). Het Box-Behnken oppervlakte-respons design omvatte vier variabelen uitgetest op drie niveaus: bindmiddel-concentratie (HPMC: 3, 4.6 en 6 %, w/w), sorbitol-concentratie (0, 11.25 en 22.5 %, w/w), sferonisatiesnelheid (650, 850 en 1050 rpm) en watergehalte (afhankelijk van de sorbitol-concentratie in de formulatie). De procesopbrengst, sfericiteit (aspect ratio, AR en tweedimensionale vormfactor, e_R) en grootte (gemiddelde Feret diameter) van de pellets werden gemodelleerd als responsfactoren. De friabiliteit, desintegratie-eigenschappen en geneesmiddelvrijstelling van de pellets werden eveneens bepaald. Voor pellets op basis van UNI-PURE® EX zetmeel was het mogelijk een hoge pelletopbrengst (>90%) te bekomen en alle variabelen van het design (evenals hun interacties) hadden een significante invloed op de pelletopbrengst ($P<0.05$). De sfericiteit van de pellets was aanvaardbaar (AR<1.2), waarbij de sferonisatiesnelheid, de waterconcentratie en hun interacties significante parameters waren. De gemiddelde pelletgrootte bedroeg tussen 900 en 1200 µm, met de sferonisatiesnelheid, water- en sorbitolconcentratie als significante parameters. Alle pelletformulaties hadden een lage friabiliteit (<0.01%), een snelle desintegratie (<10 min) en een volledige geneesmiddelvrijstelling in minder dan 20 minuten.

4 In Hoofdstuk 4 werd UNI-PURE® EX zetmeel geëvalueerd als voornaamste bestanddeel van pellets met onmiddellijke geneesmiddelvrijstelling die slecht wateroplosbare geneesmiddelen bevatten (hydrochlorothiazide en piroxicam). Een 2^4 factorial design met centraal punt werd gebruikt om de invloed van de concentratie aan hydrochlorothiazide (10 en 50%, w/w), HPMC (bindmiddel, 4 en 7%, w/w), sorbitol (0 en 10%, w/w) en water (granulatievloeistof, laag en hoog gehalte) te evalueren op de pelletopbrengst, grootte (gemiddelde Feret diameter) en sfericiteit (aspect ratio, AR en tweedimensionale vormfactor, e_R). Het optimale gehalte van de granulatievloeistof was afhankelijk van de geneesmiddel- en sorbitol-concentratie in de formulatie. Alle factoren (behalve de sorbitol-concentratie) en de interacties tussen de geneesmiddel- en bindmiddel-concentratie en tussen de geneesmiddel-concentratie en het watergehalte waren significant ($P<0.05$) voor de pelletopbrengst. Het model was niet significant voor de pelletvorm, terwijl de hydrochlorothiazide-concentratie en het watergehalte (alsook hun interacties) een significante invloed ($P<0.05$) hadden op de pelletgrootte. De friabiliteit, desintegratie, residueel vochtgehalte en *in-vitro* vrijstelling van de pellets werden bepaald. Pellets met 2.5% (w/w) piroxicam werden eveneens geëvalueerd. Voor beide modelgeneesmiddelen was het mogelijk om pellets met een hoog rendement (>90%), aanvaardbare sfericiteit (AR<1.2) en lage friabiliteit (<0.01%) te produceren. Ten gevolge

van de snelle pelletdesintegratie werden zowel hydrochlorothiazide als piroxicam snel vrijgesteld uit de pellets: meer dan 80% van de geneesmiddeldosis in 30 minuten. De biologische beschikbaarheid van pellets (beladen met 50 mg hydrochlorothiazide) werd bepaald na orale toediening aan 6 honden. Via een HPLC methode (gevalideerd volgens de ICH-richtlijnen) werden de plasmaconcentraties aan hydrochlorothiazide bepaald. Er werd geen interferentie vastgesteld tussen hydrochlorothiazide, de interne standaard en endogene plasmacomponenten. De calibratiecurves waren lineair binnen het gehele concentratiegebied (r^2=0.99986 ± 0.00026; n=10). De recovery van hydrochlorothiazide (10-2000 ng/mL range) na extractie varieerde tussen 77.1 en 96.3 %, terwijl 84.8 % van de interne standaard (hydroflumethiazide) werd teruggevonden. De methode was precies binnen dezelfde concentratierange, aangezien de variatie-coëfficienten voor de herhaalbaarheid en intermediaire precisie respectievelijk tussen 1.01 en 8.63 % en tussen 2.05 en 8.97 % lagen. De detectie- en kwantificatie-limieten bedroegen respectievelijk 4.45 en 1.49 ng/mL. De biologische beschikbaarheid ($AUC_{0\rightarrow 24h}$ en C_{max}) van hydrochlorothiazide pellets bij honden was niet significant verschillend ten opzichte van sneldesintegrerende hydrochlorothiazide-tabletten met onmiddellijke vrijstelling (P>0.05).

5 In Hoofdstuk 5 werden pellets op basis van UNI-PURE® EX zetmeel enterisch omhuld met een Eudragit® L30 D-55 dispersie. De gewichtstoename aan polymeer na coating was 15 tot 30% (w/w). De pellets bevatten piroxicam (2.5 % (w/w), slecht wateroplosbaar) of anhydrische theophylline (2.5 en 25 % (w/w), grof en gemicroniseerd poeder, medium wateroplosbaar) als modelgeneesmiddelen. Naast de wateroplosbaarheid, deeltjesgrootte en concentratie van het modelgeneesmiddel werden ook de invloed van sorbitolconcentratie (0 en 10 %, w/w) en de droogmethode (oven en fluid-bed) op de pelletopbrengst, grootte (Feret gemiddelde diameter), sfericiteit (aspect ratio, AR en tweedimensionale vormfactor, e_R), oppervlakte-morfologie en geneesmiddelvrijstelling geëvalueerd. Het bindmiddel (HPMC) en het gehalte aan granulatievloeistof (water) werden geoptimiseerd om een maximale opbrengst (fractie tussen 900 en 1400 µm) en een aanvaardbare sfericiteit (AR<1.2) te bekomen. De pelletfriabiliteit was lager dan 0.01% voor alle formulaties, terwijl de gemiddelde pelletdiameter lager was voor pellets met sorbitol en voor formulaties die in de oven werden gedroogd. Kwikporosimetrie in combinatie met scanning electron microscopie toonde de invloed aan van de droogmethode en van de sorbitolconcentratie op de oppervlaktestructuur: het oppervlak van de pellets gedroogd via fluid-bed zonder sorbitol en met 2.5% aan modelgeneesmiddel vertoonde barsten, dit was gecorreleerd met een Hg-intrusiepiek voor poriën binnen een range van 6-80 µm. Als gevolg van de betere

mechanische eigenschappen van de vochtige massa in aanwezigheid van sorbitol, werden pellets met een vlak oppervlak bekomen aangezien de belangrijkste piek van Hg-intrusie verschoof naar een kleinere poriegrootte. Door het gebruik van een hogere geneesmiddelconcentratie en gemicroniseerde theophylline verschoof de Hg-intrusie nog verder in de richting van kleinere poriën. Na ovendroging werd geen Hg-intrusie in de pellets gedetecteerd en visueel werden geen barsten waargenomen. Indien de dikste polymeerfilm werd aangebracht (30 % gewichtstoename) werden alle pelletformulaties geformuleerd met theophylline succesvol gecoat (<10% geneesmiddelvrijgave na 2 u in zuur midden, 0.1N HCl), terwijl pellets met de laagste coatingsdikte (15% gewichtstoename) 5 tot max. 30 % theophylline vrijstelden. Hierbij was de geneesmiddelvrijstelling afhankelijk van de samenstelling van de pellets en van de droogmethode aangezien deze factoren de oppervlakte-structuur van de pellets bepaalden. De vrijstelling van piroxicam in een zuur medium bedroeg minder dan 1% anafhankelijk van de oppervlakte-eigenschappen van de pellets, wat te wijten is aan de slechte wateroplosbaarheid van piroxicam. In een basisch medium (fosfaatbuffer pH 6.8) stelden de pellets de geneesmiddeldosis vrij in minder dan 45 min. De biologische beschikbaarheid van gecoate en niet-gecoate piroxicam-pellets werd bepaald na orale toediening aan 6 honden. Voor de bepaling van het geneesmiddelgehalte in hondenplasma werd gebruik gemaakt van een HPLC-methode die gevalideerd was in overeenstemming met de ICH-richtlijnen. Er werd geen interferentie waargenomen van piroxicam en meloxicam (interne standaard) met endogene componenten. De calibratiecurves waren lineair (R^2= 0.99655 ± 0.00247; n=11) in een concentratiegebied van 0.2 tot 2.4 µg/mL. De recovery van piroxicam na extractie varieerde afhankelijk van de concentratie tussen 77.3 en 82.4 %, terwijl 81.6 % van de interne standaard werd gerecupereerd. De methode was exact: de variatie-coëfficiënten voor de herhaalbaarheid en intermediaire precisie varieerden respectievelijk van 7.2 tot 9.9 % en van 1.8 tot 11.9 %. De detectie- en kwantificatieimieten bedroegen respectievelijk 0.05 en 0.16 µg/mL. De $AUC_{0\rightarrow 24h}$ en C_{max} waarden na orale toediening van piroxicam pellets aan honden vertoonden geen significant verschil met de waarden bereikt na toediening van piroxicam-capsules met onmiddellijke vrijstelling (P>0.05).

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Aleksandra DUKIĆ-OTT
Gent, 28th November 2007

CURRICULUM VITAE

Aleksandra DUKIĆ-OTT
Born on 08.12.1973 in Belgrade, Serbia
Serbian nationality
Married

Education

11/2003 – present	Doctoral studies, Laboratory of Pharmaceutical Technology, Gent University, Belgium (Thesis: Modified starch as an excipient for pellets prepared by means of extrusion/ spheronisation; Promoters: Prof. Dr. C. Vervaet and Prof. Dr. J. P. Remon)
10/2002 – 10/2003	Master in Pharmaceutical Sciences, Gent University, Belgium (Thesis: Inulin as filler-binder for tablets prepared by direct compression; Promoters: Prof. Dr. J. P. Remon and Prof. Dr. C. Vervaet)
10/1992 – 10/1998	Bachelor of Pharmacy, Belgrade University, Serbia (Thesis: Importance of particle size in pharmaceutical formulations and determination methods; Promoter: Prof. Dr. M. Primorac)

Work Experience

09/2001 – 10/2002	Head of department for production of syrups and solutions at HEMOFARM Group, Vršac, Serbia (since 2006 part of STADA Arzneimittel AG, Bad Vilbel, Germany)
10/1999 – 09/2001	Pharmacist in production facilities of solids, semi-solids, syrups and solutions at HEMOFARM Group, Vršac, Serbia
01/2000	Obtained the license as pharmacist, Belgrade, Serbia
10/1998 – 10/1999	Pharmacist in local pharmacy, Pančevo, Serbia
09/1998 – 02/1999	Teaching pharmacology at Medical High School, Pančevo, Serbia

Publications and Patents

2007

• A. Dukić-Ott et al., In-vivo and in-vitro evaluation of enteric-coated starch-based pellet formulations – *Submitted to Eur. J. Pharm. Biopharm.*

• A. Dukić-Ott et al., Immediate release of poorly soluble drugs form starch-based pellets prepared by extrusion/spheronisation, *Eur. J. Pharm. Biopharm. 67 (2007) 715-724*

• A. Dukić et al., Development of starch-based pellets via extrusion /spheronisation, *Eur. J. Pharm. Biopharm. 66 (2007) 83-94*

2006

•Use of debranched starch in extrusion-spheronisation pharmaceutical pellets *(Patent published as: EP1719503, US2006246192, JP2006312630)*

Oral and Poster Presentations (a selection)

2007

• A. Dukić-Ott et al., Influence of pellet core properties on drug release from enteric-coated starch-based pellets prepared by extrusion/spheronisation – *Poster presentation at AAPS Meeting and Exposition, San Diego, USA*

2006

• A. Dukić-Ott et al., Immediate release of poorly soluble drugs from starch-based pellets prepared by extrusion/spheronisation – *Poster presentation at AAPS Meeting and Exposition, San Antonio, USA*

• A. Dukić-Ott et al., Development of enteric coated starch-based pellets prepared by extrusion/spheronisation – *Poster presentation at AAPS Meeting and Exposition, San Antonio, USA*

• A. Dukić et al., Immediate release of poorly soluble drugs from starch-based pellets prepared by extrusion/spheronisation – *Oral presentation at Biopharmacy Day, Beerse, Belgium*

• A. Dukić et al., Modified starch as an excipient for pellets prepared by extrusion/spheronisation – *Poster presentation at 5th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Geneva, Switzerland*

2005

• A. Dukić et al., Influence of wet massing and re-extrusion on the quality of starch-based pellets produced by extrusion/spheronisation– *Poster presentation at AAPS Meeting and Exposition, Nashville, USA*

• A. Dukić et al., Modified starch as an excipient for pellets prepared by extrusion/spheronisation – *Poster presentation at AAPS Meeting and Exposition, Nashville, USA*

Workshops and Courses Attended

2007	• 3rd International Granulation Workshop, Sheffield, UK
	• Workshop no. 114: Fluid bed drying, granulating and coating, Technology Training Center, Binzen, Germany
2006	• Course in Statistics 2006-2007, Center for Statistics, Gent University, Belgium: Module 4 - Design and Analysis of Clinical Trials
	• Tablet tech Seminar: Solid dosage form manufacturing, FMC Biopolymer, Brussels-Genval, Belgium
	• 51st Pharma Polymers International EUDRAGIT® Workshop, Röhm Pharma Polymers, Darmstadt, Germany
2005	• Courses in Statistics 2005-2006, Center for Statistics, Gent University, Belgium: Module 3 - Introductory Statistics, Module 4 - Analysis of Variance
	• Differential Scanning Calorimetry (DSC) Training Course, TA Instruments, Etten-Leur, The Netherlands
	• Modulated®Differential Scanning Calorimetry (MDSC) Training Course, TA Instruments, Gent, Belgium
2004	• AnalySIS® Extended Basic Seminar LifeScience, Soft Imaging System, Münster, Germany
2003	• Tablet tech Seminar: Solid dosage form manufacturing, FMC Biopolymer, Brussels, Belgium
	• Workshop no.74: Pellets, Technology Training Center, Binzen, Germany

Grants and Travelships

2006	AAPS Travelship for AAPS Annual Meeting and Exposition, San Antonio, USA
2005	AAPS Travelship for AAPS Annual Meeting and Exposition, Nashville, USA
2003 – 2007	Doctoral grant (BOF), Gent University, Belgium
2002	FIP Fellowship for Master studies

Languages

Serbo-Croatian: mother-tongue

English: fluent

German: conversation level