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Partial Depolymerization of Hydroxypropylmethyl Cellulose for Production of Low Molar Mass Polymer Chains

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ABSTRACT. Low molar mass (LMM) biopolymers are highly required to design functional nanomaterials, which mainly find application in biomedical fields. However, the synthesis of LMM polymer is a challenging task. In this work, we report a partial enzymatic depolymerization process which allows to produce a series of LMM hydroxypropylmethyl cellulose (HPMC) polymer, with a weight average molar mass (M_w) under and over 10000 g.mol⁻¹ and low dispersity ($D < 1.5$). Variation of the starting HPMC grade, reaction time, and enzyme concentration were the key parameters to control the M_w and yield of the target molecules. This approach provides a versatile way of producing LMM HPMCs with varying degrees of substitution, and having a single reactive aldehyde function at one chain extremity. LMM HPMC can find for instance application as building blocks for the development of new functional molecular architectures.

KEYWORDS. Hydroxypropylmethyl cellulose, HPMC, endoglucanase, controlled polymer, low molar mass polymer.

1. INTRODUCTION

Depolymerization of bio-based polymers in particular polysaccharides, proteins and lignin is an attractive field of research to yield high-value chemicals and biofuel from renewable resources (Azadi, Inderwildi, Farnood, & King, 2013; Chalamaiiah, Dinesh Kumar, Hemalatha, & Jyothirmayi, 2012; Wahlström & Suurnäkki, 2015). Most studies aim at producing low molar mass compounds including monosaccharides and/or other platform chemicals to replace petroleum based chemicals. This can typically be done through a more or less complete

depolymerization of polymers with the notable exception of protein hydrolysis from which bioactive peptides are often sought.

Hydroxypropylmethyl cellulose (HPMC), a linear polysaccharide, is a nonionic biopolymer randomly substituted with *O*-methyl and *O*-2-hydroxypropyl groups in their anhydroglucose units (AGUs) as presented in Fig. 1 (Fitzpatrick et al., 2006; Ford, 2014). The average number of substituents is generally characterized by the degree of substitution (*DS*) and the molar substitution (*MS*) (Fitzpatrick et al., 2006; Ford, 2014; Dow, 2002). *DS* is the average number of substituted hydroxyl groups per AGU and the values can range between 0 – 3 whereas *MS* refers to the average number of substituents per AGU. As propylene oxide can react with the hydroxypropyl substituent, chain propagation can take place and *MS* becomes higher than *DS*. However, the value of hydroxypropyl *MS* is generally small (0.13 - 0.82) in commercially available HPMCs (Dow, 2002). As a cellulosic polymer, HPMC hold an exclusive reducing hemiacetal group at the C1 position at one extremity of the chain while the other one has an alcoholic hydroxyl (OH⁻) group at the C4 position (Pinkert, Marsh, Pang, & Staiger, 2009; Schagerlöff et al., 2006). To date, several HPMC grades based on their *DS*, *MS* and weight average molar mass (M_w) are commercially available. The *DS*, *MS* and M_w are important parameters to determine HPMC properties such as water-solubility, retention, thickening and thermos-responsiveness (Adden, Melander, Brinkmalm, Gorton, & Mischnick, 2006; Adden, Muller, & Mischnick, 2006; Levina & Rajabi-siahboomi, 2014).

HPMC is one of the widely used cellulose ethers as hydrophilic matrix in drug delivery system (Ford, 2014; Jain et al., 2014). The reasons relate mainly to its biocompatibility, film forming ability, tabletability and compactability (Dong, Zhou, Lin, Shen, & Feng, 2018; Chang & Zhang, 2011; Hoo et al., 2013; Van Vlierberghe, Dubruel, & Schacht, 2011). Other properties such as

1 water retention, adhesive, surface activity, emulsifying, stabilizing, etc. make HPMC relevant in
2 formulation systems going from construction materials, cosmetic and food industries (Clasen &
3 Kulicke, 2001).

4 Although, HPMC is available in numerous grades their broad dispersity (\bar{D}), high M_w and
5 variation of DS (batch to batch) limit the range of application (Khatri, Katikaneni, Desai, &
6 Minko, 2018; Oh, Heng, & Chan, 2015; Viriden, Wittgren, Andersson, & Larsson, 2009). An
7 effective control over the macromolecular features including M_w and \bar{D} is then highly desirable
8 to enlarge the scope of HPMC application. This issue is especially true for the design of new
9 polymeric materials and nanomaterials (Flory, 1953; Hillmyer, 2007; Spinnrock & Colfen,
10 2018). Recently, polysaccharides have been proposed to become part of nanomedicine platform
11 where low M_w polymers ($< 22000 \text{ g.mol}^{-1}$) with low \bar{D} are generally required (Camacho,
12 Menegatti, & Mitragotri, 2016; Chae, Jang, & Nah, 2005; Gao & Edgar, 2019; Gray, Mulloy, &
13 Barrowcliffel, 2008; Mizrahy & Peer, 2012; Zhang et al., 2018). It was demonstrated that only
14 low M_w polymer can easily penetrate into cell wall; it is recognized that increasing the M_w
15 drastically reduces the penetration rate (Chae et al., 2005; Mizrahy & Peer, 2012).

16 Polysaccharides with M_w below 10000 g.mol^{-1} have been proven to be useful for the
17 preparation of nanostructures that can self-assemble as previously described for amphiphilic
18 glycoprotein analogues with numerous potential applications in drug delivery, biomedical
19 devices and sensors (Breitenbach, Schmid, & Wich, 2017; Li et al., 2018; Lu, Wang, Caceres, Li,
20 & Deratani, 2019; Schatz, Louguet, Le Meins, & Lecommandoux, 2009; Christophe Schatz,
21 2010; Wang, Caceres, Li, & Deratani, 2017). Furthermore, studies have shown that HPMC with
22 a low degree of polymerization can stabilize submicron emulsions, whereas substitution has little
23 influence (Camino & Pilosof, 2011). Other effects like lesser water retention, and low viscosity

1 can be also sought in given formulations (Poinot, Benyahia, Govin, Jeanmaire, & Grosseau,
2 2013).

3 Despite of its large number of applications in pharmaceuticals, cosmetics, food and
4 construction market, HPMC is not commercially available in M_w range down to about 20000
5 g.mol⁻¹ (Sepassi et al., 2007). Therefore, depolymerization is an attractive approach to produce
6 low molar mass (LMM) HPMC polymer. The vast majority of depolymerization studies on
7 polysaccharides involve acid and enzyme catalysis. Traditionally, mineral acid has been
8 extensively studied and several industrial processes have been proposed for production of sugars
9 from cellulose (Rinaldi & Schüth, 2009). The reaction is generally fast, going from 5 min to
10 longer time (> 1 h) and higher temperatures (> 100° C) (Rinaldi & Schüth, 2009). The chain
11 scission takes place stochastically so that even a partial acidic hydrolysis lead to a mixture of
12 products with varying degree of polymerization (DP) as exemplified in the case of xylan
13 (Akpınar, Erdogan, Bakir, & Yilmaz, 2010). Other treatment such as supercritical water
14 hydrolysis proceeds in the same way even in a much shorter time (less than 1 second) (Buffiere
15 et al., 2018). Nonetheless, both mineral acids and supercritical process can generate by-products
16 like furfural or levulinic acid (Rinaldi & Schuth, 2009; Tolonen et al., 2015) . By contrast,
17 enzymatic depolymerization proceeds gradually and selectively in mild conditions, with reaction
18 times higher than 1 h and less formation of by-products (Wahlström & Suurnäkki, 2015).
19 Enzymatic depolymerization also secure the presence of a unique aldehyde group at one end of
20 the hydrolyzed carbohydrate polymer (Melandar, Adden, Brinkmalm, Gorton, & Mischnick,
21 2006).

22 Two main groups of enzymes can be considered for cellulose depolymerization: oxidative
23 enzymes and cellulases (Horn, Vaaje-Kolstad, Westereng, & Eijsink, 2012). In the cellulase

family, endoglucanases (EG) and exoglucanases (EX) catalyze the internal chain scission and at the chain ends, respectively (Horn et al., 2012; Wahlström & Suurnäkki, 2015). So far, most of the studies done on the enzymatic depolymerization of cellulose derivatives have been performed to determine the structural features of the polymer, including the degree and position of substituents on AGU and their distribution along the polymer chain (Adden, Melander, Brinkmalm, Knarr, & Mischnick, 2009; Diao et al., 2017; Fitzpatrick et al., 2006; Momcilovic et al., 2005; Richardson & Gorton, 2003; Viriden, Larsson, & Wittgren, 2010). It was described that substitution hinders the enzymatic depolymerization since the active site of EG needs to bind to a certain number of lower substituted or unmodified AGUs to cleave the cellulosic polymer (Adden et al., 2009; Momcilovic et al., 2005). It is then expected that LMM HPMC can be produced using this route. Aside from enzymatic depolymerization, enzymatic polymerization could be another option but still the polymers are generally obtained in a limited yield with $\bar{D} > 2.5$ (Grimaud et al., 2018; Yamashita, Yamamoto, & Kadokawa, 2015).

In this study, we report a facile top-down approach for producing LMM HPMCs by using partial enzymatic depolymerization and phase separation of the polymer. Apart their M_w , the resulting HPMCs were characterized with respect to their chemical composition in terms of average level of methyl substitution (DS_{Me}) and of 2-hydroxypropyl groups (MS_{HP}) per AGU.

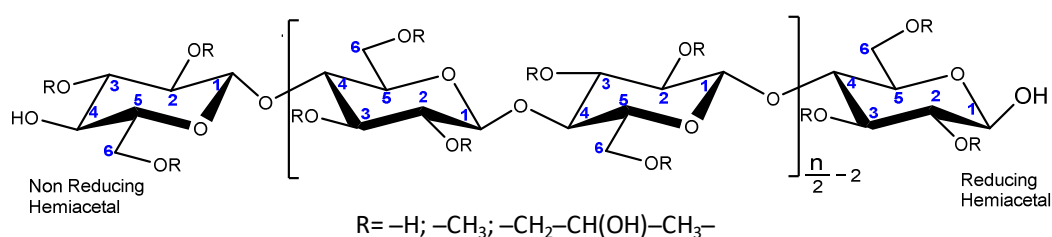


Fig. 1. Molecular structure of hydroxypropylmethyl cellulose (HPMC).

2. EXPERIMENTAL SECTION

2.1 Materials. Four commercial HPMC were chosen for this study: Methocel K4M and Methocel K15M provided by Dow Colorcon Limited–France, and Tylose MOBS 50 G4 and Metolose 90SH 100000 by Shin-Etsu Tylose GmbH & Co–Germany.

Cellulase from *Trichoderma reesei* ATCC 26921 in aqueous solution (Celluclast® 1.5L), with enzymatic activity of ≥ 700 Endo-Glucanase Units (EGU).g⁻¹ and density of 1.2 g.L⁻¹, was purchased from Sigma Aldrich. All other chemicals of analytical grade were obtained from commercial sources and used without further purification.

2.2 HPMC depolymerization

A new procedure to produce LMM HPMC and respective fractionation and purification is schematically represented in Fig. 2. HPMC solutions at 10 g.L⁻¹ in 10 mM of phosphate citric buffer (pH=5) were depolymerized at 47 °C. The amount of enzyme added was varied from 15 to 180 µL.g⁻¹ of dry HPMC. After the desired time of reaction, the enzyme deactivation was carried out by heating the mixture at 85 °C under vigorous stirring for 15 min. During this stage, a phase separation took place by precipitation of an insoluble fraction (P) of HPMC. The mixture was rapidly hot filtered using Ahlstrom polyester paper grade 3329 by maintaining the filtration funnel at temperature ≥ 85 °C. The precipitate fraction (P) was washed with boiling water in order to remove residual salts. The soluble fraction (S) was purified by diafiltration (module Amicon 8400 equipped with a 3kDa GK membrane (GE Osmonics)). The operating pressure was fixed at 3 bar. Both products P and S were then freeze dried. Depolymerization reactions were at least duplicated.

The final products were recovered as a white solid and named as following: K4M – S (or P) – X – y. X stand for the enzyme/polymer ratio ($\mu\text{L.g}^{-1}$) and “y” for the reaction time (h). Table S1 summarizes the reaction conditions and the corresponding name of the obtained products.

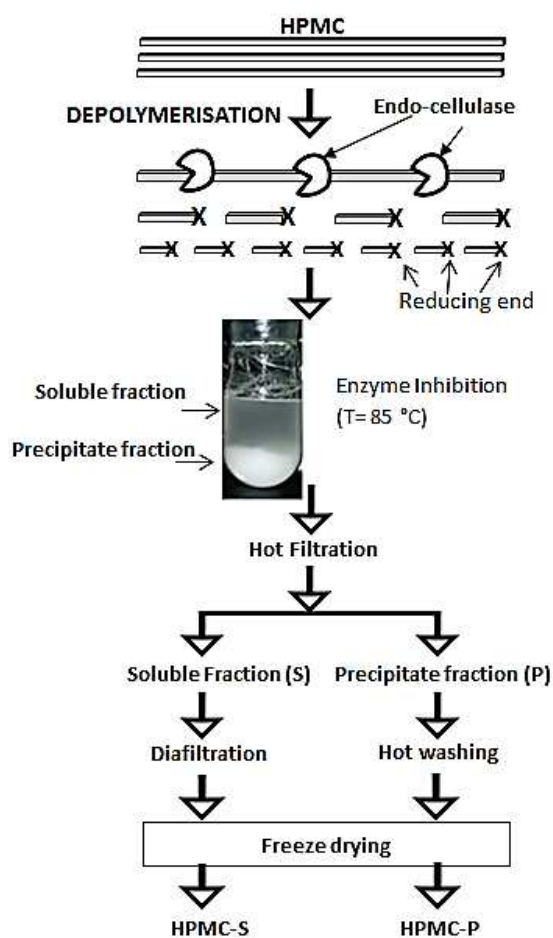


Fig. 2. Schematic procedure for the preparation of LMM HPMC exemplifying the endocellulase activity: soluble fraction (S) and precipitate fraction (P).

2.3 HPMC characterization

2.3.1 *Molar mass.* M_w and molar mass distribution (M_w/M_n) denoted as dispersity (D) were obtained by size-exclusion chromatography with multi-angle light scattering and

refractive index (SEC-MALS-RI) coupled detection (Wyatt Technology, Santa Barbara, CA, USA), using a TSK-GEL GMPWXL 7.8 x 300 mm column (TosoHaas Bioseparation Specialists, Stuttgart, Germany). The analysis conditions followed the procedure proposed by (Viriden et al., 2010). The mobile phase was 10 mM of NaCl with 0.02 % NaN_3 with a flow rate of 0.5 mL.min⁻¹. The injected volume of sample prepared at 5 mg.mL⁻¹ was 100 μL . The data were analyzed using the Zimm extrapolation method with Astra software (Wyatt technology, Santa Barbara, CA, USA). The dn/dc values were calculated using RI signal and the plot of a calibration curve of 10 different concentrations of NaCl. Afterwards several concentrations of the analysed samples were also injected and dn/dc values were directly determined from the slope.

2.3.2 Degree of substitution. The degree of methylation (DS_{Me}) and the number of hydroxypropyl groups per AGU (MS_{HP}) were determined by ¹H NMR of acetylated samples according to previously described procedures (Fitzpatrick et al., 2006; Viriden et al., 2010). 75 mg of HPMC sample were reacted with 2.25 mL acetic anhydride and 0.75 mL pyridine at 90 °C for 6 h and then dialyzed against water in a 3 kDa Spectr-Por dialysis membrane for 24 h. After drying, samples were solubilized in CDCl_3 (5 mg.mL⁻¹) and ¹H NMR spectra were acquired at 50 °C using a Bruker 300 MHz spectrometer. ¹H NMR spectra and details of the calculation of DS_{Me} and MS_{HP} coefficients are described in Fig. S1 in the Supplementary Material.

3. RESULTS AND DISCUSSION

3.1 Depolymerization and fractionation

Four different grades of HPMC were selected for characterization and depolymerization experiments. Table 1 presents the macromolecular features determined in this study as a function of their M_w and degree of substitution ranging from medium to high values. The results confirm that the G4 sample have the higher degree of substitution, in agreement with the suppliers information (Dow, 2002; Shin-Etsu, 2013). Previous studies reported that the substitution degree and their distribution along the polymer controls the hydrolysis rate of cellulose ethers, thereby feasibility of the partial depolymerization was claimed (Schagerlof et al., 2006; Fitzpatrick et al., 2006; Melander et al., 2006). Nevertheless, no further information on synthesis and characterization of LMM HPMC was provided. Hence, this is to our knowledge the first work that focuses on the production of LMM HPMC using enzymatic reaction.

Table 1

Characterization of macromolecular features and substitution degree of the starting HPMCs samples.

Sample	M_w g.mol ⁻¹	\bar{D}^a	dn/dc mL.g ⁻¹	DS_{Me}^b	MS_{HP}^c
G4	85000	1.7	0.137	1.95	0.32
K4M	230000	4.0	0.126	1.49	0.25
K15M	415000	1.9	0.126	1.51	0.26
90SH	814000	2.0	0.125	1.52	0.31

^a Dispersity. ^b Degree of methylation. ^c Hydroxypropyl molar substitution.

1 The depolymerization process starts when the enzyme cleaves the β -glycosidic bonds of the
2 HPMC polymer and the resulting hydrolysates always presented a broad molar mass distribution.
3 The obtained dispersity reported in Table 2 are quite consistent with previous studies indicating
4 that the depolymerization with EG from *Trichoderma reesei* yields a wider range of hydrolyzed
5 products (Schagerlof et al., 2006; Melander et al., 2006). For instance, the cumulative molar
6 distribution of the G4 hydrolysate obtained after 72 h of reaction using an enzyme/polymer ratio
7 of 180 $\mu\text{L.g}^{-1}$ clearly exhibits two fractions roughly one above and one below 10000 g.mol^{-1} as
8 exposed in Fig. 3a.

9 Synthesized polymer needs to be separated from the hydrolyzed solution for its application.
10 Therefore, a phase separation technique was adopted to recover the LMM HPMC. Remarkably,
11 during the inactivation of the enzyme at 85 °C, a phase separation occurs spontaneously due to
12 the thermoresponsive properties of the polymer (see Fig. 2). Notably, the top clearest part
13 denoted “fraction S” consist of lower M_w while the bottom part denoted “fraction P” corresponds
14 to the sample with the higher M_w as exemplified in Fig. 3b and 3c respectively (cumulative molar
15 distribution) and in Fig. 3d by the chromatogram of the corresponding P and S fractions. This
16 result emphasizes the importance of the M_w in the phase separation of LMM HPMC polymer.

17
18 At this point, it is worth noting that the combination effect between the enzymatic
19 depolymerization process leading to the HPMC chain formation with a broad M_w distribution and
20 the phase separation of the higher M_w by hot precipitation provides an efficient way of producing
21 two series of LMM HPMC.

Table 2

Macromolecular features of the crude hydrolysates obtained from depolymerization of the studied samples using an enzyme/polymer ratio of 180 $\mu\text{L.g}^{-1}$ for 72 h.

Sample	M_w g.mol^{-1}	M_n g.mol^{-1}	dn/dc mL.g^{-1}	\bar{D}^a
G4	15000	4300	0.137	3.6
K4M	8200	2000	0.126	3.9
K15M	8500	1800	0.126	4.7
90SH	7600	1500	0.125	5.1

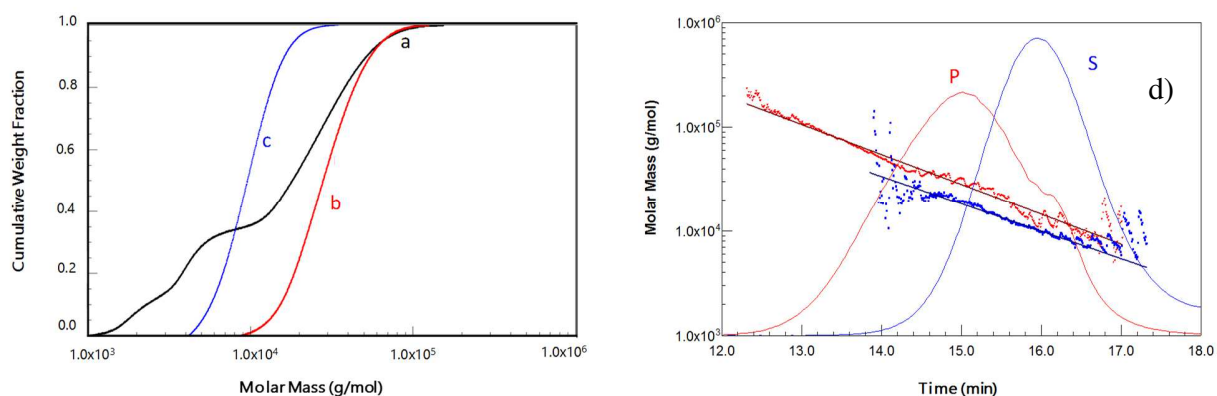


Fig. 3. G4 depolymerization using an enzyme/polymer ratio of 180 $\mu\text{L.g}^{-1}$ for 72 h. Cumulative molar mass distribution of the crude hydrolysate (a), and corresponding P (b) and S(c) fractions. d) Chromatogram of the fractions (RI traces and M_w variation).

To gain deeper insight in the depolymerization efficiency using commercial Celluclast[®], three parameters including type of starting HPMC, enzyme concentration and reaction time were investigated. The yields of P and S fraction, the total yield expressed as the sum of (P+S yields) as well the M_w and dispersity (\bar{D}) values of the fractions were taken as the reaction indicators.

3.2 Effect of HPMC M_w on depolymerization efficiency

The K4M, K15M and 90SH samples have a similar degree of substitution whereas they differ primarily by the chain length as can be seen in Table 1. It has been hypothesized that the length of starting HPMC could have influence on the production of LMM HPMC; therefore, longer starting HPMC could generate more or less a homogeneous hydrolysate. However, the chromatograms of crude hydrolysates of K4M, K15M and 90SH reveal the same profile as the G4 sample, indicating the presence of two fractions as displayed in Fig. S2. These results can be also corroborated by the higher \bar{D} of the crude hydrolysates summarized in Table 2. Then, the fractionation procedure was performed, and M_w and \bar{D} values of the corresponding fractions are summarized in Table 3. Interestingly, the S fractions exhibit a similar M_w of about 8000 ± 1000 g.mol⁻¹ and the P fragments of about 18000 ± 2000 g.mol⁻¹ for the three samples K4M, K15M and 90SH. The constant production of the two fractions with a similar M_w whatever the starting HPMC chain length suggests that these three samples could have two kinds of highly substituted zones resistant to the enzyme activity distributed along the polymer chains.

On the other hand, it can be seen that the yield of fraction S is significantly higher (54 %) when using K4M instead of K15M (41%) and 90SH (38 %). The yield values decreases when M_w of the starting HPMC increases. It was assumed that the viscosity of the reaction mixture might play a role on the reaction kinetics since M_w of K15M and 90SH are two and four fold larger than K4M. For instance, holding all other factors constant, 90SH formed a gel in tested reaction conditions where the enzyme mobility and activity might be reduced. Nevertheless, a qualitative control (visual) showed that the viscosity of the reaction mixture rapidly decreased during the first hour owing to the enzyme mediated depolymerization process. Consequently, viscosity

cannot be taken as a major parameter determining the size and the yield of the obtained fractions after 72 h of reaction.

Table 3

M_w and yield of hydrolyzed samples produced using 180 $\mu\text{L.g}^{-1}$ for 72 h.

Fraction P	M_w^* (g.mol^{-1})	\bar{D}	Yield (%)	Fraction S	M_w^* (g.mol^{-1})	\bar{D}	Yield (%)	Total yield (P+S) (%)
K4M-P-180-72	16000	1.3	32	K4M-S-180-72	7200	1.5	54	86
K15M-P-180-72	20000	1.3	54	K15M-S-180-72	8000	1.4	41	95
90SH-P-180-72	20000	1.4	54	90SH-S-180-72	9000	1.5	38	92
G4-P-180-72	34000	1.4	66	G4-S-180-72	11000	1.2	20	86

*The dn/dc values were determined to be 0.132 mL.g^{-1} for fraction P and 0.126 mL.g^{-1} for S.

Other than the M_w , starting HPMC samples display a great discrepancy in their dispersity as can be reflected by the values ranging from 1.7 to 4 in Table 1 and the GPC chromatogram on Fig. S3. For that reason, an additional control experiment following the same fractionation procedure but without adding enzyme was performed. As expected, 97-99 % of the intact starting HPMCs were recovered in the precipitate fraction independently of the dispersity and substitution degree. This result confirms that HPMC depolymerization can proceed only through the enzyme catalysis in our conditions.

The higher yield of fraction S with $M_w < 10000 \text{ g.mol}^{-1}$ appears to arise from a high dispersity value combined with the lower M_w of K4M compared to that of K15M and 90SH. The starting G4 sample with M_w about three fold smaller than that of K4M and a dispersity similar to that of

K15M and 90SH was expected to provide a high yield of fraction S. Nonetheless, data in Table 3 indicates lower value (20 %) than those obtained for the other samples (>38 %) even if it is combined with the discarded oligomers. These results indicate that M_w of the starting polymer does not have influence on the M_w of fractions, but on the total yield of fractions. On the other hand, the degree of substitution of the starting polymer controls the M_w and the yield of the resulting fractions.

A closer look at the data in Table 3 shows that 90SH and K15M samples provide total yield of hydrolyzed fractions yield more than 92 %. Assuming that the difference to 100 % was mainly due to the loss of oligomers, K4M sample seems to produce larger quantity of oligomers probably because of its dispersity. It can be noted also that the total yield for G4 reach only 86 %, which is similar to K4M. Therefore, it was concluded that a high substitution does not avoid the oligomers production (see Fig. 3a, $M_w \leq 2000 \text{ g.mol}^{-1}$).

These results can be better understood by examining the mechanism of the enzymatic depolymerization, that consists of two steps: the first refers to the binding and adsorption process of enzyme onto the cellulosic chain, whereas the second involves the cleavage of glycosidic bonds (Turon, Rojas, & Deinhammer, 2008; von Schantz, Schagerlöf, Karlsson, & Ohlin, 2014). From this mechanism, it can be inferred that a larger number of binding points on the starting HPMC must result in hydrolyzed fractions. However, productive binding and the subsequent cleavage process are assumed to be hindered by substitution on AGU, but also depends on the distribution of substituents along the polymer chain as previously mentioned. Therefore, G4 with an average number of about 2 substituents per AGU is then more resistant to the productive enzyme binding and the number of possible cleavage per chain lower than in the case of the other studied HPMC grades. This can explain the higher M_w of the G4 fractions seen in Table 3.

1 With respect to the oligomers generation, literature has reported that the Celluclast® product
2 consists, besides EG, in a mixture of EX including cellobiohydrolases and β -glucosidase (Turon
3 et al., 2008; Wikiera, Mika, Starzynska-Janiszewska, & Stodolak, 2015). Then, it is reasonable to
4 relate the reduction of total yield to the action of EX, which produce mainly cellobioses.
5 Melander et al., (2006) assumed that EX activity is hindered by the substituents on the polymer
6 chain. This may explain why the reduction of the total yield related to the EX activity proceeds
7 only at a low extent. Accordingly, fraction S mainly comes from the HPMC depolymerization by
8 EG action but also partially from EX.

9 It was expected at the beginning of this work that HPMC contain zones resistant to the enzyme
10 cleavage enabling the formation of LMM HPMC. This was confirmed by the incomplete
11 depolymerization of the studied samples in our conditions. Then, it is interesting to note that the
12 substituents and the distance between zones resistant to the enzyme binding are the main factors
13 affecting the chain length of LMM HPMC. This result also suggests that EG from *Trichoderma*
14 *ressei* is very sensitive to the distribution of substituents along the polymer chain leading to its
15 high selectivity to control the polymer length.

16 To summarize this part, K4M emerged as the better candidate among the studied samples to
17 produce LMM HPMC polymer. Moreover, fractionation process of the obtained crude
18 hydrolysate by phase separation induces the reduction of \bar{D} values below 1.5, which are suitable
19 for preparing block copolymers with well-defined architecture that is the main target of this work
20 (Lu et al., 2019; Wang et al., 2017).

21 For the sake of clarity, the remaining discussion will emphasize the use of K4M as the
22 starting HPMC. The data obtained for the other starting HPMC samples are gathered in
23 Table S2.

3.3 Effect of enzyme concentration and reaction time on yield

To highlight the impact of enzyme concentration on fraction yield, reactions with various enzyme/polymer ratios (15, 30, 50, 180 $\mu\text{L.g}^{-1}$) were synthesized for 72 h. Fig. 4a presents the evolution of yield values for P and S fractions as a function of the enzyme concentration. The optimal yield of S fraction reaches 55 % using only an enzyme concentration of 30 $\mu\text{L.g}^{-1}$. This value does not change significantly for higher enzyme concentrations. By contrast, the total yield values decreases from 99 to 88 % when the enzyme/polymer ratio increases from 15 to 30 $\mu\text{L.g}^{-1}$. In the same way, P yield drops to more than 30 % suggesting that part of S fraction and oligomers came from the further hydrolysis of P fraction. Similar results have been found for reactions using a constant enzyme/polymer ratio (30 $\mu\text{L.g}^{-1}$) and varying the reaction time (1, 24, 72, 96 h) as has depicted in Fig. 4b.

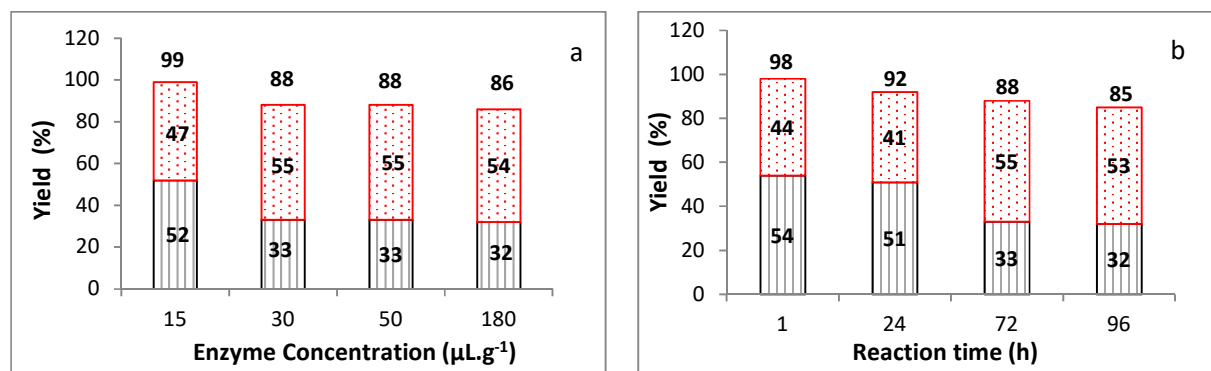


Fig. 4. Yield variation of the recovered fractions for K4M depolymerization as a function of (a) the enzyme concentration: total yield values (S+P) on the upper side, yield of S (red dashed) and P (black vertical lines) fractions after 72 h of reaction time; b) the reaction time when using 30 $\mu\text{L.g}^{-1}$ of enzyme/polymer ratio.

The following mechanism was then proposed to account for these observations. A rapid first step of depolymerization takes place leading to the formation of a high yield of fraction P. Hence, they are supposed to contain domains more hardly available to the enzyme so that the

further hydrolysis into S fragments takes longer reaction time. This second step can be enhanced by increasing the enzyme concentration, which enables the binding and the chain cleavage to occur at a greater extent. It can then be concluded that optimization of P and S yields is possible by varying the reaction time and enzyme concentration.

3.4 Effect of enzyme concentration and reaction time on M_w

Fig. 5 displays the M_w evolution of fractions P and S produced by various enzyme/polymer ratio (30, 50, 180 $\mu\text{L.g}^{-1}$) as a function of the reaction time. M_w of fractions S is about 13000 g.mol^{-1} after one hour of reaction independently of the enzyme concentration while M_w of fractions P gradually decreases by increasing the enzyme concentration. After the sharp drop observed during the first hour (Fig. S4), M_w of S and P fractions displays a gradual reduction, which is enzyme concentration dependent: the higher the enzyme/polymer ratio, the faster the M_w decrease.

M_w evolution of S fractions can be divided into two regimes: a first regime during the first hours where EG productive binding leads to a rapid depolymerization and a second one (from 24h) that might be attributed to the formation of oligomers including cellobioses (342 g.mol^{-1}) produced by EX. This interpretation is supported by the linear relationship between the M_w variation and reaction time as shown in Fig. 6a with a slope of about -62 and -20 $\text{g.mol}^{-1}.\text{h}^{-1}$ for fractions S prepared with an enzyme/polymer ratio of 30 and 180 $\mu\text{L.g}^{-1}$ respectively, and the constant diminution of the total yield in the second regime as represented in Fig. 6b.

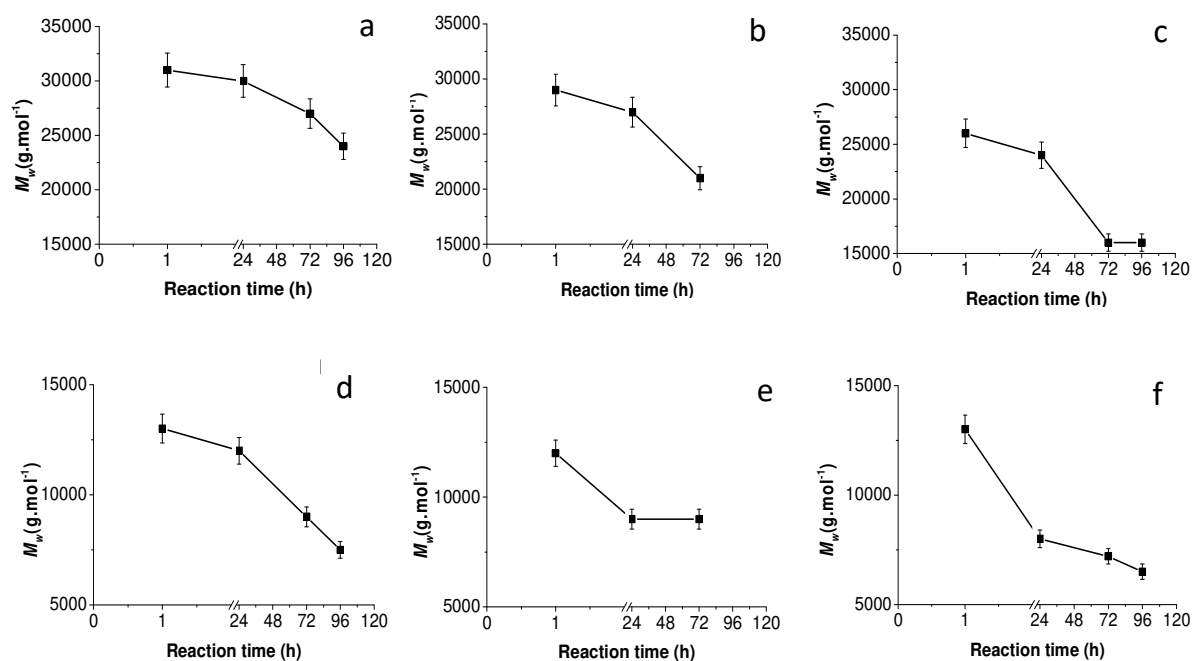


Fig. 5. Effect of enzyme concentration on M_w of P and S as a function of reaction time with the enzyme/polymer ratio of 30 $\mu\text{L}\cdot\text{g}^{-1}$ (a, d); 50 $\mu\text{L}\cdot\text{g}^{-1}$ (b, e) and 180 $\mu\text{L}\cdot\text{g}^{-1}$ (c, f).

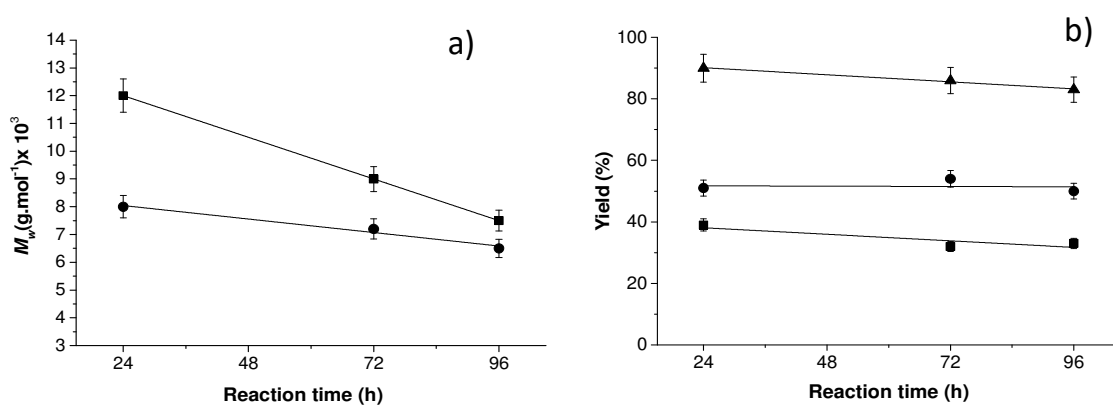


Fig. 6. a) Weight average molar mass decrease of S fractions using (■) 30 and (●) 180 $\mu\text{L}\cdot\text{g}^{-1}$ enzyme/polymer ratio as a function of reaction time. b) Yield of depolymerized HMPC fraction P (■), S (●) and total yield (▲) as a function of reaction time using 180 $\mu\text{L}\cdot\text{g}^{-1}$ enzyme/polymer ratio.

As stated above, the combination effect between the selectivity of EG and the synergistic mechanism of EG-EX towards the more accessible zones along the starting HPMC allow tuning the M_w of LMM HPMC (Table S2). To get a further insight on the effect of the cleavage mechanism on the chemical structure of the obtained LMM HPMCs, the following section analyzes the impact of operating hydrolysis on the substituent distribution.

3.5 Degree of substitution of P and S fractions

The degree of substitution by methyl (DS_{Me}) and hydroxypropyl (MS_{HP}) groups of seven representative samples are summarized in Table 4. Three trends can be identified:

i) DS_{Me} values of the P fractions were significantly higher than those of the starting K4M and of its corresponding S fraction with an average value of 1.72 ± 0.03 .

ii) DS_{Me} values of S fractions were only slightly higher than that of the starting K4M with an average value of 1.55 ± 0.06 . Although the results does not reveal a difference between the reaction time and enzyme/polymer ratio, the higher substitution values of hydrolysate fractions is in agreement with productive enzyme binding onto the less substituted domains. However, previous studies have reported lower DS_{Me} for hydrolyzed cellulose ether with very low polymerization degrees (DP) than the starting HPMC (Adden, Melander, et al., 2006). The LMM HPMCs produced in our study have much larger DP that can account for this apparent discrepancy. On the other hand, the more substituted G4 generates fractions with similar or lower DS_{Me} than the starting HPMC. It appears then that the substituents distribution (homogeneous or heterogeneous) along the chain plays also a dominant role.

iii) MS_{HP} values of the P and S fractions were slightly higher than that of the starting HPMC. However, it was difficult to put in evidence the variation between the two fractions. Other than

that, it must be noted that the standard deviation for MS_{HP} values ($\pm 10\%$) is higher than that for DS_{Me} ($\pm 4\%$). This is due to the weakness of the broad band “c” in ^1H NMR spectra compared to the other signals.

As stressed before, solubility property of HPMC is partly governed by DS_{Me} . A higher value makes the hot precipitation easier owing to the aggregation of hydrophobic methyl substituents; phase separation between P and S fractions is then facilitated. Other parameter such as the higher mobility of S fraction due to its lower M_w has been reported previously for polyisobutene-*n*-pentane by Baker, Clemson & Allen (1966).

Table 4

Number average molar mass (M_n), degree of methyl substitution (DS_{Me}) and Molar hydroxypropyl substitution (MS_{HP}) of K4M, G4 and their hydrolyzed samples.

Fraction P	M_n (g.mol ⁻¹)	DS_{Me}	MS_{HP}	Fraction S	M_n (g.mol ⁻¹)	DS_{Me}	MS_{HP}
K4M*	57500	1.49	0.25				
K4M-P-30-1	23846	1.75	0.29	K4M-S-30-1	9286	1.57	0.30
K4M-P-50-1	20714	1.66	0.26	K4M-S-50-1	9231	1.46	0.29
K4M-P-180-1	20000	1.73	0.31	K4M-S-180-1	7642	1.52	0.34
K4M-P-30-72	22500	1.74	0.34	K4M-S-30-1	5625	1.57	0.35
K4M-P-50-72	16154	1.71	0.30	K4M-S-50-72	5625	1.51	0.27
K4M-P-180-72	12308	1.76	0.29	K4M-S-180-1	4800	1.64	0.30
G4*	50000	1.95	0.32				
G4-P-180-72	24286	2.01	0.32	G4-S-180-72	9167	1.71	0.39

*Starting HPMC

It was demonstrated in the previous sections that the depolymerization during the first hour can be predominantly attributed to the EG activity since the total yield can be considered quantitative (more than 95 %). In these conditions, the following equations are proposed to quantify the average number of cleavage (z) by using the number average molar mass (M_n) listed in Table 4.

$$\frac{F^P}{M_n^P} + \frac{F^S}{M_n^S} + \varepsilon = \frac{(z+1)}{M_n^0} \quad \text{Eq. 1}$$

where: F^P and F^S denote the mass fraction of the corresponding P and S samples; M_n^P , M_n^S , M_n^0 stand for number average molar mass of P, S, and starting HPMC; ε is the mole number of oligomers which were discarded during the purification process. Assuming a 100 % recovery in S and P fractions, mass conservation equations can be written as:

$$M_n^0 = x \cdot M_n^P + y \cdot M_n^S \quad \text{Eq. 2}$$

with

$$x + y = z + 1 \quad \text{Eq. 3}$$

Where: x and y count for the respective average number of S and P fragments. Therefore, calculation of equation (Eq.1) while neglecting ε gave a total number of about 3 and 4 chain cleavages by using K4M with 30 $\mu\text{L.g}^{-1}$ and 180 $\mu\text{L.g}^{-1}$ during the first hour, respectively. This result means that there are between 3 and 4 domains along the K4M chain where EG can readily adsorb leading to productive binding. It comes from equations 2 and 3 that K4M was cleaved after 1 h into an average number of 1 P and 3 S fragments when using 30 $\mu\text{L.g}^{-1}$ and 1.5 P and 3.5 S fragments when using 180 $\mu\text{L.g}^{-1}$. It is obvious that this calculation is only indicative as the part of oligomers ($\leq 5\%$) is neglected. Nevertheless, it can be concluded that the zones of possible EG adsorption are more or less evenly distributed giving rise to the S fragments.

From this section, we can mention that the presence of two co-existing domains with different degrees of substitution distributed along the HPMC chain can explain the production of the P and S fractions. In addition, the produced HPMC polymer hold an aldehyde group on the reducing end position as a result of the enzymatic depolymerization, and this is an advantage especially important for further coupling reaction for preparation of conjugated molecules and AB block copolymers as mentioned before (Lu et al., 2019; Wang et al., 2017).

4. Conclusions

This work reported the production process of LMM HPMC with M_w lower than 20000 g.mol⁻¹ and low dispersity ($\bar{D} < 1.5$). The adopted production strategy was based on the enzymatic reaction, fractionation process and the substitution pattern of commercially available HPMCs that led to the formation of a series of LMM HPMC. The experimental data demonstrated that only after 1 h of reaction, two groups of LMM HPMC with respective M_w of about 13000 and 37000 g.mol⁻¹ can be recovered with a total yield as high as 98 %. Furthermore, the M_w values can be gradually decreased down to about 6000 and 16000 g.mol⁻¹, while maintaining high total yields (>85%), by extending the reaction time and/or increasing the enzyme concentration. This novel procedure can attract attention in the field of nanomedicine and nanoparticles production, where LMM HPMCs are highly required. Compared to other routes for the LMM polysaccharide production, such as supercritical water hydrolysis, the approach using enzymatic reaction enables to monitor easily M_w of the obtained LMM HPMCs and to maintain the reactive aldehyde group at one end of the LMM polymer.

ASSOCIATED CONTENT

Supplementary Material.

Additional information regarding the operating conditions of the HPMC depolymerization, ^1H NMR spectra and analysis of degree of substitution, SEC traces of the crude hydrolysates, molar mass distribution of starting HPMC polymers, molar mass kinetic for K4M depolymerization using an enzyme/polymer ratio of $30 \mu\text{L.g}^{-1}$ vs reaction time, and the list of all short HPMC polymers produced in this research (PDF).

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Notes

The authors declare no competing financial interest.

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