Pharmaceutical Research, Vol. 18, No. 11, November 2001 (© 2001) *Research Article*

Microcrystalline Cellulose-Water Interaction—A Novel Approach Using Thermoporosimetry

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Received May 8, 2001; accepted August 3, 2001

Purpose. To study the physical state of water in microcrystalline cellulose (MCC) and in silicified microcrystalline cellulose wet masses and the effect of granulation on different water fractions.

Methods. Thermoporosimetry, together with the solute exclusion technique, was used to measure different water fractions and pore size distributions of wet granules. To understand the effect of granulation on the physical state of water, both ungranulated and granulated wet masses were studied. In addition, dynamic and isothermal step melting procedures were compared.

Results. Four distinct fractions of water (nonfreezing, freezing bound, free, and bulk water) could be detected in MCC wet masses. Granulation decreased the volume of bulk water and increased the volume of freezing bound and free water. Consequently, granulated wet masses were able to hold more water inside the particles compared to ungranulated wet masses. Thus, granulation had a similar effect on MCC as beating has on cellulose fibers in the papermaking process.

Conclusions. Thermoporosimetry and solute exclusion increased the understanding of MCC-water interaction and showed how the physical state of water in MCC wet masses changes during granulation.

KEY WORDS: thermoporosimetry; isothermal step melting; microcrystalline cellulose; pore structure; effect of granulation; physical state of water.

INTRODUCTION

The interaction of microcrystalline cellulose (MCC) with water was previously examined by sorption isotherms (1–4), microcalorimetry (2,5), and thermal methods (5). Recently, near infrared (NIR) spectroscopy was also used to study the state of water in MCC (4,6). Water is known to exist in at least three thermodynamic states in MCC: tightly bound, relatively unrestricted, and in an intermediate state (7). In general, water associated with amorphous solids has been classified into the mono- and multilayer water adsorbed at the surfaces, and to the water absorbed into the internal structure of solid (8).

MCC is manufactured from wood pulp by acid hydrolysis. After being washed with water the suspension is spray dried into microcrystalline powder. The manufacturing process shortens the cellulose chains and removes the dissolving parts of cellulose fibres, i.e., hemicellulose and lignin. The structure of cellulose is shown in Fig. 1. Because interesting parallels exist between the properties of cellulose fibers and MCC, the interaction of cellulose fibers with water is considered below.

Various definitions of the different categories of water in cellulose fibers can be found in the literature. Nakamura *et al.* (9) divided water in wood pulps to three different categories: nonfreezing water, freezing bound water, and free water. At low relative humidity, water is adsorbed only on the cellulose fiber surfaces and bound to the cellulose chains by hydrogen bonds (nonfreezing water). Further layers of water molecules, which are still significantly influenced by the cellulose surface, are also included in the same category (10). At high relative humidities approaching saturation, capillary condensation occurs, and the porous cellulose fibers can hold appreciable amounts of water in submicroscopic pores, cracks, and crevices between fibrils. This water has a depressed melting temperature, and it is called freezing bound water (FBW). Free water is the unbound water in cellulose fibers with thermodynamic properties similar to those of pure bulk water.

Differential scanning calorimetry (DSC) has been traditionally used to measure the nonfreezing water in starch, cellulose, and pulp fibers (9,11,12). The amount of nonfreezing water is calculated by subtracting the total freezing water in the sample (determined from the measured heat) from the total water in the sample (determined gravimetrically). Freezing bound water is much more difficult to quantify than nonfreezing water because the DSC melting peak overlaps with that of bulk water (9,13). However, water held in the capillaries of porous materials has a depressed melting point because of the higher pressure of water in cavities with a curved interface. The melting temperature depression of the material has been used to measure a pore size distribution by thermoporosimetry. Generally, thermoporosimetry has been applied to systems in which the pores are sufficiently small, so that separate bulk and pore water peaks are formed in DSC melting endotherms (14). For water-saturated pulp fibers, the melting temperature of the water in the cell wall is depressed only slightly below that of bulk water, so the pore and bulk water peaks overlap, making analysis difficult.

The pore structure of dry MCC powder has traditionally been determined by using either nitrogen adsorption or mercury porosimetry (1–3). In the interaction with water, new pores are generated as the cellulose particles swell, but during drying most of the pores collapse. Consequently, the pore structure of MCC should be measured with techniques directly applicable to water-saturated particles. The methods previously used in pulp fiber research are solute exclusion (15), water retention value (16), nuclear magnetic resonance spectroscopy (NMR) (10), and thermoporosimetry (17). To measure the pore size distribution of pulp fibers with DSC, Maloney *et al.* (17) developed an isothermal step melting procedure.

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ABBREVIATIONS: DSC, differential scanning calorimetry; FBW, freezing bound water; FSP, fiber saturation point; MCC, microcrystalline cellulose; NFW, nonfreezing water; NIR, near infrared; NMR, nuclear magnetic resonance; PSD, pore size distribution; SMCC, silicified microcrystalline cellulose; TBW, total bound water; TFW, total freezing water.

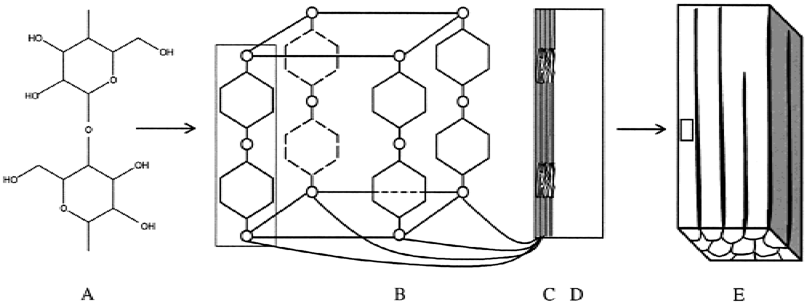

Fig. 1. The structure of cellulose. The cellobiose units (A) are linked together into larger unit cells (B), which form crystallites (C). Crystallites with crystalline and amorphous regions are combined into microfibrils (D) and finally fibrils (E).

To understand how water influences the properties of wet powder masses, it is important to study the physical state of water in pharmaceutical wet masses. Most of the earlier studies with MCC used relatively low moisture contents, which do not include the moisture contents used in wet granulation and extrusion-spheronization. The aim of this study was to use thermoporosimetry, together with solute exclusion, to measure the water fractions in silicified microcrystalline cellulose (SMCC) wet granules over a wide moisture range. To understand the effect of granulation on the physical state of water in the wet masses, both ungranulated and granulated samples were studied. In addition, three different MCC and SMCC grades were compared.

MATERIALS AND METHODS

Materials

Three different grades of MCC were studied: Avicel PH 101 manufactured by FMC Corp. (Cork, Ireland) and Emcocel 50 and Prosolv 50 from Penwest Pharmaceuticals Co. (Nastola, Finland). Prosolv 50 is SMCC with a 2% w/w silicon dioxide concentration. The mean diameter of the cellulose particles is around 60 μm.

Preparation of Wet Masses

The wet granules were made in a planetary mixer (Kenwood Chef Classic, KM 400, UK). The required quantity of water (0.05–1.5 g g^{-1}) was added at 200 g/min, and after water addition the wet masses were mixed for 10 min. Ungranulated wet masses were prepared by adding the water (1.0 g g^{-1}) to the dry powder in a mortar. The wet masses were allowed to equilibrate overnight in plastic bags before experiments.

DSC Measurements

DSC measurements were carried out on a Mettler 821° DSC (Mettler, Toledo, Switzerland) equipped with an intracooler. The samples of approximately 3.5 mg were weighed into hermetically sealed aluminum pans. The weight of the samples was checked before and after the DSC measurements to ensure that the pan was well sealed. The water content of the samples was determined by drying samples 24 h at 105°C.

Calibration

Calibration was done by using 15 mg of 99.9999% mercury and 2 mg of ultrapure distilled water. The pan that held the mercury was treated in an autoclave to form an oxide-hydroxide layer that prevents interaction of the aluminum with the mercury. Three separate samples from each material were used in calibration.

The temperature calibration was done for both standards by applying the step procedure over the range of the melting transition, using 0.02°C steps and 10-min isothermal segments between each step. The temperature at which all the sample melted was taken as –38.80°C and 0.00°C for mercury and water, respectively.

The heat calibration was done by using the same samples and melting them three times each dynamically at 5°C/min. The average melting heat was used to adjust the instrument calibration parameters to H_m = 11.4 J/g and 334.5 J/g for mercury and water, respectively. The temperature precision was ±0.02°C, and the heat measurement precision was ±1.0%.

Isothermal Step Melting Procedure

The principle of the isothermal melting technique is to raise the temperature in a frozen sample to a present value where it is held constant until the melting transition is completed (Fig. 2a). The melting of the sample is then repeated at a slightly higher temperature. The heat absorbed at each temperature is measured by integrating the endotherm. The melting heat is assumed to correlate directly with the amount of melted water, and, consequently, the pore size distribution (PSD) is calculated from the melting temperature depression of the imbibed water (Table I).

The relationship between pore diameter (D) and the melting temperature depression is described by the Gibbs-Thomson equation [Eq. (1)]

$$D = \frac{-4 v_{\mathrm{m}} T_0 \gamma_{\mathrm{ls}}}{H_{\mathrm{f}} \Delta T} \qquad (1)$$

where v_m is the molar volume of ice (19.6 10^{-6} m^3/mol), T_0 is the melting temperature of water at normal pressure (0°C), γ_{ls} is the surface energy at the ice-water interface (12.1 mJ/m^2), H_f is the specific heat of fusion of bulk water (334 J/g) and ΔT is the melting temperature depression (°C).

In the beginning of the measurement, the sample was cooled at 10°C/min from +25°C to –45°C and then heated to –35°C where it was held for 2 min. The isothermal step melting procedure was run at 1°C/min to the set points shown in Table 1. The duration of isothermal segments varied from 2 min at –33°C to 22 min at –0.2°C, depending on the time sample needed to complete the melting (Fig. 2a). To determine the total freezing water (TFW) [Eq. (2)], the sample was

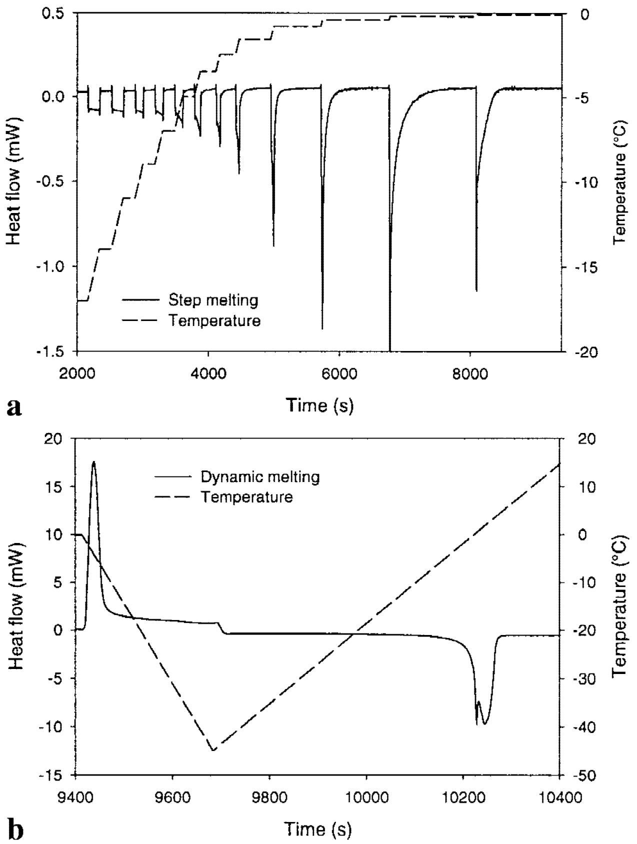

Fig. 2. The melting of water in a wet mass (a) with a isothermal step melting program as a function of time (2000–9000 s), (b) with a dynamic program as a function of time (9400–10400 s). A negative heat flow indicates an endothermic transition.

dynamically cooled at the end of the measurement to −45°C at 10°C/min and then heated to +25°C at 5°C/min (Fig. 2b). Four measurements per test point were averaged. These were analyzed with the Student's *t* test.

$$TFW = \frac{H_t}{H_f \times W_{dry}} \text{ (g g}^{-1}\text{)} \tag{2}$$

where H_t is the energy transferred according to the melting peak of total freezing water (J) and W_{dry} is the mass of dry solids (g).

The amount of nonfreezing water was calculated by subtracting the total freezing water (*TFW*) in the sample (determined from the measured heat) from the moisture content (*MC*) in the sample (determined gravimetrically) [Eq. (3)].

$$NFW = MC - \frac{H_t}{H_f \times W_{dry}} \text{ (g g}^{-1}\text{)} \tag{3}$$

Total Bound Water Measurements

The principle of the total bound water (TBW) measurement is to raise dynamically the temperature in a frozen sample to slightly below 0°C, where it is held constant until the melting transition is completed. With this method, the pore size distribution cannot be determined, but the total amount of water with depressed melting temperature can be measured.

In a TBW measurement, the sample was cooled from +3°C to −30°C and then heated to −0.2°C at 5°C/min (Fig. 3a). The temperature was kept at −0.2°C for 12 min to ensure that the sample had enough time to complete the melting. The sample was then cooled to −30°C, and the amount of H_{tbw} was calculated by integrating this freezing peak [Eq. (4)] (Fig. 3b). In the end of the measurement, the sample was heated to +25°C to determine H_t (Fig. 3c). The measurements for SMCC wet granules were performed in duplicate, and the average is reported. The results for ungranulated and granulated of different cellulose grades are the mean of four measurements.

Table I. Thermoporosimetry Melting Temperatures and Calculated Pore Diameters

T (°C)	D (nm)
−33.0	1.3
−20.0	2.2
−17.0	2.5
−14.0	3.1
−11.0	3.9
−9.0	4.8
−7.0	6.2
−5.0	8.6
−3.5	12.3
−2.6	16.6
−1.6	27.0
−0.8	53.9
−0.4	107.8
−0.2	215.6

$$TBW = \frac{H_{tbw}}{H_f \times W_{dry}} \text{ (g g}^{-1}\text{)} \tag{4}$$

where H_{tbw} is the energy transferred according to the freezing bound water (J).

Solute Exclusion Technique

Because part of the water inside the particles has similar thermodynamic properties to water outside the particles, these water fractions cannot be differentiated with DSC alone because some of the water inside the particles may be in pores so large that it has properties very similar to bulk water. The solute exclusion technique was used to measure the total amount of water inside the cellulose particles, i.e., fiber saturation point (FSP). Solute exclusion is a technique that has been used to measure the swelling of wood pulps since its inception in the 1960s (15). The measurement is based on the concentration change when a noninteracting probe, which is too large to penetrate the MCC particles, is added to a quantity of the wet cellulose. Stone and Scallan (15) found that the inaccessibility of pulp fibers reached a maximum around 30 nm, and, consequently, 2 × 10^6 dextran polymer with molecular diameter of 56 nm is usually used to determine the FSP value.

For the measurements, 2 g of the wet sample was dispersed with water to obtain a 20% dispersion. Thirty-five milliliters of a 2% solution of 2 × 10^6 Dalton dextran polymer (T2000, Amersham Pharmacia Biotech AB, Uppsala, Sweden) was added to the dispersion and stirred for 1 hr. After the stirring, most of the solution outside the particles was removed by centrifugation after which the solution was filtered. The concentration of dextran solution was determined by using polarimeter (Autopol IV, Rudolph Research, Flanders, New Jersey).

Terminology

In this study, terminology similar to Nakamura *et al.* (9) was used, i.e., water fractions were divided to nonfreezing water (NFW), freezing bound water (FBW), and free water. The sum of NFW and FBW is referred to as the total bound water (TBW), which shows the amount of water in micropores. By combining thermoporosimetry with solute exclusion, it is possible to differentiate free water inside and outside the particles. Consequently, water in macropores inside the particles is called free water, and water outside the particles is called bulk water. The total freezing water (TFW) is the sum of FBW, free, and bulk water.

RESULTS AND DISCUSSION

Effect of Moisture Content

Endotherms of the TFW of SMCC wet granules are shown in Fig. 3c. With increasing water content the intensity of water peaks increased and the FBW and free/bulk water peaks overlapped in the thermograms. The results agree with those obtained for pulp fibers (9,13) and hydroxypropyl methylcellulose (18). The amount of TFW and NFW were calculated from these endotherms according to Eqs. (2) and (3). The amount of TBW was determined from the FBW exotherm according to Eq. (4) (Fig. 3b). By this means it was possible to differentiate FBW from free and bulk water, which overlapped in the melting endotherm.

At low moisture contents (0.05–0.2 g g^{-1}), all the water was bound on the surface of cellulose, i.e., water was nonfreezing (Fig. 4). The amount of nonfreezing water increased with increasing moisture content up to 0.26 g g^{-1}. The results are consistent with the amounts of nonfreezing water found for starches and pulp samples (11,12,19). There is still not a well-accepted thermodynamic theory to explain why some of the water in hydrated systems does not freeze. However, it has been shown that there is a correlation between the number and type of available hydration sites and the quantity of nonfreezing water (9,20). Nonfreezing water is sometimes divided into a tightly bound monolayer water and an intermediately bound multilayer water, which corresponds to about three water layers next to the surface (4,7,10).

Froix and Nelson (21) studied the interaction of water with cotton linters by using NMR. They found that up to 0.09 g g^{-1} "primary bound" water existed. From 0.09 to 0.2 g g^{-1} there also exists a type of "secondary bound" water. "Primary bound" water molecules were described as those sorbed onto nonhydrogen-bonded hydroxyl groups, whereas "secondary bound" were those that penetrated the fiber bundles and formed hydrogen bonds with internal surface hydroxyls in the amorphous regions. The authors equated both primary and secondary bound water with nonfreezing water.

The other water fractions of SMCC wet granules can be seen from Fig. 4. The sum of NFW and freezing bound water FBW is referred to as the TBW, which shows the amount of water in micropores. The amount of TBW increased with increasing moisture content and reached a peak at 1.1 g g^{-1} after which it decreased slightly (Fig. 4). Free water, which cannot be detected by DSC alone, is located in larger macropores. It is probable that the pores of the wettest granules are larger than 216 nm, and they cannot be separated from free water with thermoporosimetry. Fiber saturation point

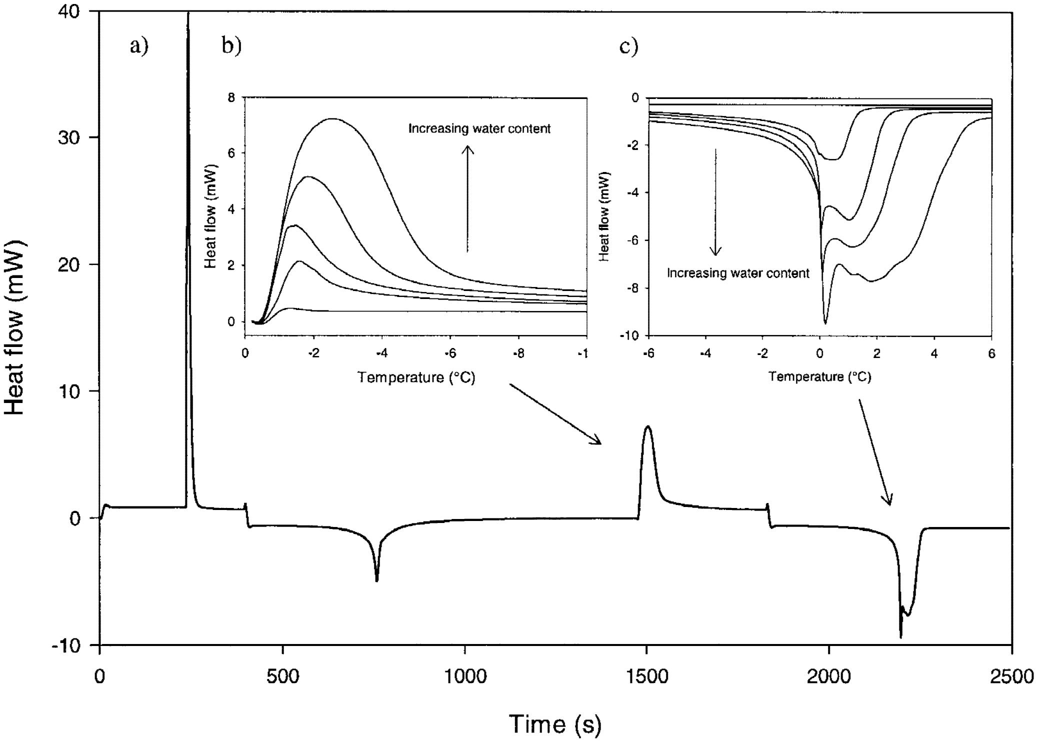

Fig. 3. Total bound water (TBW) measurement of SMCC wet granules (a). Exotherm of freezing bound water (FBW) (b). Endotherm of total freezing water (TFW) at the moisture range 0.2–1.0 g g^{-1} (c).

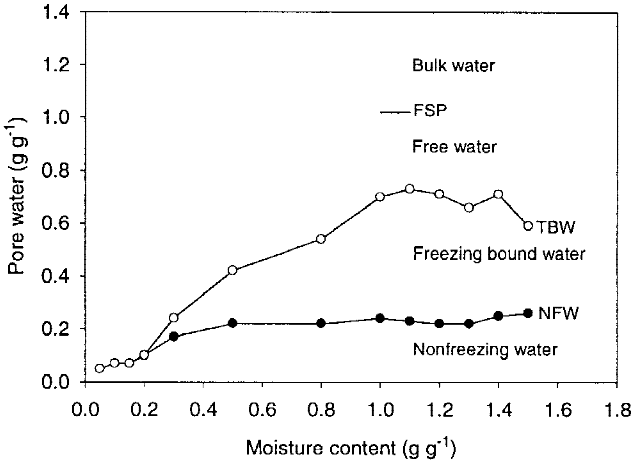

Fig. 4. Different water fractions of SMCC wet granules as a function of moisture content.

(FSP) measures the total amount of water inside the cellulose particles, i.e., swelling of the particles. When the moisture content of the sample exceeds the FSP, the additional water is retained between particles as bulk water.

Effect of Granulation

Different water fractions of granulated and ungranulated wet masses obtained by the isothermal step melting measurement are shown in Table II. Granulation or cellulose grade had no effect on the amount of nonfreezing water, because they were the same for the ungranulated and granulated wet masses (Tables II and III). The results are consistent with Maloney and Paulapuro (22) who found that the amount of nonfreezing water in cellulose fibers was independent of beating. This means that beating, as well as granulation, has a negligible impact on the total quantity of hydration water in the wet mass.

With ungranulated wet masses the amounts of NFW and FBW were almost equal, and only a very small amount of water was in macropores as free water. The FSP values of ungranulated wet masses were between 0.41 and 0.46 g g^{-1}; hence, most of the water was as bulk water outside the particles. With granulated wet masses, the amount of FBW was twice as much as the amount of NFW and free water. In general, granulated wet masses contained no bulk water, because the FSP values of the wet granules were very close to the moisture content. Consequently, all of the water was inside the particles. Most of the water was in micropores (TBW) and a smaller part in macropores (free water).

The pore size distributions of different cellulose grades are shown in Fig. 5. The amount of FBW was slightly greater with Avicel and Prosolv compared to Emcocel, but there were no significant differences between cellulose grades (Table III). Instead, the difference in the pore volume of granulated and ungranulated wet masses can be seen clearly in Fig. 5. The pore volumes of wet masses were quite similar with the smallest pores, but the difference between granulated and ungranulated wet masses was greatest with the biggest pores (54–216 nm).

Granulation had the greatest effect on the amount of bulk water outside the particles. Both micro- and macropore volume of MCC and SMCC wet masses increased remarkably during granulation, and the granulated wet masses were able to hold more water inside the particles compared to ungranulated wet masses. The differences between the water fractions of ungranulated and granulated wet masses were also statistically significant (Table III).

Granulation seemed to have the same effect on MCC as beating has on cellulose fibers in the papermaking process. Beating is known to cut cellulose fibers and cause morphologic changes. A primary effect of beating on cellulose is to open larger pores in the cell wall and, hence, increase the swelling of the pulp fibers (10,23). Consequently, the lamellar structure of fibrils inside the cellulose fibers is loosened, which increases the water penetration into the cell. It is expected that the granulation process works in the same way; bonds between microfibrils are loosened and the particles swell.

As Froix and Nelson (21) stated, the amount of water contained in a cellulose system is a direct function of the microstructure of that system. Consequently, one should not expect to obtain identical results with different samples without normalization of water accessibility into the structure, i.e., granulated vs. ungranulated wet masses. This suggests that the mixing time and the shear forces applied to the wet mass during granulation affects the location of water in the MCC or

Table II. Moisture Contents, Fiber Saturation Points, and Different Water Fractions Measured by the Isothermal Step Melting Method

	MC[a] (g g^{-1})	FSP[b] (g g^{-1})	NFW[c] (g g^{-1})	FBW[d] (g g^{-1})	TBW[e] (g g^{-1})	Free water[f] (g g^{-1})	Bulk water[g] (g g^{-1})
Avicel PH 101 granulated	1.00 ± 0.07	1.07 ± 0.02	0.25 ± 0.01	0.51 ± 0.04	0.75 ± 0.05	0.24 ± 0.09	—
Emcocel 50 granulated	1.04 ± 0.06	1.11 ± 0.01	0.24 ± 0.01	0.48 ± 0.02	0.72 ± 0.02	0.32 ± 0.05	—
Prosolv 50 granulated	1.06 ± 0.05	1.02 ± 0.00	0.25 ± 0.02	0.51 ± 0.04	0.76 ± 0.02	0.25 ± 0.03	0.04 ± 0.05
Avicel PH 101 ungranulated	0.96 ± 0.08	0.46 ± 0.02	0.24 ± 0.03	0.21 ± 0.02	0.44 ± 0.03	0.02 ± 0.04	0.50 ± 0.08
Emcocel 50 ungranulated	0.93 ± 0.04	0.42 ± 0.02	0.22 ± 0.02	0.19 ± 0.01	0.41 ± 0.01	0.01 ± 0.03	0.52 ± 0.06
Prosolv 50 ungranulated	0.96 ± 0.03	0.41 ± 0.04	0.23 ± 0.01	0.21 ± 0.00	0.44 ± 0.01	—	0.53 ± 0.03

[a] Moisture content determined gravimetrically.

[b] Fiber saturation point.

[c] Nonfreezing water.

[d] Freezing bound water.

[e] Total bound water = NFW + FBW.

[f] FSP − TBW, in case $MC < FSP$ = MC − TBW.

[g] MC − FSP.

Table III. Student's *t* Test for the Results Shown in Table II

	FSP	NFW	FBW	TBW	Free water	Bulk water
Granulated wet masses						
Avicel/Emcocel	*	NS	NS	NS	NS	ND
Avicel/Prosolv	**	NS	NS	NS	NS	ND
Emcocel/Prosolv	***	NS	NS	*	NS	ND
Granulated vs. ungranulated						
Avicel PH 101	***	NS	***	***	*	***
Emcocel 50	***	NS	***	***	***	***
Prosolv 50	***	NS	***	***	***	***
Ungranulated wet masses						
Avicel/Emcocel	*	NS	NS	NS	NS	NS
Avicel/Prosolv	NS	NS	NS	NS	ND	NS
Emcocel/Prosolv	NS	NS	***	*	ND	NS

ND, not determined; NS, not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

SMCC wet mass, i.e., the water fraction inside or outside the particles.

Role of MCC and SMCC in Extrusion-Spheronization

MCC is an essential excipient in extrusion-spheronization. Fielden *et al.* (5) suggested that MCC can be described as a molecular sponge, because MCC is able to trap a large volume of water within its porous structure and retain it despite the application of high pressures. During extrusion, the sponges are compressed until water is squeezed out and lubricate the particles flowing through the extruder. Variations in the performance of different sources of MCC are associated with the different interactions between the particular grade of MCC and water due to different pore structures (24).

In the crystallite-gel model proposed by Kleinebudde (25), MCC particles are broken down into smaller particles by shear forces acting on the particles during granulation and extrusion. It was suggested that finally single crystallites of colloidal size occur, and these single particles in the presence of water are able to form a gel and immobilize water. Recently, Kleinebudde *et al.* (26) postulated that the sponge model is more appropriate for the cellulose type with high degree of polymerization (powdered cellulose), whereas the gel model is more applicable to cellulose types with lower degree of polymerization (MCC and SMCC).

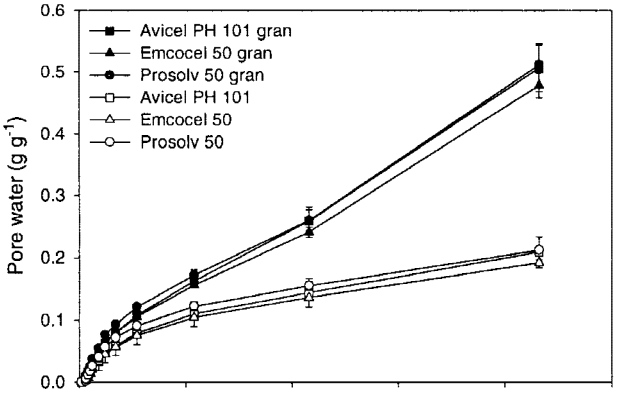

Fig. 5. The cumulative pore size distribution of granulated and ungranulated MCC and SMCC wet masses (moisture content 1.0 g g^{-1}).

Every step in the process of extrusion-spheronization affects the properties of the resulting pellets (27). However, only few authors have reported studies concerning the impact of the granulation step. Schmidt and Kleinebudde (27) found that higher shear forces during granulation resulted in a higher water content necessary for successful pelletization. Because the results were in contrast to conventional granulation, they explained their results with the crystallite-gel-model. According to the crystallite-gel-model, high shear during granulation and extrusion should result in a more delicate network, which requires more water to obtain the same plasticity compared to a coarser network of the gel.

The granulation phenomenon has been traditionally explained by the liquid saturation model suggested by Newitt and Conway-Jones (28). The liquid saturation expresses the volume of liquid relative to the volume of voids and pores between particles in the agglomerate. The transition between different states of liquid saturation is usually induced by increasing the level of binder liquid, but an identical effect can be obtained also by consolidating the agglomerates by further mixing (28). However, the liquid saturation model has been developed for nonporous, spherical sand particles, which do not have the ability to take water into the internal structure. It was shown in this study that MCC has a porous structure and the volume of pores increases remarkably during granulation, when bulk water outside of the particles moves inside the particles. These findings support the sponge model and are able to explain the increased requirement for water content when higher shear forces are used during granulation.

Comparison of the Methods

Different water fractions of granulated and ungranulated wet masses obtained by TBW measurements are shown in Table IV. The results were compared with those obtained by isothermal step melting method in Table II and analyzed with the Student's *t* test. The relative standard deviations were around 5% with both methods. In general, the results were parallel, and the difference between step melting and TBW measurements was around 7.5%.

Table IV. Different Water Fractions Obtained by the TBW Measurement

	NFW[a] (g g^{-1})	FBW[b] (g g^{-1})	TBW[c] (g g^{-1})
Avicel PH 101 granulated	0.27 ± 0.01*	0.52 ± 0.04	0.79 ± 0.04
Emcocel 50 granulated	0.25 ± 0.01	0.53 ± 0.02*	0.78 ± 0.03**
Prosolv 50 granulated	0.27 ± 0.01	0.51 ± 0.04	0.78 ± 0.03
Avicel PH 101 ungranulated	0.22 ± 0.01	0.19 ± 0.01	0.42 ± 0.01
Emcocel 50 ungranulated	0.22 ± 0.01	0.16 ± 0.01**	0.38 ± 0.01*
Prosolv 50 ungranulated	0.23 ± 0.01	0.18 ± 0.00***	0.41 ± 0.01**

[a] Nonfreezing water.
[b] Freezing bound water.
[c] Total bound water.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Stepwise isothermal measurements were previously used in detecting drug-excipient incompatibilities (29). In this study, the isothermal step melting procedure proved to be a suitable method for measuring the physical state of water and the pore size distribution of wet cellulose masses. If one is only interested in the amount of different water fractions in the wet mass, the dynamic TBW measurement can be used. The precision of the isothermal melting measurement depends on the sample and the melting temperature (17). Precision decreases as the melting temperature approaches 0°C and is worse for samples, which display a high degree of melting in a narrow temperature range. Maloney (19) found that the enthalpy measured in a step melting experiment was nearly equivalent to that measured dynamically, the precision of melting enthalpy for water being 2–3% and the accuracy 5–6%.

Evaluation of the Method

Deodhar and Luner (30) proposed 4 nm (corresponding to −4°C) being the minimum pore diameter below which water is nonfreezing in pulp fibers. However, it has been shown in this and other studies that melting is detected in cellulose samples and in pulp fibers at much lower temperatures (17,19,20). An average NFW of 0.26 g g^{-1} suggests that the average thickness of NFW layer in the pulp fibers is around 0.9 nm (19). The thickness of NFW layer in porous silica standards varies from 0.4 to 0.8 nm (14). For evaluation of materials containing primarily larger pores, in 10- to 100-nm range, it is acceptable to neglect the thickness of the NFW layer on the PSD. Consequently, the effect of the nonfreezing water layer on the pore diameters was not taken into account when measuring the PSD of MCC and SMCC samples.

Freezing the sample is an essential part of the thermoporosimetry. However, crystallization of water within the cell wall may affect the structure of the sample and, hence, the measured pore size distribution. In fact, Maloney *et al.* (17) noted considerable hysteresis if the freezing-melting cycle was repeated several times, suggesting that freeze damage to the pores can occur. Although thermoporosimetry has been shown to be in good agreement with other pore measurement techniques for porous glasses, it is possible that other materials, especially those containing “soft pores” are more difficult to evaluate with thermoporosimetry. This question should be dealt with in future studies.

The pore size distribution could also have been determined by solute exclusion method by using a series of different size probes. However, interpreting the PSD by this method is not straightforward. In the solute exclusion method, the probe diffuses into the pores, so the measured pore diameters are strongly affected by the size of the pore openings (15). Thus, water trapped in large pores with small openings may bias the PSD. In addition, water on the pore wall is not available for diluting the dextran solution. These factors may explain why the pore volume measured with a 56-nm dextran probe is considerably larger than the pore volume below 216 nm as measured with thermoporosimetry.

In the solute exclusion method, it is only possible to determine the FSP under saturated conditions. Thus, the effect of moisture content on the FSP remains unclear. One should also keep in mind that depression of the interparticle water’s melting temperature due to pore effects can contribute to the measured PSD. This means that in some cases the amount of TBW can exceed the FSP.

Until now, it has been possible to determine the pore structure of MCC only from dry powder or granules by using nitrogen adsorption or mercury porosimetry. However, the pore structure of MCC changes in the interaction with water, because the MCC swell during wetting and shrink during drying. For the first time it was possible to actually measure the pore size distribution of wet MCC and SMCC masses by using thermoporosimetry. Thermoporosimetry combined with solute exclusion was also able to differentiate freezing bound water from free and bulk water, which normally overlap in melting endotherms. Even though it may be difficult to ascertain the actual size of the pores of MCC and SMCC wet masses with thermoporosimetry, the methods can be used to measure the different water fractions in wet powder masses and make relative comparisons between different samples.

CONCLUSIONS

By using thermoporosimetry it was possible to determine the pore size distribution of wet MCC and SMCC masses and differentiate water with depressed melting temperature from free and bulk water. Four distinct fractions of water (nonfreezing, freezing bound, free, and bulk water) could be detected in the MCC and SMCC wet masses with a combination of solute exclusion and thermoporosimetry. At low moisture contents, all the water in SMCC was nonfreezing and there was no water in micropores. Granulation or cellulose grade had no measurable effect on the total quantity of nonfreezing water. The amount of NFW and FBW increased with increasing moisture content. Granulation increased the volume of micropores and formed macropores, which contained free water. Granulation had the same effect on MCC as beating has on cellulose fibers in the papermaking process. Bonds between microfibrils were loosened, and the bulk water outside the particles was absorbed into the internal structure of MCC and SMCC. Consequently, wet granules were able to hold more water inside the particles compared to ungranulated wet masses. Thermoporosimetry and solute exclusion increased the understanding of how water is bound to the MCC and how the pore structure of MCC and SMCC particles changes when wet granulated.

ACKNOWLEDGMENTS

We thank Béatrice Marquez for help to prepare the wet masses and Leena Nolvi (Helsinki University of Technology) for the DSC and solute exclusion measurements.

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