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Mechanical properties and structure of swollen crosslinked high amylose starch tablets

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Abstract

Crosslinked high amylose starch (CLHAS) has been introduced as an excipient for controlled oral drug delivery. The kinetics of delivery is governed by the diffusion of the medication through a hydrogel matrix, which forms as water penetrates the CLHAS tablet. The formation of the gel from the compressed spray-dried starch powder comes from pseudo-crosslinking of particles by the self-assembly of amylose and amylopectin segments into double helices. Tablets reach equilibrium swelling after more than 24 h and contain about 200% water. They demonstrated nearly reversible non-linear viscoelastic properties. Under compression, water flows out and vice-versa for decompression suggesting a sponge-like behaviour. A series of tablets fabricated under increasing compression force in the dry state showed a decrease of wet equilibrium stiffness with increasing fabrication pressure. Scanning electron microscopy on freeze-dried tablets reveals a fine porous texture at the surface of the tablets, which forms, in the first minutes of water penetration. On the other hand the internal texture which develops over longer periods has much larger pores which account for the high equilibrium water content and the observed sponge-like behaviour.

Keywords: Crosslinked high amylose starch; Swelling; Stress-relaxation measurements; Mechanical tester; Hydrogel; pores; self assembly; Hylon VII

1. Introduction

Starch is a natural semi-crystalline polymer composed of two different polysaccharides: amylose ($M_w \approx 500\,000$), a linear α-(1,4) glucan and amylopectin ($M_w \approx 50–100 \times 10^6$), a dendritically branched version. The respective proportion of each in industrially used starch is usually ca. 30% amylose–70% amylopectin. However, the high amylose starch mentioned throughout this paper is a hybrid containing ca. 70% amylose–30% amylopectin. For both macromolecules, there exists two helical polymorphs: the native A and B type arrangements are double helices made of two linear strands of amylose and/or amylopectin. The difference between the A and B organization is in the unit cell hydration of the crystalline structure (Wu & Sarko, 1978a; Wu & Sarko, 1978b). Starch is also known to complex with molecules like DMSO, acetone, alcohols and lipids to form a V-type helix, where a single strand of amylose or amylopectin organizes into a 6-fold right handed helix as described by Buleon in a recent review (Buleon, Colonna, Planchot & Ball, 1998). The crosslinked high amylose starch commercialized under the trademark Contramid™ is a disordered V polymorph (Le Bail, Morin & Marchessault, 1999; Szabo, Ravenelle, Hassan, Preda & Mateescu, 2000) after spray drying (Guilbot & Mercier, 1988). Contramid™ powder blended with a pharmaceutical agent and then compressed into a tablet provides a slow-release drug delivery system (Fig. 1). This is due to slow water penetration that induces development of pseudo crosslinks as neighbouring chain segments wind into double helices leading to a continuous porous matrix that exhibits equilibrium swelling.

At room temperature a Contramid™ tablet will typically swell in water over a period of days to eventually reach an equilibrium thickness and width. These dimensions increase by approximately 70–80% and 25–35%, respectively compared to the dry state. During the process of swelling, the tablet takes-up more than 200% of water (by weight). The anisotropic swelling is due to the microporous nature of the spray-dried powder, which flattens during fabrication of the tablet. Upon water penetration, particles expand preferentially in the direction opposed to the fabrication pressure, i.e. in the axial or thickness direction of the tablet. When a

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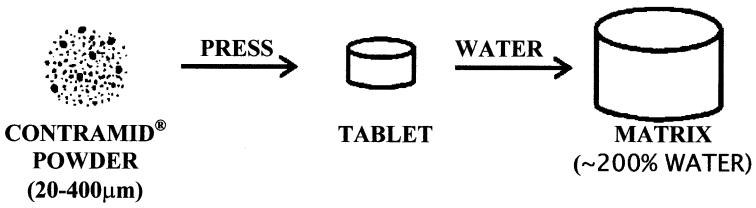

Fig. 1. Schematic representation of Contramid™ tablet fabrication and water swelling.

tablet of CLHAS is placed in water it immediately starts to swell and a radial water gradient can be detected (Moussa & Cartilier, 1996) which dissipates with time. The effect of swelling was examined by scanning electron microscopy (Le Bail et al., 1999) and it was shown that a membrane is formed at the exterior of the tablet that differs from the interior texture in the size of its pores. By following the weight and dimensions of the swollen tablets with time, we were able to observe that it takes up to 4 days of swelling at 25°C to reach an apparent equilibrium. At that point, the tablets start to decrease in weight due to diffusion of small particles out of the tablet. The particulate weight loss is observable macroscopically when the supernatant water in which the tablet was immersed is examined. The degree of swelling increases with temperature in keeping with the well known properties of gelatinized starch (Buleon et al., 1998).

2. Materials and methods

2.1. Fabrication of the tablets

The materials and methods used to fabricate the CLHAS tablets are described in a US patent (Mateescu, Lenaerts & Dumoulin, 1991; Dumoulin, Clement, Mateescu & Cartilier, 1994). Typically, Hylon VII from National Starch Inc. (70% amylose) is gelatinized in 1% NaOH solution at 50°C prior to being crosslinked with sodium tri-metaphosphate (STMP) (Le Bail et al., 1999). The degree of crosslinking is the amount of crosslinking agent added relative to the dry starch expressed as %w/w. The samples studied here are crosslinked in aqueous sodium hydroxide at 3.25% i.e. 3.25 g. STMP/100 g. Hylon VII. After washing, the powder obtained by spray drying is further agglomerated in an H_2O vapour fluidized bed to give a gross particle size of about 400 μm, which is then pressed into tablets using commercial equipment. The material and tablets used in this study were obtained as lot #336 through the courtesy of Labopharm Inc, Blvd Chomedey, Laval, Québec, Canada, H7V 3Z3.

2.2. Swelling of the tablets

Before mechanical testing, the tablet is swollen for 96 ± 3 h at 37°C followed by a 48 h room temperature wait. However, the samples presented in Fig. 5 were swollen at room temperature for a period of 72 ± 3 h. After such a period, a satisfactory equilibrium swelling was attained according to previously investigated mechanical properties as a function of time. Under these conditions, the CLHAS hydrogel tablets are in a satisfactory equilibrium state for stress-relaxation measurements as explained below. Dimensions (diameter, thickness and weight) of the tablets are recorded before and after observations, i.e. swelling, stress-relaxation measurements, solvent changes, etc. The measurement of the mechanical properties of swollen tablets was done in two testing solutions: distilled water and phosphate buffered saline solution (PBS). The PBS solution is composed of 148 mM NaCl, 2.5 mM KCl, 0.8 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2{\cdot}(6H_2O)$.

2.3. Hardness of the tablets

The hardness of the dry tablets was measured using a compressive force up to the point where the tablet would collapse. The peak force needed to break the tablet is referred to as the hardness.

2.4. Mechanical properties of the swollen tablets

Force/temperature curves of the hydrogel tablets have been studied and these are predominantly elastic in behaviour. However, natural polysaccharide gels are usually partly crystalline, viscoelastic and non-reversible as regards their stress–strain behaviour. Mechanical properties of Contramid™ tablets were studied using a mechanical testing system, the Mach-1™ (Biosyntech Inc., Montreal, Quebec). This mechanical testing system consists of an actuator for precise control of displacement (25 nm resolution) and a load cell for measurement of the load (10 mg resolution)

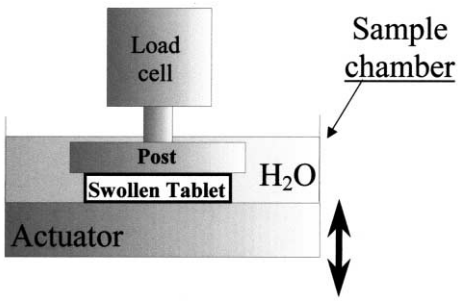

Fig. 2. Schematic representation of the Mach-1™ rheology instrument.

during compression of the sample (Fig. 2). The Mach-1 has been extensively used for the mechanical characterization of biomaterials and biological tissues (Dumont, Ionescu, Reiner, Poole, Tran-Khanh & Hoemann, 1999; Garon, Légaré, Guardo, Savard & Buschmann, 2001; Légaré et al., 1998; Thibault et al., 2000)

Prior to testing, the diameter and thickness of swollen tablets are measured using a current-sensing micrometer. The swollen tablet is then placed on the base of a testing chamber mounted on the actuator platform of the Mach-1 (Fig. 2). The testing chamber was designed for uniaxial unconfined compression, thus permitting the expansion of the swollen tablet in the radial direction and the expulsion or absorption of water during compression. The contact between the compression post fixed to the load cell and the surface of the tablet is precisely controlled by the displacement of the actuator until a load criteria of 0.3 g is reached. The testing chamber is then filled with the testing solution and the position and the load are set to zero.

Because of the biconcave shape of the swollen tablets a first slow compression (0.2 μm/s) accounting for the heterogeneity of the thickness (5–8% of the thickness depending on the sample) is applied to compress the tablet into a regular geometric cylinder. This first step corresponds to the difference between the highest and the lowest thickness of the sample measured prior to the test with the current-sensing micrometer. This first step (few hundreds of microns displacement) is applied slowly in order to minimize disruption from the existing equilibrium and to avoid damaging the texture of the tablet. After the first step, four compression ramps of an amplitude corresponding to 1% of the tablet thickness are applied. A fixed relaxation time is allowed after each compression ramp until equilibrium is reached.

3. Results and discussion

3.1. Viscoelastic behaviour of the swollen tablets

In this paper, rheology represents the study of flow of a liquid through a porous media under compression. For a uniaxial unconfined compression, assuming an elastic behaviour and a uniform strain, the stress (σ) and the deformation (ϵ) are expressed as

$$\sigma = \frac{F}{A}, \qquad \epsilon = \frac{\Delta L}{L}, \tag{1}$$

where F is the force measured by the load cell, A the area or surface of the tablet, L its initial thickness and ΔL represents the amplitude of the compression. The modulus, or intrinsic stiffness, is equal to the ratio of the equilibrium strain over the deformation as

$$E = \frac{\sigma}{\epsilon}. \tag{2}$$

Fig. 3 shows typical stress-relaxation curves obtained for a swollen Contramid™ tablet with a thickness of 5.2 mm, a diameter of 11.5 mm and a powder fabrication pressure 6.3 kN. This tablet with a hardness of 175 N was tested after 72 h of swelling in distilled water. The latter curves demonstrate a Maxwell type behaviour for the material. For ease of comprehension, Fig. 3 is discussed in terms of: (A) the amplitude of the step compression of 1%, (B) the peak stiffness followed by the relaxation (between B and C) and (C) the equilibrium stiffness. Fig. 3 records four compression ramps of 1% followed by four decompression ramps of 1%. Those four compression ramps were applied after a first low velocity step to account for the thickness heterogeneity. For each compression ramp, the load rapidly increases to a maximal value corresponding to the maximal deformation and then relaxes to an equilibrium value. During decompression the procedure is similar but opposite with a rapid decrease of the load to a minimal value followed by an increase of the load with time to reach equilibrium. This sequence of compression and decompression can be repeated to study reversibility.

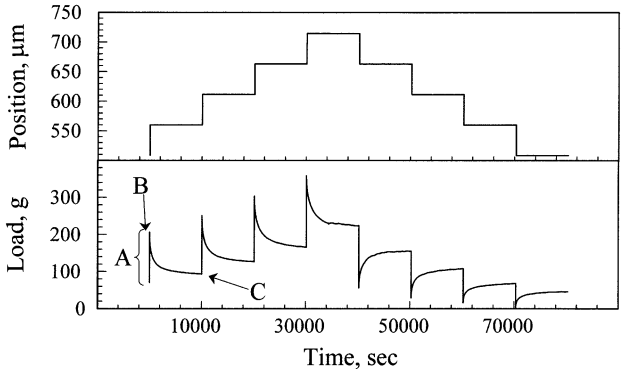

Fig. 3. Stress-relaxation curve corresponding to a Maxwell model obtained upon the application of a sequence of four 1% compression steps followed by four 1% decompression steps and finally by another four 1% compression steps.

3.2. Reversibility and linearity of the mechanical properties of swollen tablets

Stress-relaxation curves provide information on the reversibility of the mechanical properties of the material. Fig. 4 shows the variation of the equilibrium stiffness with the sample deformation for a tablet with a thickness of 4.5 mm, a diameter of 7 mm and a powder fabrication pressure of 10–12 kN. This tablet with a hardness of 123 N was tested after 94 h of swelling in distilled water. During this test, a sequence of four compression and four decompression ramps of 1% followed by a second sequence of four compression ramps of 1% were applied to the swollen tablet to study the reversibility of the mechanical properties. We clearly see that there is hysteresis behaviour, i.e. the material is modified irreversibly during the compressions. This provokes a reduction of the stiffness of the swollen tablet. This could be explained by a textural rearrangement taking effect during compression. Still, these results show

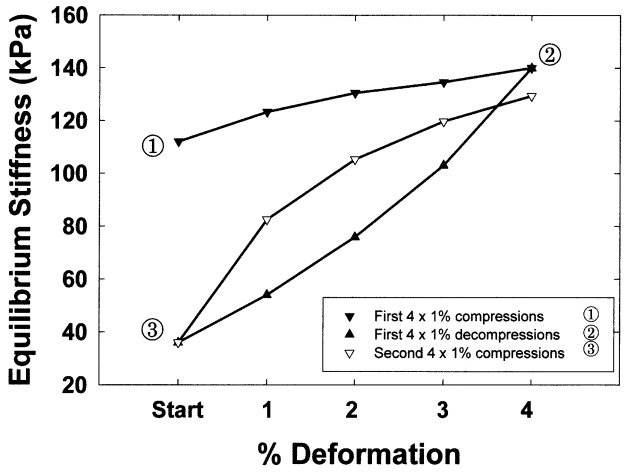

Fig. 4. Hysteresis manifested during compression–decompression–compression sequence.

that the system demonstrates a good capacity to deform and recover and can be characterized as a quasi-reversible system.

Stress-relaxation curves also provide information about the linearity of the mechanical properties of swollen tablets. For the viscoelastic properties to be linear, the equilibrium stiffness should be independent of the deformation. Figs. 3–5 show that the equilibrium stiffness is non-linear. During compression, the equilibrium stiffness increases slightly as the deformation increases from one step to the other. During decompression, the non-linearity tends to be greater.

A key feature about CLHAS is that one only needs to press the dry powder into a tablet and as water penetrates radially, a self-assembly phenomenon creates this semi-elastic gel.

The self-assembly phenomenon refers to a physical mechanism that links the powder particles together. This mechanism is the development of pseudo-crosslinks between the powder particles due to the tendency of amylose chains in adjacent particles to wind into double helices, which induces particle coalescence. The same pseudo-crosslinking must occur inside the particles, albeit at a slower rate. Solid-state NMR studies on wet Contramid™ powder and swollen tablets provided convincing evidence for the formation of the amylose double helix (B polymorph) (Shiftan, Ravenelle, Mateescu & Marchessault, 2000). It has been demonstrated that a low degree of order is put in place in Contramid™ powder by the process of chemical crosslinking and spray-drying (Le Bail et al., 1999; Szabo et al., 2000). In order for the tablet to swell uniformly and allow physical crosslinks to hold shape and functional properties of the swollen tablet, the chemical crosslinking and spray-drying process must produce a uniform distribution of amylopectin and amylose with a major non-crystalline component. The latter provides the mobile chains in the matrix material where the physical crosslinks develop during water penetration. The heterogeneity of particle coalescence inside the wet tablet results in a microporous interior that is best described as sponge-like. Clearly, the fabrication pressure of the dry tablet, which controls dry bulk density (≈1.1 g/cc) also has an important influence on mechanical properties.

3.3. Effect of the process variables for tableting on the mechanical properties

Previous studies have demonstrated how the chemical variables such as degree of crosslinking and solubility of the crosslinking agent influence the drug release properties of Contramid™ (Lenaerts, Moussa, Dumoulin, Mebsout, Chouinard & Szabo, 1998; Sourty & Marchessault, 1998). In this section we use the important tableting force variable to demonstrate how particle packing in the dry state influences self-assembly, i.e. hydrogel properties. The viscoelastic properties of a series of tablets fabricated with compression forces ranging from 6.3 to 13.6 kN were investigated. Fig. 5 shows the equilibrium stiffness measured during four compression ramps of 1% amplitude. First, we observed that the non-linearity of the equilibrium stiffness increases with the deformation. We also observe that the equilibrium stiffness of the swollen tablet decreases with increasing fabrication pressure for the dry tablet. The reasons for this behaviour are thought to be related to the increasing homogeneity of he matrix under increasing fabrication pressure. It was earlier shown that the effect of the compression force had no noticeable effect on drug delivery properties (Lenaerts, Dumoulin, Cartilier & Mateescu, 1992), at least in the range where the tablet swells uniformly, but had a significant effect on the hardness of dry tablets (Szabo et al., 2000). It was stated that an increase in compression force increases the hardness of the tablet up to a certain limit where the hardness would start decreasing again. The inverse behaviour of the dry tablet hardness and equilibrium stiffness of the swollen tablet is shown in Fig. 6. Since equilibrium stiffness decreases with compression force used to make the tablet, this can be explained by

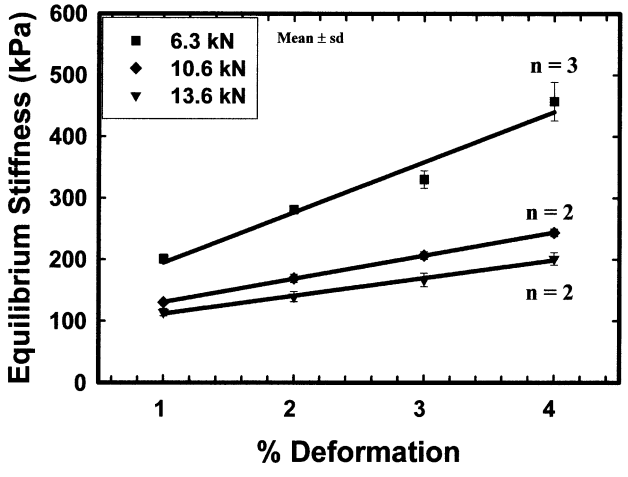

Fig. 5. Increase in stiffness with a decreasing compression force at fabrication of the dry tablet.

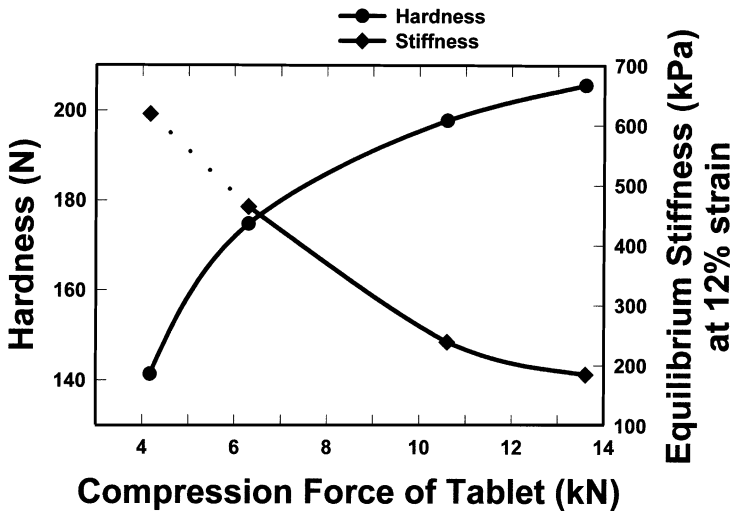

Fig. 6. Variation of hardness and equilibrium stiffness as a function of compression forces used to fabricate the tablets. Dotted line represents an estimated value from results obtained without satisfying equilibrium stiffness conditions.

analogy with the role of solid fillers in composites, e.g. gravel in cement or carbon black in rubber reinforcement. Hardness increases thanks to a heterogeneous matrix. Thus, a smaller pressure upon fabrication from the dry powder leaves the tablet in a heterogeneous state. As the tablet swells in water, this heterogeneity is maintained in the sense that hard particles are linked to a soft matrix. As the fabrication pressure is increased, the swollen tablet loses the features just mentioned, becoming a more homogeneous matrix and the equilibrium stiffness decreases. This is true up to a certain pressure (13.6 kN) where the CLHAS granules are crushed under too great a fabrication pressure. At this pressure the self-assembly process creates a soft unfilled starch gel. Even more, the tablets lose cohesion in the thickness direction along the centre and the tablet breaks-up. As explained earlier, they are thus worthless for drug delivery and/or for stress-relaxation measurements like the ones performed here. The inverse mechanical behaviour of the dry tablet hardness and equilibrium stiffness of the swollen tablet demonstrates the difference in the forces holding the particles together in the dry and wet systems: van der Waals forces and H-bonds in one case and physical hydrated double helix interactions in the other.

3.4. Texture of swollen tablet

The viscoelastic behaviour of the CLHAS swollen matrix has been demonstrated. To relate this behaviour to the fine structure of the swollen tablet a study of the material texture using scanning electron microscopy (SEM) was attempted. To investigate the swollen tablets, freeze-drying was used to prevent the surface and internal texture from collapsing due to capillary drying stresses. Fig. 7A shows a 3D view of a freeze-dried swollen tablet, i.e. a section parallel to the tablet surface at mid-point of the thickness. This view distinguishes between the interior (large pores) and the cylindrical periphery (smaller pores). From this micrograph it is clear that a skin structure less than a hundred microns thick, envelops the tablet. Observations on freshly wetted tablets show that it is formed during the first minutes of water penetration and its role in the overall kinetics of drug delivery is still not understood. The material response to pressure applied at the surface of the tablet during tablet fabrication is different from that in the interior and thus creates a different texture. The observed difference between the swollen outer layer and the inner texture is the average size of the pores. However the pore sizes observed from SEM are not necessarily the exact pore size of the material which controls the hydraulic flow since resolution of SEM and artifacts due to freeze-drying are possible. Nevertheless, the respective sizes are representative of the honeycomb texture in the swollen state. These pores should be considered as low-density domains in the matrix rather than tunnels or canals but the domains are interconnected. Fig. 7B shows that the denser periphery is composed of pores of 1–10 μ while Fig. 7C shows that the inner structure is composed of pores ranging from 10 to hundreds of microns. These observations, combined with the observed equilibrium swelling and the near-elastic behaviour under strain, suggest a self-assembly reticulation of particles producing coalescence to form a sponge-like material during water penetration as model. The observed polymorphic changes on wetting (Le Bail et al., 1999; Szabo et al., 2000) indicate that the formation of the double helix crystal structure of starch is activated on wetting of the tablet. It has been proposed that this ‘Velcro’ effect between amylose chains is responsible for the self-assembly (Le Bail et al., 1999; Lenaerts et al., 1998).

3.5. Determination of hydraulic permeability and average pore size

Stress-relaxation curves from mechanical tests can be analysed to obtain structural information concerning the

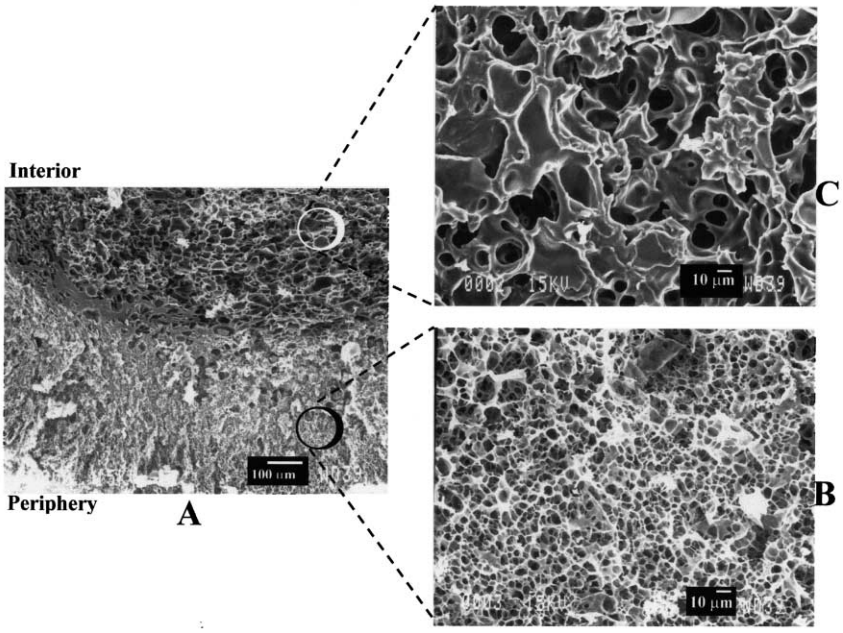

Fig. 7. Scanning electron microscopy of swollen tablets: (A) transverse section and periphery of freeze-dried CLHAS tablet; (B) membranous periphery (small pores); (C) interior section of tablet (larger pores).

swollen tablets. Using a fibril-network reinforced poroelastic model developed for tissue mechanics (Li, Soulhat, Buschmann & Shirazi-Adl, 1999; Soulhat, Buschmann & Shirazi-Adl, 1999), the stress-relaxation curves can be processed to obtain several theoretical parameters including the hydraulic permeability. This poroelastic model was developed for biological tissues including cartilage. Since CLHAS tablets demonstrated similar rheological behaviour as the cartilage, the model was applied to the stress-relaxation curves presented in the previous sections.

The hydraulic permeability (k) is directly related to pore size and thus drug transport of swollen tablets. The pore size of the matrix can be estimated using the permeability and the Navier–Stokes equation. For this particular case, the Navier–Stokes equation is reduced to

$$\mu\nabla^2 v = \nabla P, \tag{3}$$

where v is the fluid velocity, P the pressure and μ the viscosity. The pressure gradient is directly related to the fluid velocity by the permeability (Darcy's Law)

$$v = k\nabla P. \tag{4}$$

Using a dimensional approximation

$$\nabla = \frac{1}{l}, \tag{5}$$

the pore size can be estimated to be

$$l = \sqrt{(k\mu)}. \tag{6}$$

The hydraulic permeability was estimated for tablets swollen in distilled water and in a PBS. Tablets were first allowed to swell to equilibrium in distilled water then a sequence of two compression ramps of an amplitude corresponding to 1% of the thickness was applied to the tablets. The tablets were then placed in PBS until a new equilibrium was reached (at least 24 h) and a second sequence of two compression ramps of 1% amplitude was applied to the tablets. It was observed that the swelling of the tablets was decreased by around 7% in thickness and 5% in width due to the ionic force of the solvent. Stress-relaxation curves measured during the two ramp compressions of 1% were analysed with the model to obtain the permeability. Using Eq. (6) and the hydraulic permeability, an approximate pore size for the swollen CLHAS matrix was calculated. An average pore size of about 1.6 nm in distilled water with a decrease of 16% to about 1.3 nm in PBS was found. The difference in the size of the pores is significant. More interestingly, the average pore sizes calculated are of the order of magnitude of that observed for the water-swollen cellophane membranes calculated from permeability measurements (Kuzmak, 1953). This suggests that a continuous membrane structure is the matrix system wherein the fine pores resides. Nevertheless, these values do not correlate with the large pores observed in the SEM pictures (see Fig. 7).

4. Conclusions

CLHS swells in water to form a sponge-like material with non-linear viscoelastic properties. With successive compression–decompression–compression sequences the viscoelastic properties demonstrate non-reversibility, thus suggesting that the matrix is never in a totally stable

equilibrium. Physical stress forces rearrangements in the matrix itself since the pseudo-crosslinks are non-covalent. The stress-relaxation curve for compressions of a few percent shows Maxwell behaviour, i.e. elasticity and viscosity of the matrix are joint responses to the applied compression. The latter expels water from the hydrogel as in flow through a porous bed of linked particles.

The equilibrium stiffness of a swollen tablet as a function of compression decreased as dry fabrication pressure increased. This was interpreted in terms of a reinforced matrix where the size of the filler particles and their content decreases as the compression variable for dry tableting increases. This is tantamount to saying that the overall homogeneity of the microporous gel is greatest for the most strongly compressed tablet for a given Contramid™ starting powder. As mentioned above, the drug release kinetics are not greatly affected by this tableting variant (Lenaerts et al., 1992) and the practical interest is to use this variant in order to obtain a tablet with the desired dry hardness as well as cohesion at the swollen equilibrium.

The Mach-1™ mechanical tester has potential for monitoring the reversibility and linearity of the mechanical properties of swollen tablets. The influence of tableting process variables on mechanical properties can also be studied. For the crosslinked high amylose starch: degree of crosslinking, extent of particle aggregation, tablet thickness, etc. are manufacturing variables which can affect drug release. The viscoelastic properties of the swollen state can provide information for modelling drug release with respect to the variables.

The heterogeneity of the internal structure revealed in the scanning electron micrographs of the freeze-dried tablets is noteworthy. Water swollen Contramid™ is not at all like a molecular level hydrogel with molecular tetrafunctional crosslinks. Rather, it is like a sponge with average pore size of about 30–40 μm but with a macroscopic skin-core distribution of these large pores. By fitting the hydrogel observed data with a fibril-network reinforced poroelastic model developed for tissue mechanics, we calculated an average pore size three orders of magnitude smaller than the large pores. This suggests that the swollen matrix contains micropores, which collapse into higher density regions of the matrix due to freeze-drying or were beyond the resolution of SEM. These high-density regions being the observed texture on the SEM micrographs. Embedding hydrogels and starches is a good technique to 'freeze' the texture of a system in the swollen state in order to cut thin slices observable by TEM (Helbert & Chanzy, 1996). Such TEM observation of thin sections of swollen Contramid™ tablets exchanged in solutions of water soluble and polymerisable melamine resin (NANOPLAST) (Bachuber & Frösch, 1983), have revealed the presence of nanometer scale canals after negatively staining (Sourty & Marchessault, 1998). This technique permits an in situ polymerization of the resin thus providing high resolution of TEM by trapping the hydrogel structure and permitting ultramicrotome thin sections for observation. Comparing the calculated average pore size using the poroelastic model and observations on negatively stained thin sections (not shown), proved the existence of a continuous membranous texture with pores of nanometer size. This continuous fine pore matrix is most probably the rate controlling sieve for the slow release of the pharmaceutical agent.

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