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The effect of ball milling and rehydration on powdered mixtures of hydrocolloids

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ABSTRACT

In many applications, particularly in food related work, it is assumed that ball milling merely serves as a means of reducing crystallinity by the steady attrition of crystals. In this work mixtures of cellulose with other biopolymers have been co-ball milled in the dry state typically at moisture contents of <12% (w/w) and the effects of recrystallizing these mixtures studied. We have found that recystallizing the mixtures under a humid (97%RH) atmosphere increases the crystallinity of the cellulose fraction in a fashion governed by the other hydrocolloid present in the mixture. Some of the measured effects occur during ball milling of the dry powders. A relative method of fitting mixtures of type I and type II cellulose is described. Progressive transition between these forms with time was discovered for eucalyptus and microcrystalline cellulose at 97%RH. Locust bean gum (LBG) appeared to exert a protective effect on both eucalyptus and microcrystalline cellulose against the destruction of crystallinity by ball milling. For eucalyptus cellulose high levels of type I were produced during recrystallization with LBG under humid conditions. Both cellulose samples crystallized in the type I form in the presence of LBG whereas type II was produced in the presence of other hydrocolloids. Possible mechanisms for these unusual observations are proposed.

1. Introduction

The crystalline state of cellulose is a much discussed, controversial and industrially important topic. Common ways of changing the crystallinity include the ball milling (BM) of samples which reduces the crystallinity and ultimately produces an amorphous material (Howsmon & Marchessault, 1959). Conversely the presence of a humid atmosphere can cause a recrystallization of amorphous material normally to the stable type II polymorph of cellulose. Cellulose can exist in different polymorphic forms. The main distinction is between types I and II. Type I consists of single crystal fibrils where the cellulose chains are parallel, while in an adjacent fibril the chains are oriented in the opposite direction. Type II cellulose exhibits an antiparallel packing. This will be discussed in more detail later.

In the present experiments the ball milling of mixtures of cellulose with other hydrocolloids has been investigated. At first sight it might seem surprising that any effects other than the reduction of crystallinity would be observed, particularly as the ball milling is carried out in the dry powdered state where any interactions might be expected to be small even if the subsequent crystallization is carried out in a humid atmosphere. However substantial and contrasting effects of different hydrocolloids on the crystallinity of cellulose were observed and these form the basis of this paper.

Cellulose crystallization has been directly related to the formation of intermolecular hydrogen bonds (Matsuda, Kowsaka, Okajima, & Kamide, 1992). It was reported that amorphous cellulose recrystallization to cellulose II occurred after contact with water or at high relative humidities (RH) (Kimura, Hatakeyama, & Nakano, 1974; Kocherbitov, Ulvenlund, Briggner, Kober, & Arnebrant, 2010). Hatakeyama and Hatakeyama (1981) suggested that hydrogen bond formation occurs simultaneously with the rearrangement of cellulose molecules by heat treatment. Heat was also found to significantly increase the crystallinity of wood cellulose (Bhuiyan, Hirai, & Sobue, 2000).

However, Howsmon and Marchessault (1959) reported that under conditions of slow recrystallization or after short times of ball milling, amorphous cellulose could be made to return to the cellulose I configuration, although cellulose II is the more thermodynamically stable. They suggested from energy and entropy considerations that in the amorphous state the chains return to the cellulose I configuration under conditions of moderate swelling. This allows only slow recrystallization initiating from points of residual order and so favours the cellulose I lattice. Similar results concerning recrystallization of ball milled cellulose have been reported by other workers (Ago, Endo, & Hirotsu, 2004;

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Table 1
Acetate and pyruvate levels for the different xanthan samples.

Xanthan	Abbreviation	Acetate (%)	Pyruvate (%)
Standard acetate (SA)/standard pyruvate (SP)	SA/SP	6.19	3.85
Standard acetate (SA)/low pyruvate (LP)	SA/LP	6.02	2.20
Standard acetate (SA)/high pyruvate (HP)	SA/HP	5.10	6.49

Iyer, Sreenivasan, Chidambareswaran, & Patil, 1984; Paes et al., 2010).

In order to probe cellulose recrystallization, ball milling of cellulose alone or in 1:1 mixtures of polysaccharides containing β 1–4 linked backbones, and showing low or no crystalline content themselves, has been carried out and the recrystallization of cellulose in the presence of these polysaccharides compared with cellulose on its own.

2. Materials and methods

2.1. Hydrocolloids

Galactomannan (LBG) and a range of xanthan samples (see Table 1), having variable pyruvate substitutions; (SA/SP) standard acetate/standard pyruvate, (SA/LP) standard acetate/low pyruvate and (SA/HP) standard acetate/high pyruvate were kindly provided by Danisco. The glucomannans Propol RS and low molecular weight konjac mannan (Rheolex) were supplied by the Shimizu Chemical Corporation and xyloglucans 3 and 8 (3 higher M_w and 8 lower M_w) by the Dainippon Pharmaceutical Corporation.

Two types of celluloses have been used in this work; Avicel PH-101, also known as microcrystalline cellulose (MCC DP [degree of polymerization] ≈ 200) from Sigma chemicals and eucalyptus pulp (DP ≈ 600).

Polysaccharides were used directly as supplied with no further treatment or purification.

2.2. Ball milling

Ball milling was carried out on a Planetary Mill (Pulverisette 5, Fritsch, Germany). Balls and bowls were made of agate (ball diameter: 20 mm, bowl volume: 250 ml). Ball milling was carried out at 200 rpm over 4 h with 5 min of ball milling followed by 5 min of rest to allow for heat dissipation. The effective ball milling time was therefore 2 h. Eucalyptus cellulose was pre-ground for 1–2 min in a coffee grinder prior to ball milling.

2.3. X-ray diffraction and curve fitting

Samples were prepared by filling plastic holders with randomly oriented powders. X-ray measurements were carried out on a Bruker D5005 diffractometer (Bruker AXS, UK) using copper K alpha (CuKα) radiation of wavelength 1.5418 Å. Slit focus reflection geometry and rotational averaging were used.

Relative crystallinity determination was performed by firstly applying a linear background over the range of the main diffraction features. The diffraction peaks were then simulated as a series pure Gaussian functions. For cellulose I these represent the $1\bar{1}0$, 110 and 200 crystallographic planes whilst for cellulose II they represent $1\bar{1}0$, 110 and 020 planes. One broad pure Gaussian feature was used for the amorphous component. This method is based on the work of Carrillo, Colom, Sunol, and Saurina (2004). In order to reduce the number of variables in the fitting regime, positions, relative intensities and widths were restricted to typical values for cellulose I and II diffraction peaks, whilst single variables describing the overall pattern position and pattern intensity were allowed to vary independently. Any group position which was deemed to be outside acceptable limits was flagged and a statistical parameter giving the goodness of fit was noted (see Table 2). The statistical parameter determined whether the sample was predominantly type I, II or a mixture. Slight variations in overall pattern position were due to small errors in the height of the samples as well as reflecting probable genuine small differences in the sample.

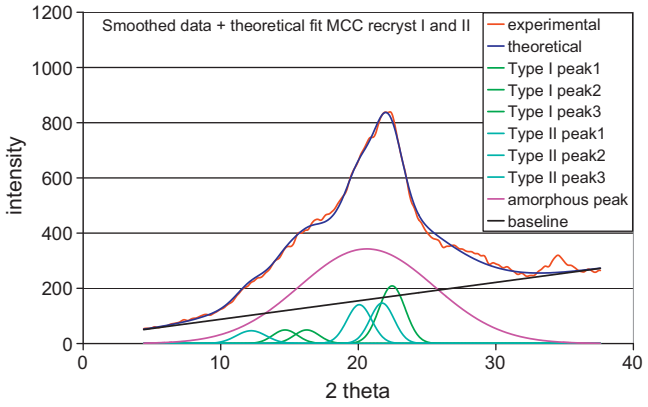

Fig. 1. The recrystallization in microcrystalline cellulose after 7 days over a relative humidity of 97%. The percentages of crystallinity based purely on area for type I and type II were 12.3 and 13.5, respectively. The feature at $2\theta = 35$ is commonly observed in these type of samples. It is sometimes ascribed to water related structures but may be a genuine weak diffraction peak. It is not used in these fits. The method is an extension of that of Carrillo et al. (2004).

In this way an estimate of the relative contents of cellulose I and II allomorphs could be obtained for the amorphous as well as recrystallized samples. However this method must be regarded as only relative and will only give values comparable between samples assessed using the same method. It is not an absolute method principally due to the unknown background scatter and the limited angular range of the X-ray measurement.

The data manipulation was carried out using intensity vs. 2θ data in the *Microsoft Excel software* package with the *Solver add in*. Fig. 1 shows the results for fits using a type I and type II mixed pattern. For some hydrocolloids (in particular the xanthans) features were observed in the pattern which rendered fitting extremely difficult. In this case the values can only be regarded as very approximate.

3. Results and discussion

Ball milling carried out over a long period leads to complete disruption of the cellulose I crystalline pattern and upon recrystallization only the cellulose II polymorph is formed (Howsmon & Marchessault, 1959). The present study however is performed over moderate ball milling times which, due to the remaining small crystals of type I polymorph, results in the production of both type I and II polymorphs upon recrystallization and raises the possibility of transformations between these forms and the effect on these transformations of the other cellulose-like polysaccharides present in the mixture.

Fig. 1 illustrates the fitting method to obtain estimates of type I and type II polymorph levels. The recrystallization in Microcrystalline Cellulose after 7 days in a relative humidity of 97% is shown and the percentages of crystallinity returned for type I and type II

Table 2
Selected fits to the crystalline component in hydrocolloid mixtures and cellulose alone. The goodness of fit parameter measures the difference between the experimental and theoretical data, hence the lower the value the better the fit. A starred value indicates that this is a poor fit due to either a high sum of square difference value or the positions of the peaks being too far away from acceptable cellulose type I and II values. Abbreviations: MCC, micro-crystalline cellulose; EU, eucalyptus cellulose; LBG, locust bean gum; XYLO, xyloglucan; SA/LP, standard acetate/low pyruvate.

Hydrocolloid system	Type I %age	Type II %age	Goodness of fit parameter
MCC day 7			
Type I fit	16.6	–	792*
Type II fit	–	19.7	453*
Type I + II fit	12.3	13.5	200
EU day 7			
Type I fit	15.1	–	535*
Type II fit	–	18.6	260
Type I + II fit	8.9	13.0	137
MCC + LBG day 7			
Type I fit	9.0	–	241
Type II fit	–	8.3	385*
Type I + II fit	11.1	3.7	191*
EU + LBG day 7			
Type I fit	24	–	150
Type II fit	–	19.9	1245*
Type I + II fit	25.8	4.1	140*
MCC + PROPOL day 7			
Type I fit	7.9	–	340.4*
Type II fit	–	9.9	121
Type I + II fit	2.6	10.0	118
MCC + XYLO3 day 7			
Type I fit	−9.7	–	399*
Type II fit	–	10.2	392
Type I + II fit	−7.0	5.4	190*
MCC + XANTHAN (SA/LP) day 7			
Type I fit	0	–	395
Type II fit	–	8.4	220
Type I + II fit	10.0	7.7	119*

were 12.3 and 13.5, respectively. This was regarded as a good fit based on two criteria. Firstly the sum of the squares of the difference between the experimental and theoretical curves was lower than for a fit using a type I or type II pattern alone (200 as opposed to 792 [I] and 453 [II]).

Secondly the shift multipliers which allowed the whole patterns to shift either to the right or left were close to 1 for both type I and type II. In other words the positions of the peaks were close to the optimal type I and type II patterns chosen as references.

Fig. 2a and b shows the X-ray patterns for selected polysaccharides individually before and after ball milling. Ball milling is commonly used to decrease crystallinity of cellulose and other materials by mechanical attrition (Ago et al., 2004; Maier, Zipper, Stubicar, & Schurz, 2005; Ouajai & Shanks, 2006). The X-ray patterns for most of the polysaccharides except cellulose and the xyloglucans exhibited simple broad scattering halos as would be expected for amorphous polymers. Cellulose (both eucalyptus and MCC) is the only polymer with a reasonably sharp and distinctive crystalline pattern (Fig. 2a). The xyloglucans (both 3 [shown] and 8) have a semicrystalline pattern. The individual polysaccharides after ball milling (Fig. 2b) all showed a broad amorphous pattern. Cellulose samples with initially different crystal structures (cellulose I and II) have been found to yield virtually the same amorphous X-ray scattering curves after ball milling as reported by Maier et al. (2005).

3.1. Crystallinity correction factors

Fig. 2c shows scattering curves for selected mixtures of MCC with several polysaccharides before ball milling. Table 3 gives the optimal multiplying factors for the individual scattering curves (Fig. 2a) required to mimic these 50/50 mixtures of hydrocolloids. There was concern that the diffraction curves from the various hydrocolloids varied substantially in intensity. This can be due to several factors such as different holder packing density and scattering power. Thus a 50/50 wt./wt. mixture of 2 components will not necessarily give a 50/50 division of areas. Hence correction factors may have been necessary for the mixtures and these values used to correct apparent crystallinities when these are calculated from the areas under the curves. Table 3 which uses the data shown in Fig. 2a and c gives a list of these values. Despite the fact that the packing densities of the original polysaccharides were probably different the factors were close to 0.5 for the mixtures. Only in the case of the MCC/xanthan (SA/LP) mixture was a substantial deviation observed. Even in this case changing the factor to 0.5 still produced a reasonably close match to the mixture as judged by eye. This gives confidence in the relative order of the subsequent calculated crystallinity.

3.2. Xanthan

In the case of xanthan, there is limited work in the literature about its conformation in the powder state with controversial data about the level of crystallinity. Hartley, Chevance, Hill, Mitchell, and Blanshard (1995) indicated that the X-ray pattern of xanthan obtained from Sanofi Bio-industries shows a broad scattering halo (expected for amorphous polymers) at low water content. However, Su, Ji, Lan, and Dong (2003) reported that dry xanthan gum purchased from Shandong Food and Fermentation

Table 3
Calculated multiplying factors from the theoretical fits to 50/50 microcrystalline cellulose (MCC)/polysaccharide mixtures (see Fig. 2a and c).

Microcrystalline cellulose/polysaccharide mixture	MCC factor	Polysaccharide factor
MCC/locust bean gum	0.509	0.458
MCC/Propol	0.522	0.447
MCC/xanthan (SA/LP)	0.47	0.579
MCC/xyloglucan3	0.521	0.447

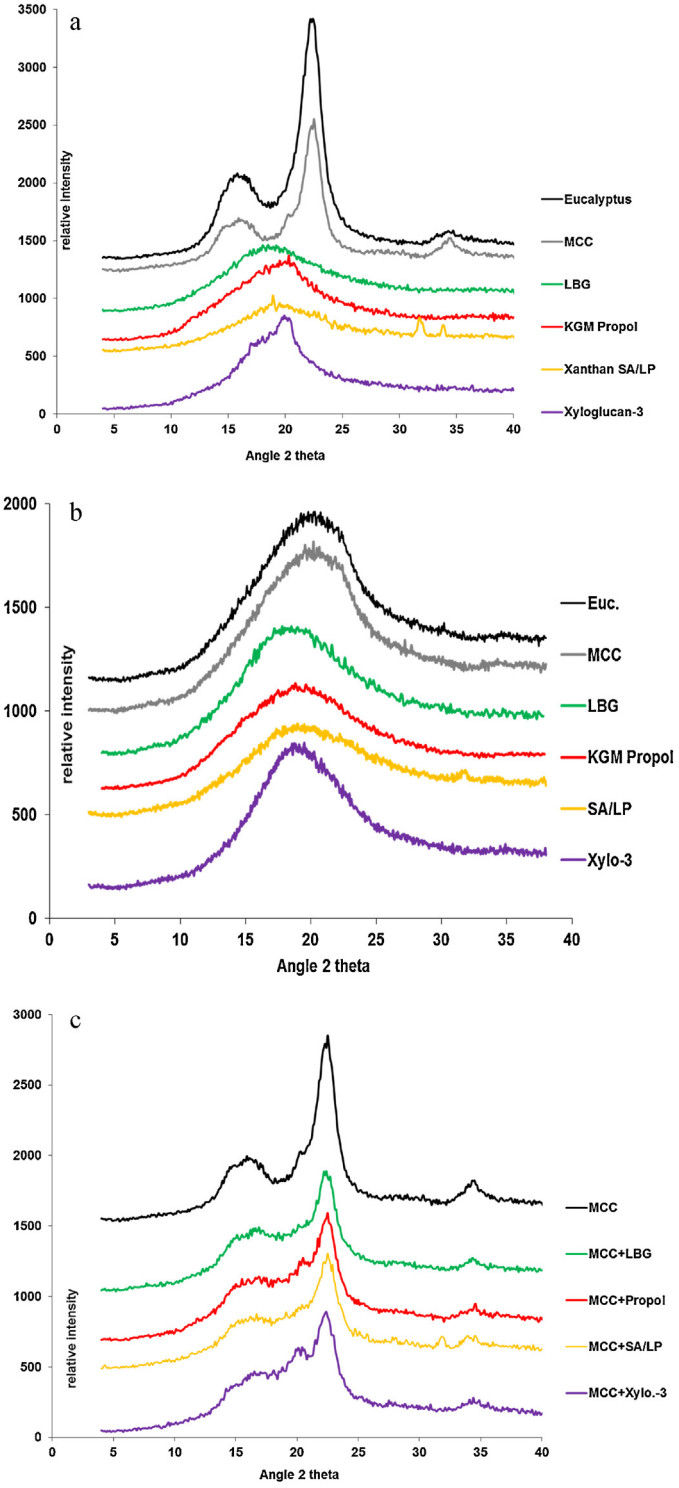

Fig. 2. The X-ray scattering patterns of the individual polysaccharides (a) before and (b) after ball milling. (c) Experimental scattering curves for 50/50 mixtures of hydrocolloids. See Table 2 for the best theoretical fits using an optimal combination of the scattering patterns for the individual polysaccharides in a. The polysaccharides in c have not been ball milled. Once again note the feature at $2\theta = 35$ in many of these traces.

Engineering Research Major Laboratory is highly crystalline and its X-ray pattern exhibited two sharp peaks.

In the present work we do not observe the crystallinity seen by Su et al. (2003); however, small crystalline peaks are observed. These peaks could be due to presence of different salts in the broth,

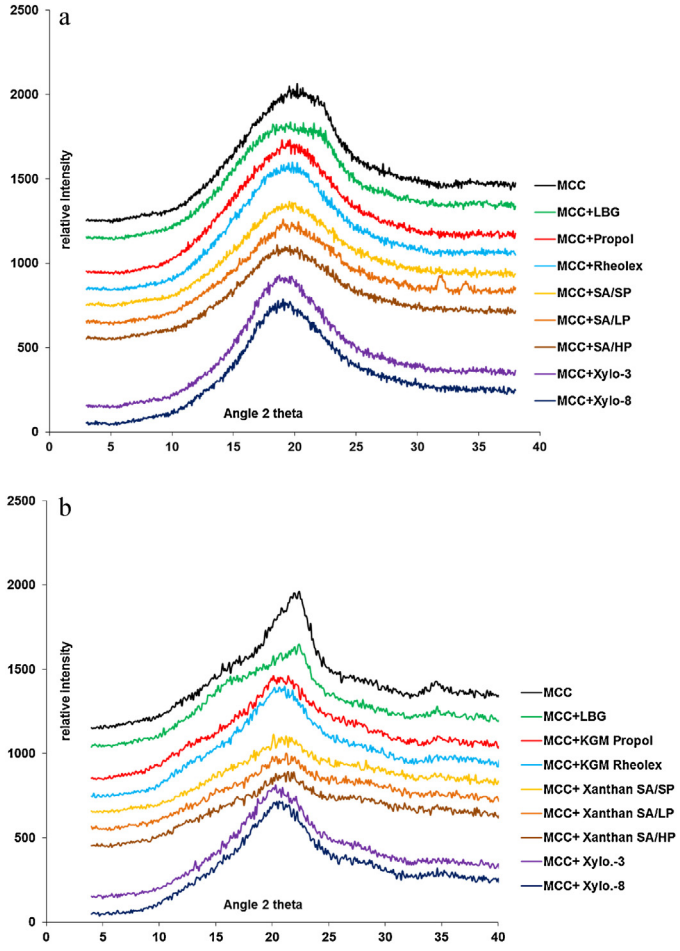

Fig. 3. The X-ray scattering patterns of the co-ball milled MCC/polysaccharide mixtures (a) (top) immediately after ball milling and (b) (bottom) after 7 days storage over P_2O_5 and 7 subsequent days storage over a saturated K_2SO_4 solution which gave an RH of 97%.

remaining after the fermentation process (Raschip, Vasile, Ciolacu, & Cazacu, 2007). On increasing the water content (at a relative humidity of 97%) these peaks decrease in magnitude possibly due to solvation. For a more thorough discussion of sharp peaks in the diffraction patterns of xanthan and in particular the peaks observed here in the region of 30–35 2θ, see Lad et al. (2013).

3.3. Co-ball milling

Fig. 3 shows the X-ray patterns of selected cellulosic backbone polysaccharides co-ball milled with MCC before and after storage for seven days in a sealed chamber over a saturated salt solution (K_2SO_4 RH 97%). The patterns show a protective effect of LBG for both Eucalyptus (see Fig. 4b) and MCC, in that the LBG containing samples are not completely amorphous compared for example with the SA/SP xanthan containing sample which produces an amorphous halo. The recrystallization of both celluloses is retarded in the presence of xanthan (and also konjac mannan and xyloglucan). An obvious explanation for this observation would be that the presence of foreign polysaccharide chains is a barrier for the cellulose to form an ordered crystalline structure; an effective poisoning of the crystal. However, mixing locust bean gum with the celluloses seems to enhance recrystallization in terms of the eventual crystal content, in comparison with other mixtures.

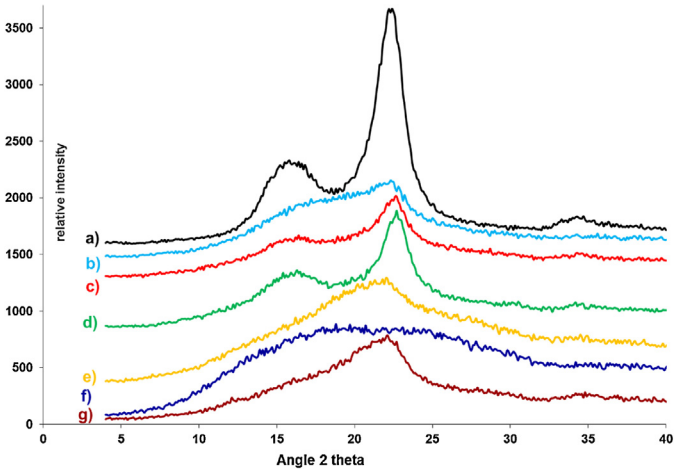

Fig. 4. Detailed effects of LBG on the recrystallization properties of eucalyptus cellulose. (a) Original native eucalyptus non-ball-milled; (b) co-ball milled eucalyptus and LBG immediately after ball milling; (c) co-ball milled eucalyptus and LBG after 7 days drying over P_2O_5 and subsequent transfer to RH 97% for 7 days; (d) co-ball milled eucalyptus and LBG stored for 6 months over P_2O_5 and then transferred to RH 97% for 7 days; (e) as c but ball milled separately and then mixed; (f) ball milled LBG after 7 days drying over P_2O_5 and then subsequent transfer to RH 97% for 7 days and (g) ball milled eucalyptus after 7 days drying over P_2O_5 and subsequent transfer to RH 97% for 7 days.

3.4. Detailed effects of locust bean gum

The effect of locust bean gum on the recrystallization of eucalyptus cellulose in co-ball milled mixtures is complex. As can be seen in Fig. 4b the mixture of eucalyptus and LBG shows a relatively large crystallinity immediately after ball milling. Fig. 4c (crystallization after 7 days at RH 97%) shows that the recrystallization in the presence of LBG is far more rapid than in the presence of the other polysaccharides (compare with Fig. 3b).

Fig. 4 shows some detailed effects of LBG on the recrystallization properties of eucalyptus cellulose. The protective effect of LBG against ball milling can be seen by eye in that trace b (immediately after co-ball milling) exhibits more crystallinity than trace e (immediately after separate ball milling and mixing). Also shown on this trace are several other control experiments. There is a suggestion of a basal level of crystallization occurring during storage over phosphorus pentoxide; compare the traces for 6 month storage with the X-ray pattern taken immediately after ball milling where the crystalline peaks for the former are more intense and sharper.

3.5. Mechanism for the effect of locust bean gum

The difference between the effects of LBG (a galactomannan) and the other β 1–4 linked polysaccharides on the recrystallizaton of celluloses, particularly konjac mannan (Rheolex) (a glucomannan), appears to be due to the peculiar structural properties of LBG.

Typical galactomannans contain a β 1–4 linked D-mannan backbone, to which are attached α-D-galactose units at the O6-position of the D-mannose residues. The main chain is structurally similar to that of cellulose which is also β 1–4 linked. Mannose is an epimer of glucose at the 2-position, therefore glucose and mannose differ only in the orientation of the OH group at position 2. The compatibility of the cellulosic and mannan backbones explains why galactomannans can bind to xanthan and cellulose (Cairns, Miles, Morris, & Brownsey, 1987).

Substitution of the mannan chain by more than 12 wt.% of galactose makes the galactomannans water soluble. The galactose substituents can also influence the crystalline association of mannan chains (Marchessault, Buleon, Deslandes, & Goto, 1979; Newman & Hemmingson, 1998). The large galactose side chain content prevents strong cohesion of the main backbones of different neighbouring macromolecules, so that no extensive crystalline regions can be formed between the galactomannan chains. Water at room or elevated temperatures can thus easily penetrate between the single molecules to hydrate or dissolve the accessible gum.

Intermolecular associations between galactomannans also occur in water. The degree of association is increased as galactose content decreases and is alkali sensitive, implying that association is via unsubstituted mannan regions in the backbone (Whitney, Brigham, Darke, Reid, & Gidley, 1998; Wielinga, 2000).

The influence of the galactose substituents on the mannan chain associations may be the reason for the difference between glucomannan and galactomannan effects on cellulose recrystallinity observed here. The polysaccharide backbone in konjac mannan consists of mannose and glucose units linked in the same way (β 1–4) as the glucose residues in cellulose. However, unlike the mannan backbone of LBG, it does not have sugar side-chains which can block association with cellulose during and after ball-milling. It is proposed that the interaction between konjac mannan and the cellulose interferes with recrystallization whereas the more limited association between cellulose and LBG allows the recrystallization of the cellulose but may prevent destruction of the crystals during ball milling by crosslinking the chains strongly at particular sites.

The crystallization and precipitation of cellulosic backbone polysaccharides on the cellulose fibre surface has been reported previously. Chanzy, Dube, and Marchessault (1978), Chanzy, Imada, Mollard, Vuong, and Barnoud (1979), and Chanzy, Grosrenaud, Joseleau, Dube, and Marchessault (1982) used suspensions of microfibril cellulose in aqueous crystallization media for co-crystallization of mannan backbone polysaccharides to form "shish-kebab" structures, in which mannan adopts a two-fold screw conformation closely matching that of cellulose. In this form mannan chains act as cross linking bridge "kebab" between cellulose microfibril "shish".

Whitney, Brigham, Darke, Reid, and Gidley (1995, 1998) used fermentation cultures with control incubations of *Acetobacter aceti* ssp. *xylinum* which additionally contained xyloglucan, LBG fractions or konjac glucomannan for the production of cellulose. They reported that whilst xyloglucan, konjac glucomannan and low galactose galactomannan induced a coalescence of cellulose fibrils and a dramatic reduction (30–60%) of crystallinity, higher galactose galactomanan only slightly reduced (15%) the crystallinity, and the cellulose molecular organization was much less disrupted. They indicated that the polysaccharides in the cellulose matrix were less mobile when aligned with or incorporated in the cellulose fibril which occurred to a greater degree for konjac mannan and low galactose galactomanan. Polysaccharides were more mobile when acting as cross linking bridges which occurred for high galactose galactomanan samples.

In this study, similar experiments were also carried out between mixtures of celluloses and selected polysaccharides (data not shown) after ball milling each polymer separately, in order to investigate the effect of the absence of "mechanical contact" forces between the two types of polymers chain on recrystallization. These are not considered in detail here; however, interestingly, the X-ray patterns of MCC/LBG and eucalyptus cellulose/LBG mixtures were dramatically different from co-ball milled samples after 7 days storage at an RH of 97% and now showed no protective effects or enhanced rates of recrystallization relative to the other polysaccharides (compare Fig. 4c and e). Therefore, it seems that the presence of mechanical forces between celluloses and LBG during ball milling is required to see the effects of LBG-cellulose binding.

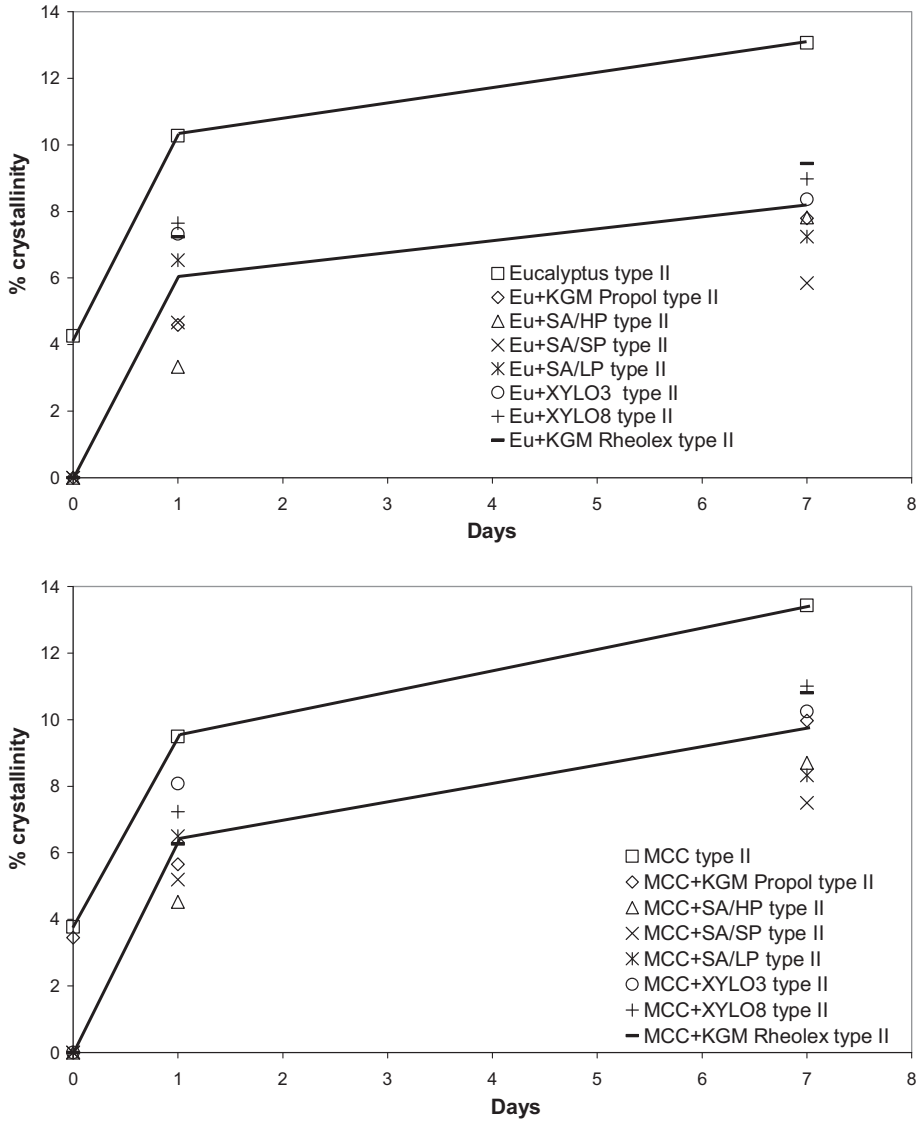

Fig. 5. Type II crystallinity for (a) eucalyptus cellulose and (b) MCC; individually and in mixtures with other polysaccharides as a function of time. Samples were stored over a saturated salt solution at RH 97%. Added lines are merely an aid to the eye and are not based on any fit.

3.6. Crystallization to type II cellulose

Fig. 5a and b shows the crystallinity of eucalyptus cellulose and MCC individually and with other polysaccharides as a function of time. These plots show the recrystallization to type II cellulose. LBG is not shown here as the crystallization which it promotes is to type I (see Fig. 7). As expected the absolute levels of crystallinity of the pure cellulose samples are higher than for the mixtures with other polysaccharides as the mixtures have only 50% of the absolute amount of cellulose present and hence we would expect a 50% reduction. As can be seen on the plots this varies greatly for the different polysaccharides. It must be borne in mind in this comparison that the pure systems also have type I present as well as type II. There is a clear order of the effectiveness for the different polysaccharides. The ranking for the effectiveness of a polysaccharide at reducing the level of crystallinity of cellulose is shown in Fig. 6. This is a correlation between the effects of the polysaccharides on MCC and Eucalyptus. As the MCC and eucalyptus experiments and analyses are independent, these correlations give us confidence in the validity of the results. The xyloglucans, being the least effective at reducing crystallinity, lie at the bottom of the curve; the xanthans, being the most effective, lie at the top, with the konjac mannans in

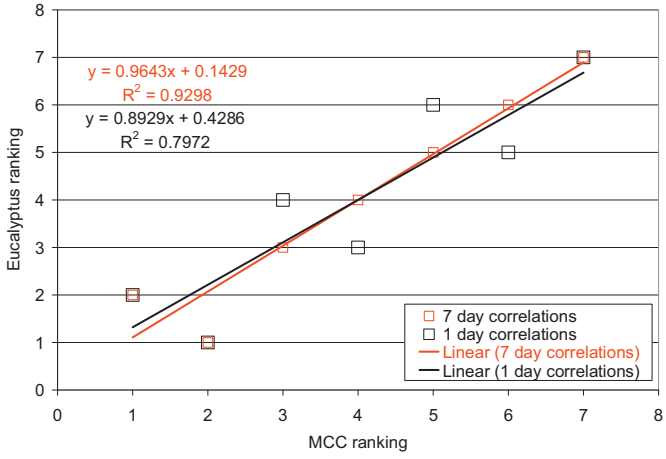

Fig. 6. The correlation between the ranking order of the effectiveness of a polysaccharide on the crystallization levels of MCC and eucalyptus cellulose. Lowest numbers correspond to the highest crystallinity of the cellulose and hence the weakest effect on the recrystallization. See text for sample allocation.

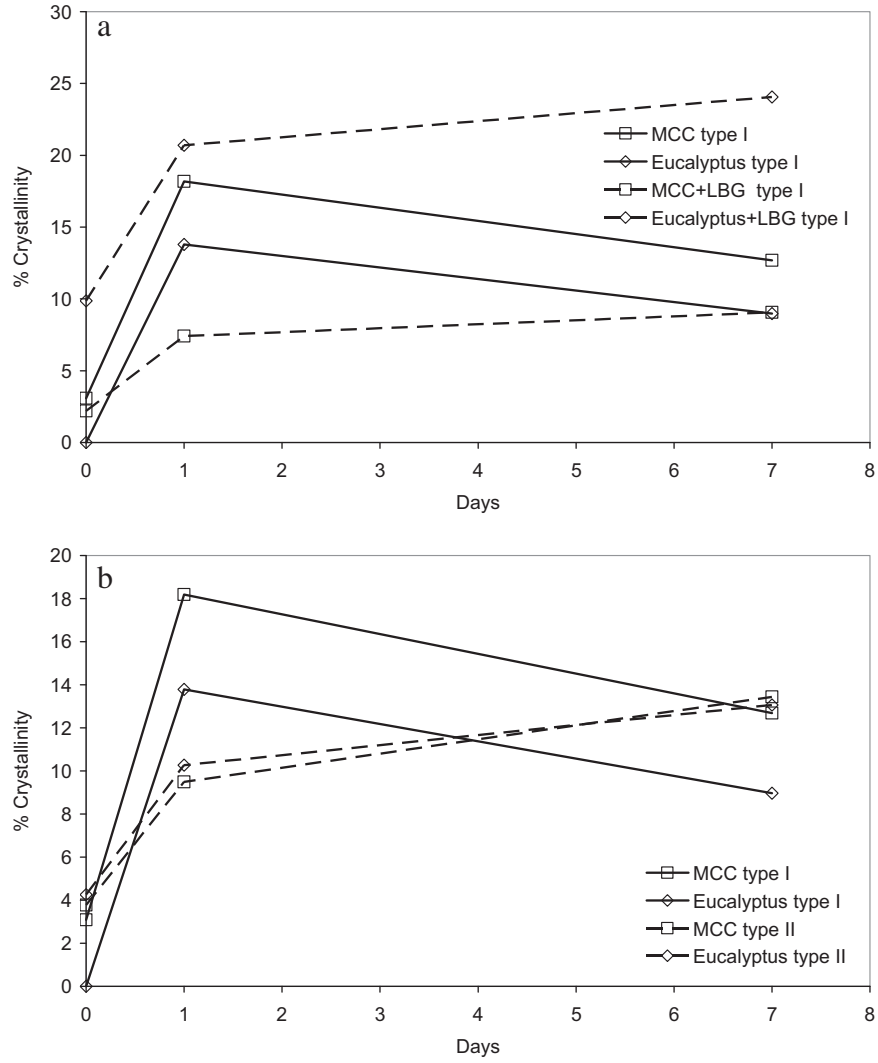

Fig. 7. (a) (top) Type I crystallinity of eucalyptus and MCC cellulose individually and in mixtures with LBG as a function of time. Samples were stored over a saturated salt solution giving an RH value of 97%. (b) (bottom) Type I and Type II crystallinity of eucalyptus and MCC cellulose as a function of time. Samples were stored over a saturated salt solution giving an RH value of 97%. Notice the apparent slow conversion of Type I to Type II in b.

the middle. The calculated degree of type II crystallization is listed in Table 4. These results are not easily explained by either binding arguments presented above or charge arguments related to the charge on the xanthans.

Both ball milled celluloses, i.e. MCC and eucalyptus individually have degrees of crystallinity of about 13% after 7 days storage over RH 97%. Generally the recrystallization of MCC to cellulose type II was slightly higher by about 1–2% in presence of different polysaccharides in comparison with eucalyptus. This could be due to lower molecular weight of MCC and consequently greater ease of recrystallization and less interaction with the other hydrocolloids.

3.7. Crystallization to type I cellulose

Fig. 7a shows the degree of crystallinity of ball milled MCC and eucalyptus cellulose individually and co-ball milled with LBG as a function of time. Once again the recrystallization occurs in a sealed chamber over a saturated salt solution (K_2SO_4 RH 97%). The observed rates (slopes of lines) are similar in the presence of LBG and there is no conversion to type II with time as is seen in the pure samples; in fact the degree of recrystallization to cellulose type II is negligible for both types of cellulose in the presence of LBG (Table 2).

Table 4
The calculated cellulose type II crystallization for different samples after 7 days storage over RH 97%. Abbreviations: MCC, micro-crystalline cellulose; EU, eucalyptus cellulose; XYLO, xyloglucan; SA/HP, standard acetate/high pyruvate; SA/LP, standard acetate/low pyruvate; SA/SP, standard acetate/standard pyruvate.

BM polysaccharides b	Crystallinity (Type II)	BM polysaccharides b	Crystallinity (Type II)
MCC	13.4	EU	13.1
MCC + Xylo.-8	11.0	EU + KGM Rheolex	9.4
MCC + KGM Rheolex	10.8	EU + Xylo.8	9.0
MCC + Xylo.-3	10.2	EU + Xylo.3	8.4
MCC + KGM Propol	10.0	EU + KGM Propol	8.1
MCC + SA/HP	8.7	EU + SA/HP	7.8
MCC + SA/LP	8.3	EU + SA/LP	7.2
MCC + SA/SP	7.5	EU + SA/SP	5.9

3.8. Conversion of crystalline forms

Fig. 7b shows the recrystallization in pure samples of ball milled eucalyptus cellulose and MCC as they crystallize to both forms I and II as a function of time. The degree of crystallinity of type I after 1 day of storage at 97% RH for eucalyptus cellulose and MCC is around 18% and 13%, respectively. The level of type II is approximately 10% for eucalyptus cellulose and 9% for MCC. After 7 days storage at high RH 97%, the degree of crystallinity of type I was reduced to about 9% and 12% and for eucalyptus cellulose and MCC whilst the type II level increased to about 13% for both samples. It appears that after ball milling, recently recrystallized type I cellulose can gradually transform to type II.

Cellulose I consists of micro fibrils; so called "single crystal fibrils", where the cellulose chains are parallel, while in an adjacent fibril the chains are oriented in the opposite direction. During recrystallization from the amorphous state in the presence of sufficient water, cellulose I chains may move and combine with adjacent chains to form the antiparallel packing of cellulose II. This implies that a sufficiently high water content and storage time are required for the transformation to cellulose II.

The transformation of cellulose I with a specific amount of water to cellulose II during ball milling has been reported by Ago et al. (2004). They reported that native cellulose I if ball milled for 2 h with water (30 wt.%) in the solid state transformed from the cellulose I to cellulose II polymorph during ball milling. In the work presented here, the transformation occurred after ball milling and 7 days storage over saturated K_2SO_4 solutions (RH 97%).

Generally the crystalline conversion of cellulose I to cellulose II occurs in the mercerization–regeneration treatment of native cellulose, by use of an aqueous sodium hydroxide solution with a concentration of more than 11 wt.% (Kiessig, 1939). In this process, cellulose molecules exist in a highly swollen state in alkaline solution, due to the formation of alkali–cellulose complexes ($C_6H_{10}O_5{\cdot}NaOH{\cdot}3H_2O$), and recrystallize in an antiparallel manner to form the energetically favourable cellulose II polymorph after washing with water (Hayashi, 1989; Okano & Sarko, 1985).

4. Conclusions

The main conclusion of this work is that polysaccharides can affect recrystallization rates of cellulose when co-ballmilled in mixtures.

Passage through the solution state appears not to be necessary in the case of LBG, the ball milling of dry samples being sufficient to observe the effects. LBG is particularly effective at both protecting cellulose against reduction of crystallinity by ball milling and in increasing subsequent recrystallization.

There is a well-defined order among the other polysaccharides tested here in their ability to reduce recrystallization of cellulose to type II in a humid atmosphere; however from the data presented it is difficult to argue conclusively whether the polysaccharides exert their effect by protecting to various degrees against crystallization during ball milling or by altering the subsequent recrystallization under the humid atmosphere.

After ball milling, newly recrystallized type I cellulose gradually converts to type II at an RH of 97% and ambient temperature.

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