Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 1525–1529
Carbohydrate RESEARCH

Note

Study of hydration of cross-linked high amylose starch by solid state ^{13}C NMR spectroscopy

Héloïse Thérien-Aubin,[a] Florence Janvier,[a] Wilms E. Baille,[a] X. X. Zhu[a,*] and Robert H. Marchessault[b]

[a]*Département de Chimie, Université de Montréal, CP 6128, Succursale Centre-ville, Montréal, Québec, Canada H3C 3J7*
[b]*Department of Chemistry, McGill University, 3420 University Street, Montréal, Québec, Canada H3A 2A7*

Received 23 January 2007; received in revised form 12 April 2007; accepted 22 April 2007
Available online 29 April 2007

Abstract—Starch is subjected to chemical treatments such as cross-linking or hydroxypropylation to meet the material requirements for food uses or controlled release in the pharmaceutical industries. In this work, two types of cross-linking formulations have been employed for the preparation of high amylose starch for use as an excipient for sustained drug release. The structural differences and chain dynamics of the modified starches in the dry and hydrated states have been compared by the use of variable contact time cross polarization-magic angle spinning solid state ^{13}C NMR spectroscopy.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: High amylose starch; Cross-linking; ^{13}C solid state NMR spectroscopy; Contact time

Cross-linked high amylose starch (CHAS) used as an excipient in drug tablet preparation has shown a zero-order release of drug over a period of 2–24 h[1–5] and is used in once-daily formulation of drugs tablets.[5–7] Starch is a naturally occurring semi-crystalline polysaccharide mixture composed of amylose and amylopectin. Amylose is a linear polysaccharide, whereas amylopectin is highly branched. The three dimensional crystalline conformations of the single and double helix polymorphs[8–13] have been identified from X-ray fiber diffraction,[14,15] that is, the double helices A and B and the single helix V. Native corn starch with high amylose content mainly shows a B-type crystalline conformation.[16]

Molecular order in starch can also be probed by ^{13}C NMR. The repeating unit of starch is shown as an inset in Figure 1. The multiplicity of the C1 resonance peak provides information on the crystallinity, the conformation of starch, and the double helix symmetry.[8–13,17] For the B-type conformation, the C1 resonance exhibits two peaks at 101 and 100 ppm and the A-type has three

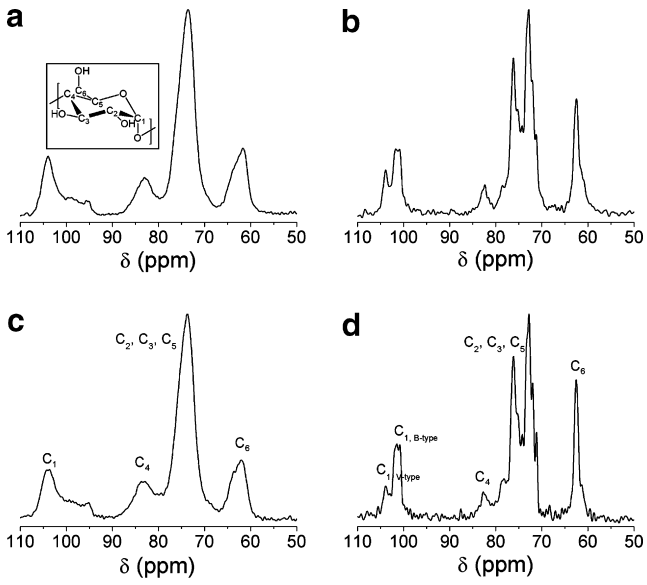

Figure 1. ^{13}C CP/MAS spectra at a contact time of 1.5 ms for (a) dry CHAS I, (b) hydrated CHAS I, (c) dry CHAS II, and (d) hydrated CHAS II. The inset represents the chemical structure of α-(1→4)-glucopyranose repeating unit of amylose with $^4C_1$ chair conformation.

* Corresponding author. E-mail: julian.zhu@umontreal.ca

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.04.014

peaks at 102, 101, and 100 ppm.[11,13,18] The broad peak of C1 at 103 ppm is typical of a single helix organized in a V-type crystalline phase or dispersed in an amorphous phase.[19] NMR can detect short range order and allow the evaluation of helix content while X-ray crystallography can detect long range order and measure crystalline domain content.[8]

We have studied the effect of temperature, tablet size, and processing method on the swelling and water uptake of CHAS tablets by NMR imaging.[20–23] The preparation method of CHAS affects the penetration of water into the tablets[23] and thus their drug-release properties. This behavior is ascribed to the limited mobility of starch chains in highly cross-linked CHAS. Experimentally, it was found that a higher degree of cross-linking in the preparation of CHAS I leads to higher degree of swelling and faster water uptake by the tablets,[24] which is opposite to the expected effect of an increased number of cross-links. In fact, the formation of B-type double helices in starch has an important pseudo cross-linking effect[2] and limits the swelling of starch. The cross-linking of starch limits the chain mobility of the polymer and reduces the formation of the B-type double helices. The aim of this study is to investigate the dry and hydrated states of two CHAS samples made by two different preparation methods. ^{13}C solid-state NMR spectroscopy with cross-polarization and magic angle spinning (CP/MAS) was used to study the correlation of the behavior of tablets with the molecular ordering of starch in such samples.

Figure 1 shows typical spectra of CHAS in both dry and hydrated states. There is no major spectral difference between CHAS I and CHAS II (prepared differently as described in the Experimental section). Both dry starches contained mainly non-crystalline domains and V-type single helices. The broad peak between 70 and 80 ppm is related to carbons 2, 3, and 5 of the glucose unit in a poorly ordered system.[8,9,25] The C1 peak at 104 ppm is associated with single helices in both amorphous[10,25] and V-helix structures.[25,26] Upon hydration, both narrowing and splitting of the peaks are observed. This is attributed to a decrease in the size of the non-crystalline domains and to an increased order in crystalline domains.[9] The typical spectral features of B-type helices are clearly visible in the spectra of hydrated starch, showing the two peaks of C1 at 100 and 101 ppm[9,11,27] and the peaks of C2, C3, and C5 between 70 and 80 ppm. A decrease of the unordered C4 peaks at 83 ppm[12,28] is also observed upon hydration, and the ordered C4 shows a signal in the 70–80 ppm region.[12,27,28]

Variable contact time ^{13}C CP/MAS NMR experiments of the dry and hydrated samples were analyzed in terms of their relaxation parameters and of a proton spin diffusion constant (T_{df}) to account for spin diffusion ^{1}H–^{1}H homonuclear dipolar interactions using the following equation:[29]

$$M(\tau_{CP}) = M_0 \exp\left(-\frac{\tau_{CP}}{T_{1\rho}^{H}}\right) \times \left\{1 - \lambda \exp\left(-\frac{\tau_{CP}}{T_{df}}\right) - (1-\lambda)\exp\left(-\frac{3\tau_{CP}}{2T_{df}}\right) \times \exp\left(-\frac{\tau_{CP}^2}{2T_2^2}\right)\right\} \quad (1)$$

where $M(\tau_{CP})$ is the NMR signal at a contact time τ_{CP}, $T_{1\rho}^{H}$ the longitudinal relaxation time in the rotating frame, T_2 the transverse relaxation time, and $\lambda = 1/(n+1)$ with n being the number of hydrogen atoms on the carbon atom observed.

Figure 2 shows typical fits of Eq. 1 to experimental data for both the dry and hydrated CHAS. The $T_{1\rho}^{H}$ values obtained in the dry state (Table 1) are coherent with previously reported values.[8] For both samples, we observe an increase in $T_{1\rho}^{H}$ upon hydration of the starch. It is clear that the longer $T_{1\rho}^{H}$ values have larger errors than the lower $T_{1\rho}^{H}$ values, which is caused, in part, by the scale of contact times used. Longer contact times may lead to somewhat improved precision on the $T_{1\rho}^{H}$ values, but the trend is clear under the experimental conditions used. For all the signals studied, the signal attenuation reached at least 20%. A slight decrease of T_2 is also observed upon hydration. This change in chain dynamics is attributed to the hydration of the starch and the conversion of non-crystalline domains or V-type single helices to B-type helices.

Only small differences in the relaxation parameters between the two CHAS are observed. In the dry state $T_{1\rho}^{H}$ is slightly higher for CHAS I than for CHAS II, but upon hydration no statistically significant difference is observed between the two CHAS. The amount of cross-linker used in the preparation of CHAS I is more than 20 times higher in molar ratio than that used for

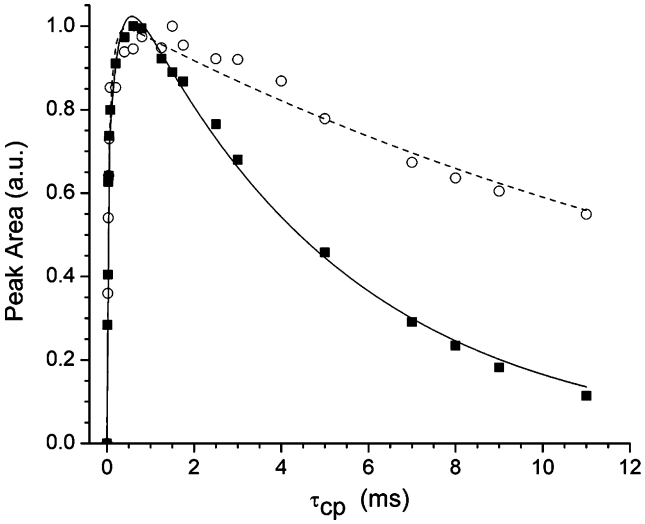

Figure 2. Analysis of the C6 peak areas as a function of the contact time used in the CP/MAS NMR experiments. Dry CHAS II (■); hydrated CHAS II (○). Lines are fits to Eq. 1.

Table 1. Relaxation parameters obtained from variable contact time ^{13}C CP/MAS NMR experiments with the dry and wet CHAS samples

	CHAS I			CHAS II		
	$T_{1\rho}$ (ms)	T_2 (μs)	T_{df} (ms)	$T_{1\rho}$ (ms)	T_2 (μs)	T_{df} (ms)
Dry state						
C1	7.4 ± 0.8	28 ± 4	0.8 ± 0.3	6.7 ± 0.4	22 ± 2	0.5 ± 0.2
C4	7.0 ± 0.8	31 ± 5	0.7 ± 0.3	6.0 ± 0.5	25 ± 2	0.6 ± 0.1
C2,3,5	7.5 ± 0.6	20 ± 2	0.7 ± 0.2	6.7 ± 0.4	20 ± 1	0.5 ± 0.1
C6	5.8 ± 0.4	19 ± 2	0.44 ± 0.05	5.4 ± 0.3	19 ± 1	0.3 ± 0.1
Hydrated state						
$C1_{V\text{-type}}$	5.4 ± 0.8	22 ± 6	0.03 ± 0.02	5.4 ± 0.7	17 ± 3	0.4 ± 0.3
$C1_{B\text{-type}}$	23 ± 8	13 ± 4	0.2 ± 0.1	26 ± 3	21 ± 2	0.3 ± 0.1
C4	7 ± 1	16 ± 4	0.10 ± 0.07	7.9 ± 0.6	20 ± 4	0.15 ± 0.05
C2,3,5	17 ± 3	15 ± 5	0.10 ± 0.08	17 ± 1	20 ± 1	0.3 ± 0.2
C6	13 ± 1	16 ± 2	0.18 ± 0.05	15 ± 1	20 ± 4	0.3 ± 0.1

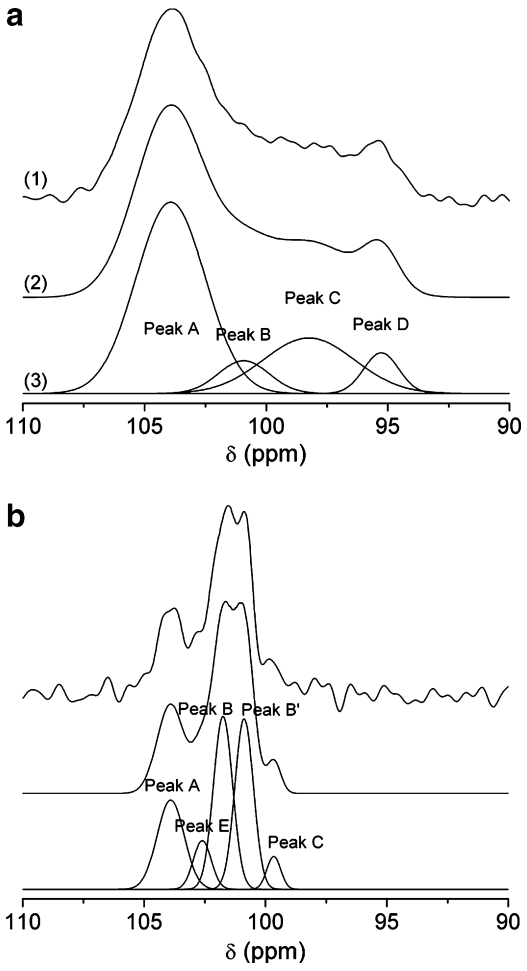

Figure 3. The CP/MAS ^{13}C NMR spectra acquired at a contact time of 1.25 ms (1), the simulated spectra (2), and the decomposed peaks (3) for (a) dry CHAS II and (b) hydrated CHAS II.

CHAS II. This could be responsible of the slightly higher rigidity of CHAS I in comparison to CHAS II as observed in the dry state with the $T_{1\rho}^{H}$ values.

Figure 3 shows the decomposition of the C1 region of the NMR spectra. In the dry starch, four peaks are needed for the decomposition of the signal observed in this region, while for the hydrated samples five peaks are needed for the best fit. Peak A, observed in all the samples, is typical of both the V-type helix[25,26] and the amorphous content.[10,28] Peaks B and B′ are characteristic of the B-type helices.[26,28] Peak C is related to the glucose units near α-(1→6) linkages[26] in amorphous domains.[10] Peak D is associated with constrained linkages in a poorly favored conformation.[26,28] Peak E is related to the interfacial region between single helices or amorphous domains and double helix regions.[10] This intermediate material retained some local order but is considered as non-crystalline.[9]

The dry samples are mainly amorphous and present type V-helix. Peak A represents more than 50% of the total area of the C1 band (Table 2). Type B double helix is present in the dry sample. This is probably related to the moisture content of 7–8 wt % in those samples. The broad peaks observed in the dry state prevent the obser-

Table 2. Analysis of the spectral components of the C1 peak of amylose in the dry and wet CHAS samples

Peaks	δ (ppm)	$W_{1/2}$ (ppm)	M_0 (%)	$T_{1\rho}$ (ms)
Dry CHAS I				
A	104.2 ± 0.1	2.4 ± 0.1	52 ± 2	6.3 ± 0.3
B	102.5 ± 0.6	4.4 ± 0.6	21 ± 1	6.0 ± 0.6
C	98.3 ± 0.3	4.3 ± 0.9	21 ± 1	5.9 ± 0.4
D	95.7 ± 0.1	1.6 ± 0.1	6.2 ± 0.6	6 ± 1
Dry CHAS II				
A	104.1 ± 0.1	3.0 ± 0.1	56 ± 2	6.8 ± 0.3
B	101.5 ± 0.2	3.6 ± 0.7	18 ± 1	6.9 ± 0.7
C	97.9 ± 0.3	4.0 ± 0.4	18 ± 1	6.4 ± 0.6
D	95.3 ± 0.1	2.5 ± 0.2	7.43± 0.5	7.1 ± 0.6
Hydrated CHAS I				
A	104.0 ± 0.1	0.9 ± 0.2	28 ± 3	7 ± 1
E	103.1 ± 0.2	1.1 ± 0.3	8 ± 1	15 ± 2
B	101.7 ± 0.1	0.9 ± 0.1	60 ± 1	40 ± 10
B′	100.8 ± 0.1	0.8 ± 0.1		
C	99.9 ± 0.2	0.6 ± 0.1	4 ± 1	20 ± 10
Hydrated CHAS II				
A	103.9 ± 0.2	1.2 ± 0.2	18 ± 1	9 ± 1
E	102.9 ± 0.4	1.0 ± 0.2	16 ± 2	24 ± 10
B	101.9 ± 0.1	0.9 ± 0.1	62 ± 1	35 ± 7
B′	100.9 ± 0.1	0.9 ± 0.1		
C	99.8 ± 0.2	0.46 ± 0.07	3 ± 1	11 ± 6

vation of the two typical peaks related to type B helices. The ordering observed in the NMR spectrum is caused by short range ordering, while X-ray diffraction needs large ordered domains to probe the ordering. The dry CHAS samples show V-type ordering and some B-type ordering by NMR, but no crystalline features were observed by X-ray powder diffraction for CHAS I samples.[19] The same results are obtained for CHAS II (data not shown).

Upon hydration, the half-height width of the peak ($W_{1/2}$) decreases by more than a factor of 2, and the two peaks type B helix is thus observed. Upon hydration the portion of type B helices in the sample increases greatly, the area of the type B helix peaks corresponds to more than 60% of the total C1 band area. The narrowing of the peaks also allows the observation of the interfacial region between the double helix domains and the unordered or single helix regions.

The decomposition of the C1 peak shows that the variations of the relaxation times between CHAS I and CHAS II are small, but the hydration of the starch leads to a large variation of the chain dynamics in the sample. The results in Table 2 show that, upon hydration, the area of peak A (amorphous domain and V-type single helix) decreases and the area of peak B (B-type helix) increases. After hydration, the proportion of B-type helices in both CHAS is about the same (ca. 60%). Thus, the variation in swelling between these two preparations[22,23] is not attributable to the increased formation of B-type helices in CHAS II. Hydrated CHAS I has a higher content of amorphous domain and V-type helices than CHAS II (28% for CHAS I versus 16% for CHAS II). This should be related to the existence of less interfacial material in CHAS I (8% for CHAS I versus 16% for CHAS II). CHAS I is gelatinized before cross-linking, which leads to a segregation between amylose and amylopectin, whereas CHAS II is cross-linked in the granular state, which leads to a more homogeneous material.

The existence of less interfacial material in CHAS I than in CHAS II is also an indication that there is less interface between B-type domains and amorphous/V-type helical domains in CHAS I. This means that the same amount of B-type helices is more evenly distributed in CHAS II than in CHAS I. Consequently, CHAS II would be a more homogeneous sample than CHAS I. The lower swelling observed for CHAS II[23] tablet seems to be related to the homogeneity of the sample.

1. Experimental

1.1. Preparation of cross-linked high amylose starch

CHAS I was prepared by gelatinizing high amylose starch (70% amylose) with 4% NaOH, and then cross-linking with 3.25% sodium trimetaphosphate (STMP) and spray-drying the sample.[1] For CHAS II, the starch was first cross-linked with 0.075% of phosphorus oxychloride in a 0.1% NaOH solution, then functionalized with 6% of propylene oxide and finally gelatinized at ca. 160 °C and spray-dried.[5] CHAS II is commercially available as Contramid® from Labopharm Inc. (Laval, QC, Canada).

1.2. Solid state ^{13}C NMR spectroscopy

Variable contact time ^{13}C CP/MAS spectra were recorded at room temperature at 150.90 MHz on a Bruker AV-600. The samples were spun at 8 kHz, and 1000–1800 scans were accumulated. The contact time varied from 0.01 to 11 ms. For the dry samples, the tablets (received from Labopharm) were crushed before the NMR experiments. For the hydrated samples, tablets were crushed and the powder was swollen in distilled water at 37 °C for 48 h.

1.3. Spectral decomposition

Spectral decomposition was performed on the C1 band because this peak is highly dependent on the starch conformation.[9,10,25,26] The dry and hydrated spectra were fitted to the sum of 4 and 5 Gaussian peaks, respectively, by the use of Origin 7.5. The iterations of the fitting procedure were stopped at the minimal value of χ^2. A first round of fitting was done while letting all the parameters (position of the peak (δ), half-height width ($W_{1/2}$) and intensity) free. Because there is no reason that the δ and $W_{1/2}$ values are different for the same sample at different contact times, a second round of fitting was performed with δ and $W_{1/2}$ values fixed to the mean value

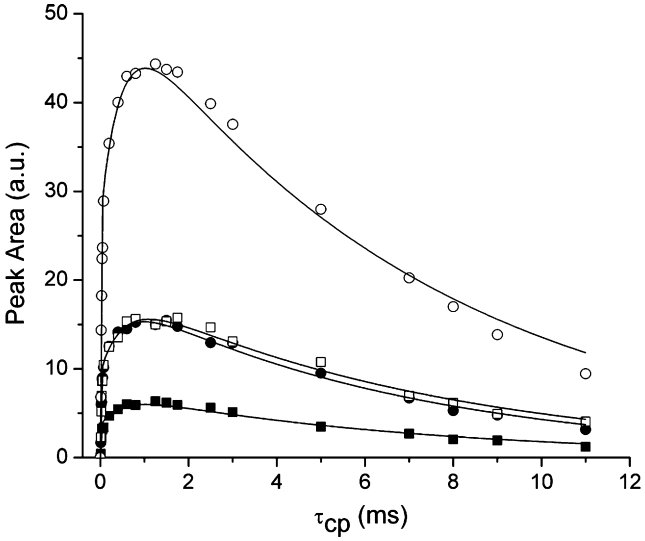

Figure 4. Analysis of the decomposed C1 peak components for dry CHAS II as a function of contact time used in the CP/MAS NMR experiments. ○ Peak A; □ peak B; ● peak C; ■ peak D.

obtained previously for a given sample, while allowing only the intensity of the peak to vary. The area of the peaks obtained was then plotted as a function of the contact time (Fig. 4).

Acknowledgment

Financial support from NSERC of Canada, the Canada Research Chair program, and Labopharm Inc. is gratefully acknowledged.

References

1. Mateescu, M. A.; Lenaerts, V.; Dumoulin, Y. U.S. Patent 5,456,921, 1995.
2. Lenaerts, V.; Moussa, I.; Dumoulin, Y.; Mebsout, F.; Chouinard, F.; Szabo, P.; Mateescu, M. A.; Cartilier, L.; Marchessault, R. *J. Controlled Release* **1998**, *53*, 225–234.
3. Mulhbacher, J.; Ispas-Szabo, P.; Mateescu, M. A. *Int. J. Pharm.* **2004**, *278*, 231–238.
4. Ravenelle, F.; Rahmouni, M. Contramid: high-amylose starch for controlled drug delivery. In *Polysaccharides for Drug Delivery and Pharmaceutical Applications*; Marchessault, R. H., Ravenelle, F., Zhu, X. X., Eds.; ACS: Washington, 2006; Vol. 934, pp 79–104.
5. Lenaerts, V.; Beck, R. H. F.; Van Bogaert, E.; Chouinard, F.; Hopcke, R.; Desevaux, C. U.S. Patent 6,607,748, 2003.
6. Mongin, G.; Yakusevich, V.; Koepe, A.; Shostak, N.; Pikhlak, E.; Popdan, L.; Simon, J.; Navarro, C.; Fortier, L.; Robertson, S.; Bouchard, S. *Clin. Drug Invest.* **2004**, *24*, 545–558.
7. Hernandez-Lopez, C.; Martinez-Farnos, L.; Karhu, D.; Perez-Campos, T.; Rovira, S.; Encina, G. *Method. Find. Exp. Clin. Pharmacol.* **2006**, *28*, 373–378.
8. Gidley, M. J.; Bociek, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 7040–7044.
9. Veregin, R. P.; Fyfe, C. A.; Marchessault, R. H.; Taylor, M. G. *Macromolecules* **1986**, *19*, 1030–1034.
10. Paris, M.; Bizot, H.; Emery, J.; Buzaré, J. Y.; Buléon, A. *Carbohydr. Polym.* **1999**, *39*, 327–339.
11. Shiftan, D.; Ravenelle, F.; Mateescu, M. A.; Marchessault, R. H. *Starch/Stärke* **2000**, *52*, 186–195.
12. Bogracheva, T. Y.; Wang, Y. L.; Hedlery, C. L. *Biopolymers* **2001**, *58*, 247–259.
13. Atichokudomchai, N.; Varavinit, S.; Chinachoti, P. *Carbohydr. Polym.* **2004**, *58*, 383–389.
14. Wu, H.-C. H.; Sarko, A. *Carbohydr. Res.* **1978**, *61*, 7–25.
15. Wu, H.-C. H.; Sarko, A. *Carbohydr. Res.* **1978**, *61*, 27–40.
16. Hizukuri, S. *Carbohydr. Res.* **1985**, *141*, 295–306.
17. Deval, F.; Crini, G.; Bertini, S.; Morin-Crini, N.; Badot, P. M.; Verbrel, J.; Torri, G. *J. Appl. Polym. Sci.* **2004**, *93*, 2650–2663.
18. Morgan, K. R.; Furneaux, R. H.; Larsen, N. G. *Carbohydr. Res.* **1995**, *276*, 387–399.
19. Le Bail, P.; Morin, F. G.; Marchessault, R. H. *Int. J. Biol. Macromol.* **1999**, *26*, 193–200.
20. Baille, W. E.; Malveau, C.; Zhu, X. X.; Marchessault, R. H. *Biomacromolecules* **2002**, *3*, 214–218.
21. Malveau, C.; Baille, W. E.; Zhu, X. X.; Marchessault, R. H. *Biomacromolecules* **2002**, *3*, 1249–1254.
22. Thérien-Aubin, H.; Baille, W. E.; Zhu, X. X.; Marchessault, R. H. *Biomacromolecules* **2005**, *6*, 3367–3372.
23. Thérien-Aubin, H.; Zhu, X. X. Water diffusion in drug delivery systems made of high-amylose starch as studied by NMR imaging. In *Polysaccharides for Drug Delivery and Pharmaceutical Applications*; Marchessault, R. H., Ravenelle, F., Zhu, X. X., Eds.; ACS: Washington, 2006; Vol. 934, pp 105–120.
24. Moussa, I. S.; Cartilier, L. H. *J. Controlled Release* **1996**, *42*, 47–55.
25. Gidley, M. J.; Bociek, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 3820–3829.
26. Paris, M.; Bizot, H.; Emery, J.; Buzare, J. Y.; Buleon, A. *Int. J. Biol. Macromol.* **2001**, *29*, 127–136.
27. Rondeau-Mouro, C.; Veronese, G.; Buleon, A. *Biomacromolecules* **2006**, *7*, 2455–2460.
28. Tang, H.; Hills, B. P. *Biomacromolecules* **2003**, *4*, 1269–1276.
29. Kolodziejski, W.; Klinowski, J. *Chem. Rev.* **2002**, *102*, 613–628.