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Comparative imaging of a slow-release starch excipient tablet: Evidence of membrane formation

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Abstract

A controlled release excipient made of chemically modified high-amylose starch (HAS) has been used to explore the surface membrane responsible for the slow release characteristics. Using scanning electron microscopy (SEM) and X-ray computed microtomography (CMT), the radial and axial surfaces of dry, swollen and freeze-dried sections observed were arranged in concentric domains. The presence of water enhances contrast in CMT imaging of the swollen tablet compared to the non-hydrated state. Freeze-dried SEM images showed a twin domain structure, thus defining membrane thickness. CMT quantified the average global porosity of the dry and swollen domains. The bird's eye view of the latter defined a central-core with high contrast and 34% porosity while the average global porosity was 19% and the dry porosity was 10%. The overall membrane after 15 min swelling is best described as concentric hydrogel domains.

Keywords: Slow-release; Controlled-release; Excipient; High amylose starch; Membrane; Scanning electron microscopy; Tomography; Hydrogel; Porosity; Imaging

1. Introduction

Controlled delivery of medication from pharmaceutical tablets improves efficiency by avoiding the "up and down" drug concentration peaks in the patient's blood resulting from multiple dose intakes. Various techniques have been used to study chemically modified high amylose starch (HAS) tablets, such as optical (Bussemer, Peppas, & Bodmeier, 2003; Moussa & Cartilier, 1996) and mechanical methods (Ravenelle, Marchessault, Legare, & Buschmann, 2002), FTIR (Dumoulin, Alex, Szabo, Cartilier, & Mateescu, 1998), X-ray diffraction (Dumoulin et al., 1998), cross-polarization magic angle spinning (CP/MAS) NMR (LeBail, Morin, & Marchessault, 1999; Shiftan, Ravenelle, Mateescu, & Marchessault, 2000) and NMR imaging (Baille, Malveau, Zhu, & Marchessault, 2002; Malveau, Baille, Zhu, & Marchessault, 2002; Therien-Aubin, Baille, Zhu, & Marchessault, 2005). However, all but the most recent work on NMR imaging (Therien-Aubin et al., 2005) has been performed on a different version HAS excipient. The objective of this study is to demonstrate how the commercial Contramid® (Lenaerts et al., 2003) excipient tablet interacts with water to organize itself into a skin-core structure. Previous work has demonstrated that the barrier skin controls outward diffusion to deliver most drugs according to a near zero-order release kinetics for about 20 h (Lenaerts et al., 1998). This work integrates information from several experimental methods to provide a more complete picture of membrane formation and how it supports drug delivery characteristics. Experimental data was derived from scanning electron microscopy (SEM) and X-ray computed microtomography (CMT). The latter is a novel method for imaging and texture analysis of wet and dry HAS excipients which swell to a limited size when put in contact with water.

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As mentioned above, HAS tablets have been previously described (Baille et al., 2002; Dumoulin et al., 1998; Lenaerts et al., 1998; Malveau et al., 2002; Moussa & Cartilier, 1996; Rahmouni, Lenaerts, & Leroux, 2003; Rahmouni et al., 2003; Rahmouni, Lenaerts, Massuelle, Doelker, & Leroux, 2002; Ravenelle et al., 2002) but a more recent process (Lenaerts et al., 2003) was used for the excipient investigated herein. All HAS tablets are noteworthy for retaining shape integrity during the drug release phase. In fact, after about an hour, the tablets have swelled to their final size which they will retain over the entire drug release phase. The trade name Contramid® should be associated with the HAS presented in this publication whose controlled release properties are opposite to a fast-release microcrystalline cellulose excipient such as Avicel™ which disintegrates in a matter of minutes when placed in water (Battista, 1975). Pioneering work by Moussa and Cartilier (Moussa & Cartilier, 1996), and collective data (Baille et al., 2002; Dumoulin et al., 1998; Lenaerts et al., 2003; Lenaerts et al., 1998; Malveau et al., 2002; Rahmouni et al., 2003; Rahmouni et al., 2003; Rahmouni et al., 2002; Ravenelle et al., 2002; Therien-Aubin et al., 2005) on swelling of pre-commercial HAS tablets, showed that tablet swelling is greater in the axial direction. The accepted explanation is that during tableting: porous, non-crystalline, spray-dried HAS particles are flattened axially and subsequent swelling response is to recover the near-spherical shape which results in predominent axial swelling.

Previous scanning electron microscopy (SEM) observations performed on freeze-dried swollen HAS tablets suggested the presence of an outer membrane or skin (Ravenelle et al., 2002). The role of a barrier membrane in controlled release excipients has been examined previously and is often referred to as a "reservoir system" where the outer gel layer acts as a semi-permeable membrane that slowly delivers the drug from the core (Bussemer et al., 2003). Moussa and Cartilier have also studied synthetic membranes by dry-coating tablets to mimic what happens in a HAS tablet upon swelling (Moussa & Cartilier, 1997). HAS tablets are swellable matrices for controlled drug delivery as reviewed by Colombo, Bettini, Santi, and Peppas (2000).

X-ray computed microtomography (CMT), a detection technique often used in medicine and material characterization, is a non-invasive method for visualizing the three-dimensional structure of various objects. Tomographic characterization was used to investigate compression effects due to tableting thus mapping density variations within a dry tablet excipient (Sinka, Burch, Tweed, & Cunningham, 2004). Similarly in this study CMT measures porosity in both the dry and the swollen state. As the sample is stepwise rotated about its axis on a turntable, sets of high definition two dimensional, 2D, images are recorded in order to reconstruct the three dimensional, 3D, structure of the analyzed tablet. The great advantage of this technique compared to a local 2D picture is access to the internal texture of the sample in terms of horizontal i.e. radial slices from which the internal porosity can be viewed and quantified.

In this regard, SEM and CMT are complementary techniques for the investigation of the morphological changes occurring in the tablet upon swelling. These techniques, capable of showing the membrane formation in HAS tablets, provide modeling information that complements and expands previous understanding. To our knowledge, this type of comparative image study of drug delivery dynamics involving a surface membrane has not been previously reported.

2. Experimental

2.1. Materials

All HAS tablets studied were prepared using modified high amylose starch material alone (provided by Labopharm, Inc., Laval, QC, Canada). The HAS was first cross-linked with 0.075% of phosphorus oxychloride in an alkaline medium, then functionalized with 6% of propylene oxide (i.e. hydroxypropylation) and finally gelatinized. The final product is a spray-dried powder with an average particle size of 80 μm (Lenaerts et al., 2003). ^{13}C CP/MAS and X-ray powder diffraction indicate that the spray-dried starting material is essentially non-crystalline (data not shown).

2.2. Tableting

This material was converted to a 25 mg miniature controlled release tablet (called pellet) using a cylindrical steel mold and a Carver laboratory press. The compression force was 6.8 ± 0.3 kN for 1 min. The final dimensions of each dry tablet were 3.2 mm in diameter and 2.1 mm in height. It was necessary to work with pellets since their small size can be entirely accommodated in the CMT X-ray chamber. These pellets were also imaged by SEM.

2.3. Scanning electron microscopy

2.3.1. Pellet treatment

The pellets were immersed 15 min in standing distilled water at room temperature. After a mild surface sponging, pellets were placed in a capped glass vial to avoid contact with liquid nitrogen during freezing. Frozen pellets were then freeze-dried for upto 24 h. Preliminary tests indicated that freeze-drying had no effect on the gross dimensions of the pellets. Radial and axial mid-cuts were performed on the freeze-dried pellets using a razor blade. Finally, the sectioned parts were deposited on SEM grids previously covered with double faced tape.

2.3.2. Imaging

Scanning electron microscopy was performed with a JEOL 840A instrument, with an EDAX Phoenix system

for image acquisition. Sample coating was performed using a Hummer VI sputter-coater with Au/Pd alloy target. SEM micrographs were recorded using an acceleration voltage of 10 kV secondary electrons.

2.4. X-ray computed microtomography

2.4.1. CMT procedure

Pellets were analyzed successively in the CMT instrument under three different conditions: the first one in the dry state, the second after being soaked in distilled water without stirring for 15 min and the third pellet was swollen 24 h at room temperature. Surface water was gently sponged from the swollen tablet prior to being placed in the CMT cell. Some pellets where freeze-dried after swelling.

2.4.2. CMT scans

Scans were performed on a standard desktop CMT instrument (Model 1072, SkyScan Inc., Aartselaar, Belgium) which has a 20–100 keV/0–250 μA sealed, air-cooled, microfocus X-ray source with a polychromatic beam derived from a tungsten target and a spot size of less than 5 μm at 4 W. For the analysis, the X-ray source was operated at 100 kV and at 98 μA (maximum power). Images were captured using a 12-bit, cooled CCD camera (1024×1024 pixels) coupled by a fiber optics taper to the scintillator. Samples were scanned at a magnification resulting in a pixel size of 10.94 μm, with a rotation step of 0.45 degree and an exposure time of 2240 ms for each step, the total scanning time was about 60 min.

2.4.3. CMT 3D-reconstruction

A set of CMT images of the pellet is necessary to get a 3D image which is obtained after reconstruction using Cone-Beam Reconstruction software (SkyScan, Inc.). The reconstruction parameter of the distance between each reconstructed cross-section is 10.94 μm and the elementary cubic volume (voxel) corresponds to a 10.94 μm edge cube.

2.4.4. CMT porosity measurements

Quantitative data such as the average porosity was derived from the computed X-ray microtomography results (CTan software, Skyscan Inc.).

3. Results and discussion

Previous SEM observations (Ravenelle et al., 2002) made on a freeze-dried 24 h swollen HAS tablet provided evidence of a skin-core texture with the surface-membrane defined by a small pore texture. Shorter swelling times were chosen in the present study since a membrane in controlled-release excipients has to rapidly control water permeability and bioactive agent release (Colombo et al., 2000), beginning in the first minutes of drug delivery. In this study imaging techniques were used to collect structural information on this surrounding skin. Furthermore, solid state NMR has previously shown that organization of starch into double helices in HAS tablets is happening in the first 5–15 min of water absorption by the tablet (Le Bail et al., 1999; Shiftan et al., 2000). HAS tablets are characterized by a swelling anisotropy (Moussa & Cartilier, 1996; Moussa & Cartilier, 1997; Ravenelle et al., 2002) which is measured by the axial/radial ratio of the pellet before and after swelling. Axial and/or radial viewing of the water imbibing tablet shows rapid development of a translucent envelope (Moussa & Cartilier, 1996) which freeze-drying converts to the microporous state. This is the beginning of the membrane structure development.

3.1. Scanning electron microscopy

Fig. 1a is a bird's eye view micrograph of the freeze-dried (15-min swollen) interior surface of a HAS pellet cut at mid-point of the thickness parallel to the tablet's face. The defined pellet edge at the mold/excipient interface suggests a complex membranous texture. Fig. 1b clearly shows three defined concentric domains inside the "whitish" outer capping shells. From right to left in Fig. 1b the three defined domains inside the whitish shells are: a honeycomb porous texture about 200 micrometer (μm) thick, and a smooth layer about 100 μm thick surrounding the core area. Fig. 1c depicts parts of the three defined domains described in micrograph 1b. Fig. 1d is magnified core which constitutes the reservoir of non-coalesced granules, as previously evidenced by NMR Imaging (Therien-Aubin et al., 2005). Compared to the proposed skin-core micrograph (Ravenelle et al., 2002) this series of images shows the sequential concentric domain created by the early water penetration and revealed in the freeze-dried tablet. Freeze-drying that prevents the surface and internal texture from collapsing due to capillary drying stresses is a time-lapse method that captures and preserves the instantaneous texture and progress of the waterfront into the tablet.

Fig. 2a is a top view of the interior surface of a dry HAS pellet and is similar to Fig. 1a except for the absence of the porous and smooth concentric layers (pseudo-membrane) which were created by the water swelling and freeze-drying treatment. The similarity of Fig. 1d and 2b strongly suggests that rather little water reaches the core during the initial 15 min of swelling. Thus, Fig. 2 is a control on the SEM images that are shown in Fig. 1a and b, where the twin concentric domains surround the slow water-penetrated "reservoir" which we call the core.

Fig. 3 is a sequence of three SEM freeze-dried radial sections which show the effect of swelling time. The X-shape cavity (Fig. 3c – 2 h 30 min) is attributed to non-uniform stresses as the core becomes more coalesced by water accumulation. However the external layers maintain their similar appearance for all three samples. Damage to the membrane caused by razor blade cuts are not representative of the pellet.

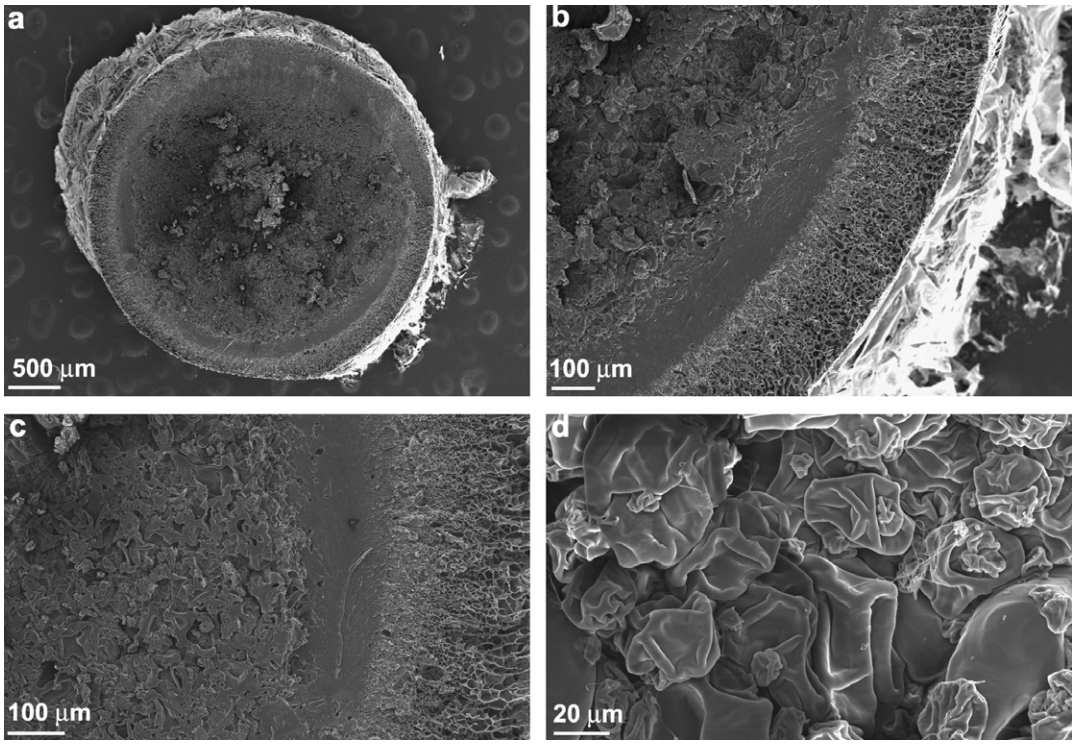

Fig. 1. SEM top view micrographs of freeze-dried radial sections of the 15-min swollen HAS pellet. The section was cut parallel to the tablet surface at mid-point of the thickness. (a) Global view of the freeze-dried pellet; (b) four concentric domains are seen from right to left: a white capping shell, a microporous surrounding layer, a smooth surrounding layer, and the core; (c) zoom in of the biphasic membrane consisting of the porous and the smooth domains; (d) close view of HAS granules.

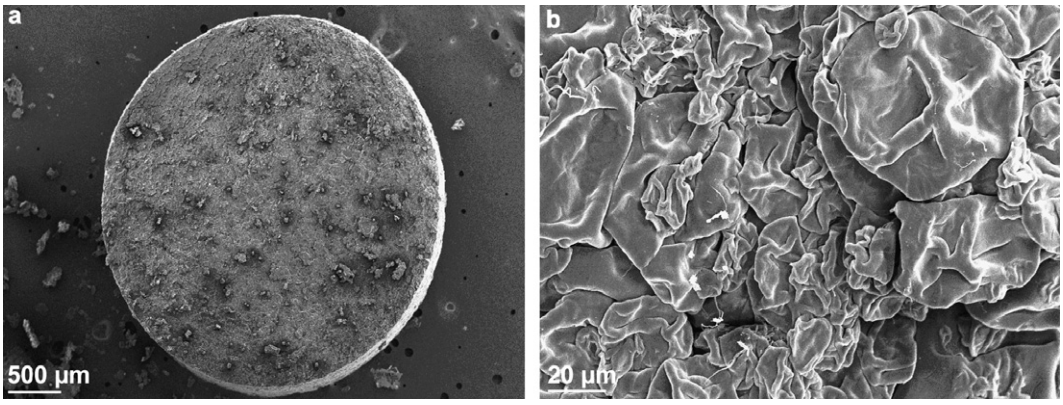

Fig. 2. SEM top view micrographs of radial sections of the dry HAS pellet. The section was cut parallel to the tablet surface at mid-point of the thickness. (a) Global view of the dry pellet; (b) close view of compressed dry HAS granules.

3.2. Tomographic texture

Variation in the internal texture can be observed by CMT in the reconstructed radial cross-sections at different heights in the tablet. Typical radial sections at the top, middle and bottom parts of the dry and swollen pellets are depicted in Fig. 4. From top to bottom the three sections of the dry HAS pellet appear relatively homogeneous and dense (Fig. 4a–c). Contrary to the dry tablet, the three sections of the swollen tablet (Fig. 4d–f) exhibit on average two concentric textures from top to bottom. These three images show a similar core which we describe as ''cross-hatched'' with more contrast over the larger area in the middle (Fig. 4e). This central cross-hatched texture is attributed to random fold lines in the granules (Fig. 1d). Fig. 4e includes the two concentric domains considered to be defined membrane, i.e. porous and smooth domains, and core. The skin cannot be distinguished as the porous and smooth layers since the pellet is swollen, resulting in a lack of contrast between these two domains. The top and bottom parts (Fig. 4d and f) of the wet tablet exhibit similar but less extensive crosshatching than in Fig. 4e. This is due to the geometry of the tablet where the circular ''arêtes'' at the top and bottom of the pellet undergo a

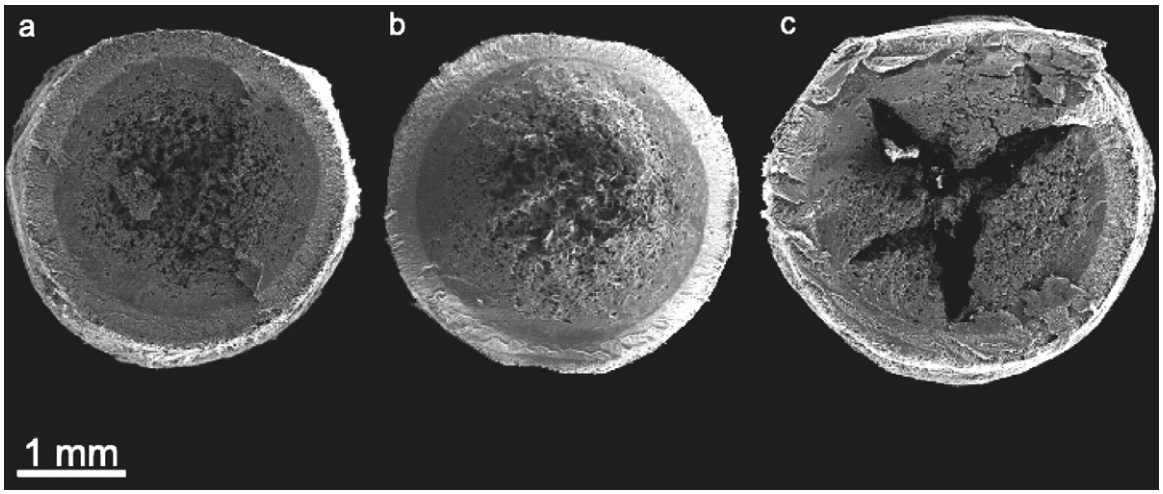

Fig. 3. SEM images of radial cross-sections of freeze-dried swollen HAS pellets: (a) 15 min swelling; (b) 1 h swelling; (c) 2 h 30 min swelling. The porous layer can be observed on the three radial sections (a–c). X-shape cavity in the internal texture is evidenced in sample (c).

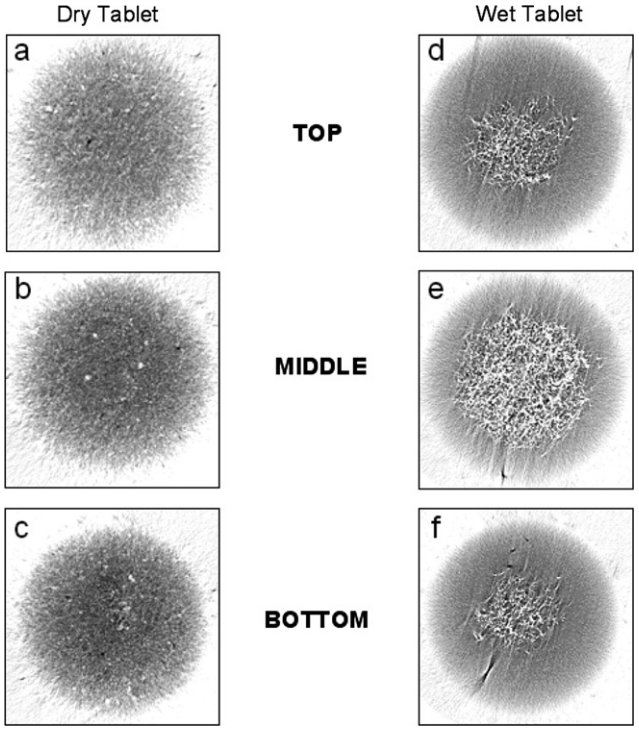

Fig. 4. CMT radial cross-sections of the dry (a–c) and 24 h swollen (d–f) HAS pellets. Top layers (a,d); middle layers (b,e); bottom layers (c,f). Diameter of the dry pellet is 3.2 mm, and 3.9 mm for the wet pellet.

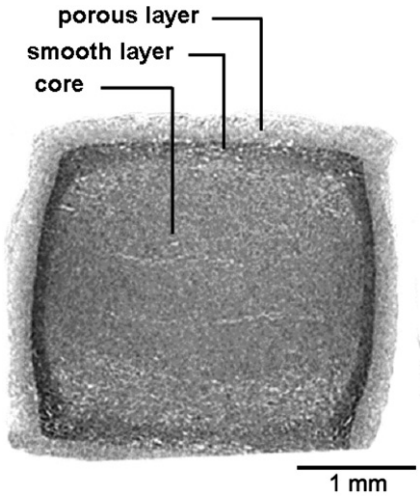

Fig. 5. CMT axial cross sections of the freeze-dried 15-min swollen HAS pellet. Pellet size is 3.0 mm in height and 3.3 mm in width. Membrane thickness is 300 μm on the right and left edges (at mid height) and 450 μm at the top and bottom sides of the pellet. The smooth layer ranges from 130 μm on the right and left edges to 200 μm on the top and bottom sides.

greater water exposure in the first instance of swelling since water can diffuse radially and axially, thus increasing the membrane thickness (Fig. 5). This supports the membrane formation mechanism where the thickness is influenced by exposure to water. Since the top and bottom pellet surfaces must have similar dual texture as the side edges, Fig. 5 was recorded to show this. The smooth layer is denser on the side edges than on the top and bottom surfaces. This is certainly due to the HAS granules intrinsic feature since they have the propensity to swell more in the compression axis direction, i.e. axial, than in the radial direction. Consequently, the smooth layer appears denser on the side edges because the granule does not swell as much along this direction. NMRI kinetics experiments evidenced this duality on the asymmetry of the tablet by focusing on the water diffusion coefficients where the radial diffusion coefficient was found to be less important than along the axial direction (Therien-Aubin et al., 2005). The greater thickness of the smooth layer at the top and bottom edges of the HAS tablets suggest a lower starch density that would favor water diffusion in this direction.

The apparent membrane thickness in the swollen pellet ranges between 750 and 850 μm in Fig. 4e, comparable to observations by NMR imaging on pharmaceutical sized tablets (Therien-Aubin et al., 2005).

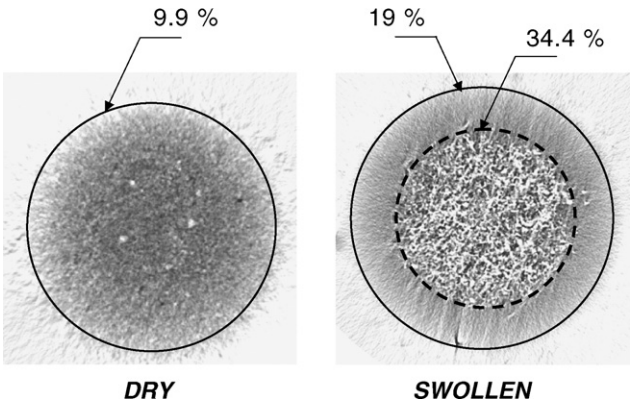

Fig. 6. Porosity in the dry (left) and swollen (right) pellets in their middle sections obtained by CMT porosity analysis. The outer circle represents the global density of the pellet and the inner dashed-circle corresponds to the tablet without the skin membrane.

Quantitative data such as the average porosity was derived from the computed X-ray microtomography results. By focusing on the middle slice of both the dry and swollen pellets (Fig. 4b and e), average porosity can be calculated based on defining the uniform grey external ring as membrane and the cross-hatched center as core. The average porosity in the whole circular cross-section of the pellet is calculated to be ca. 10% for the dry pellet and ca. 19% for the swollen pellet (Fig. 6). If the skin zone is discarded from the calculation, porosity of the core of the wet tablet is ca. 34% showing that the membrane is denser than the core, as suggested by SEM observations (Fig. 1d).

4. Conclusions

As water quenches the pellet surface, a translucent envelope surrounds the pellet while capillary forces slowly drive water into the core. The apparent membrane, formed by the rapid quenching, develops into a hydrogel of decreasing water concentration, as shown by Moussa and Cartilier (Moussa & Cartilier, 1996). Water movement becomes progressively diffusion controlled as starch retrogrades, i.e. self-assembles into double helices due to the propensity of starch to crystallize in this conformation (Le Bail et al., 1999; Lenaerts et al., 1998; Ravenelle & Rahmouni, 2006). This reorganization introduces pseudo-crosslinks and rigidifies the hydrogel texture.

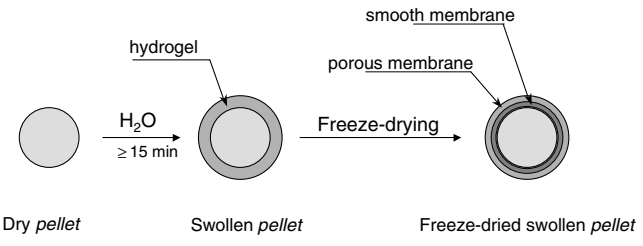

Fig. 7. Schematic of the dimensional and textural changes occurring in the HAS pellet during swelling and freeze-drying.

The combined porous and smooth domains in Fig. 1b can be considered as the membrane which NMR Imaging has shown to be a dimensionally stable element of the swollen tablet (Therien-Aubin et al., 2005). The idealized schematic shown in Fig. 7 summarizes the successive steps that create the membrane which is considered as an important feature in controlled release by HAS tablets.

The present study proposes how the combined effects of water quenching, capillary action and diffusion build the twin layer membrane revealed by our SEM results. This follows from comparing the dry HAS radial section, Fig. 2a, with the swollen freeze-dried section, Fig. 1b.

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