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1	Synthesis and Self-Assembly of AB2-type Amphiphilic Copolymers from Biobased
2	Hydroxypropyl Methyl Cellulose and Poly(L-lactide)
3	
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8	Abstract: AB ₂ -type amphiphilic (HPMC) ₂ -PLA copolymers with various hydrophilic
9	block lengths were synthesized using a three step procedure: ring-opening polymerization of
10	L-lactide initiated by propynol, amination reduction of the aldehyde endgroup of HPMC, and
11	thiol-click reaction. The resulted copolymers were characterized by NMR, DOSY-NMR, SEC
12	and FT-IR. The cloud point (CP) was determined by UV-visible spectrometer. Data show that
13	the HPMC block length has little effect on the Cp of the copolymers which is lower than that
14	of HPMC. The self-assembly behavior of the copolymers was investigated from DLS, TEM,
15	and critical micelle concentration (CMC) measurements. Spherical micelles are obtained by
16	self-assembly of copolymers in aqueous solution. The micelle size and the CMC of
17	copolymers increase with increasing HPMC block length. It is concluded that biobased and

18 biodegradable (HPMC)₂-PLA copolymers could be promising as nano-carrier of hydrophobic

19 drugs.

20

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24 1. Introduction

Micelles based on amphiphilic block copolymers have been extensively investigated as 25 drug carrier due to their favorable advantages in molecular design, long circulation, enhanced 26 drug loading and therapeutic effect, and biocompatibility (Kataoka, Harada, & Nagasaki, 27 2001). Thus, biocompatible and bioresorbable aliphatic polyesters such as polylactide (PLA), 28 polyglycolide (PGA), poly(lactide-co-glycolide) (PLGA), and poly(ɛ-caprolactone) (PCL) 29 have been commonly used as the hydrophobic block, whereas poly(ethylene glycol) (PEG), 30 poly(N-vinyl-2-pyrrolidone) (PVP), poly(N-isopropylacrylamide) (PNIPAAm), poly(vinyl 31 32 alcohol) (PVA), poly(amino acid), and more recently polysaccharides as the hydrophilic block 33 to construct amphiphilic block copolymers (Elsabahy & Wooley, 2012; Guo, Wang, Shen, Shu, & Sun, 2013; Mi, Wang, Nishiyama, & Cabral, 2017; Yi et al., 2018). PLA, as an FDA-34 approved biobased polyester, is widely used in biomedical and pharmaceutical fields in the 35 form of drug carrier, tissue engineering scaffolds, bone fracture internal fixation devices, 36 orthopedic screws and plates, etc, due to its good degradability, processability, and 37 mechanical strength (Lassalle & Ferreira, 2007; Rajendra P. Pawara & Abraham J. Dombb, 38 2014; Ramzi A. Abd Alsaheb, 2015; Rancan et al., 2009; Saini, Arora, & Kumar, 2016; Tsuji, 39 2005). Interestingly, PLA stereocomplex has been used to construct colloidal systems such as 40 41 mixed micelles and hydrogels for pharmaceutical applications (L. Yang, Wu, Liu, Duan, & Li, 2009). 42

43 PEG is also an FDA-approved polymer widely used for biomedical and pharmaceutical
44 applications. Nevertheless, PEG as a polyether is prone to peroxidation, which could

adversely affect cells (Barz, Luxenhofer, Zentel, & Vicent, 2011; Ishida & Kiwada, 2008). 45 Therefore, great effort has been made to search for hydrophilic alternatives of PEG. 46 Polysaccharides have attracted increasing attention in recent years for uses as biomaterials to 47 solve problems related to immunogenicity and toxicity associated with synthetic polymers 48 because of their biodegradability, biocompatibility and bio-based nature (Aminabhavi, 49 Nadagouda, Joshi, & More, 2014; Ganguly, Chaturvedi, More, Nadagouda, & Aminabhavi, 50 2014). Certain polysaccharides present inherent bioactivity that can help in mucoadhesion, 51 thus improving drug targeting and diminishing inflammatory response. Recent years have 52 witnessed a rapid growth on polysaccharide based micelles as drug delivery system 53 (Aminabhavi et al., 2014; Rudzinski & Aminabhavi, 2010), such as pullulan (Jeong et al., 54 55 2006), cellulose (Guo, Wang, Shu, Shen, & Sun, 2012), dextran (Jeong et al., 2011; Sun et al., 2009), chitosan (Huo et al., 2012), heparin (Tian et al., 2010), and hyaluronan (Y. L. Yang, 56 Kataoka, & Winnik, 2005). Among them, cellulose is of great importance because of its 57 outstanding properties, but its applications are restrained due to its poor solubility. Thus, 58 soluble cellulose derivatives have been studied as a hydrophilic building block to prepare 59 amphiphilic copolymers which can self-assemble in micelles, such as ethyl cellulose, 60 hydroxyl propyl cellulose (Ostmark, Nystrom, & Malmstrom, 2008), hydroxyl propyl methyl 61 cellulose (HPMC) (Ostmark et al., 2008), hydroxyl ethyl cellulose (Hsieh, Van Cuong, Chen, 62 Chen, & Yeh, 2008) etc. Graft copolymerization of PLA onto cellulose and cellulose 63 derivatives have been reported (Chen & Sun, 2000; Teramoto & Nishio, 2003; Yuan, Yuan, 64 Zhang, & Xie, 2007). However, the grafting of PLA on the side chain hydroxyl group results 65 in complex chain structure and the reaction is poorly controllable. 66

In our previous work (Wang, Caceres, Li, & Deratani, 2017), linear HPMC-b-PLA 67 diblock copolymers with various HPMC block lengths were synthesized by combination of 68 ring-opening polymerization, amination reduction and thiol-click reaction. The resulted 69 70 amphiphilic copolymers are susceptible to self-assemble into spherical micelles with a diameter of 60-120 nm. It has been shown that the geometrical structure of copolymers affect 71 the morphology, stability and size of micelles because the hydrophobic and hydrophilic 72 73 segments lead to complex spatial arrangements during the micellization process (Cao et al., 2013; Li, Kesselman, Talmon, Hillmyer, & Lodge, 2004). In this work, amphiphilic AB₂-type 74 PLA-(HPMC)₂ copolymers with various HPMC molar masses were synthesized and 75 characterized. The self-assembly properties of the copolymers were investigated from the 76 77 morphology, size and size distribution, cloud point (Cp) and critical micelle concentration (CMC) measurements. 78

79

80 2. Materials and Methods

81 2.1 Materials

L-lactide was purchased from Purac Biochem (Goerinchem, Netherlands) and purified 82 by recrystallization from ethyl acetate. HPMC (K4M) was kindly provided by Colorcon-Dow 83 chemical (Bougival, France). Propynol, stannous octoate (Sn(Oct)₂), cysteamine, sodium 84 cyanoborohydride (NaBH₃CN), 1,4-dithiothreito 2,2-dimethoxy-2-85 (DTT), phenylacetophenone (DMPA), diethyl ether and dimethyl sulfoxide were purchased from 86 Sigma-Aldrich, and used as received. Toluene was purchased from Sigma-Aldrich, dried over 87 by calcium chloride (CaCl₂) and distilled prior to use. 88

89 2.2 Synthesis of alkynyl terminated PLLA

Alkynyl terminated PLLA was synthesis by ring-open polymerization, of L-lactide using propynol as initiator and Sn(Oct)₂ as catalyst. Typically, L-lactide (7.2 g), propynol (0.112 g), and Sn(Oct)₂ (0.2 g) were introduced into a dried Schlenk tube, and 30 mL anhydrous toluene was added under stirring. The solution was degassed by performing five freeze–pump–thaw cycles. The reaction then proceeded 24 h at 80°C. Finally the product was obtained by precipitation in methanol, followed by vacuum drying.

96 2.3 Enzymatic degradation of HPMC and determination of substitution degree

97 HPMC was depolymerized to yield HPMC oligomers as reported in a previous work 98 (Wang et al., 2017). Briefly, 1 g HPMC was added in 100 mL citrate phosphate buffer (pH = 99 5), and stirred overnight at 47 °C to ensure complete dissolution. Cellulase was added to the 90 solution, and enzymatic depolymerization proceeded for various time periods. The reaction 101 was stopped by heating the solution to 85 °C, and the suspension was hot filtrated. The 102 collected solution was purified by dialysis for 3 days using a membrane of MWCO=3500 g 103 mol⁻¹, followed by freeze drying.

104 HPMC is a cellulose derivative whose hydroxyl groups are substituted by methyl and 105 hydroxypropyl groups. The average number of substituents per repeat unit is characterized by 106 the molar substitution or hydroxypropylaction (MS_{HP}) and the degree of substitution or 107 methylation (DS_{Me}). The former can be higher than 3 and the latter varies between 0 and 3. 108 HPMC with a DS_{Me} between 0.1 and 2.0 is water soluble as the substitution groups allow to 109 decrease the intra- and inter-chain hydrogen bonding. In contrast, when the DS is above 2, 110 HPMC becomes insoluble in water due to the presence of many hydrophobic methyl groups. 111 (Claes, 2006). The ¹H NMR spectra of acetylated HPMC oligomers were acquired in CDCl₃
112 at 50 °C. The DS_{Me} and MS_{HP} values were then calculated according to the method reported
113 by Fitzpatrik (Fitzpatrick et al., 2006).

114

115 2.4 Synthesis of thiol terminated HPMC

Thiol terminated HPMC was obtained by reductive amination connecting the aldehyde 116 end group of HPMC molecules and the amine group of cysteamine in the presence of sodium 117 cyanoborohydride. Briefly, HPMC with DS_{Me} of 1.43 and average molar mass of 7000 g 118 mol⁻¹ (1 g) and NaBH₃CN (0.2 g) were solubilized in a mixture (3/1 v/v) of 50 mL dimethyl 119 sulfoxide (DMSO) and 0.01 M NaCl at 60 °C. Cysteamine (0.1 g) was added, and the solution 120 121 was stirred at 60 °C for 6 days under reflux. The solution was then dialyzed against deionized water for 3 days (MWCO 3500 g mol⁻¹), followed by freeze drying. Afterwards, the product 122 was dissolved in water, and stirred overnight in the presence of excess DTT under nitrogen 123 atmosphere. Finally, the solution was dialyzed against deionized water and freeze dried. 124 Ellman assay was performed to determine the content of thiol group using 5,5'-dithio-bis-(2-125 nitrobenzoic acid) (DTNB) as Ellman's reagent. DTNB is a versatile water soluble compound 126 for quantitating free sulfhydryl groups as a measurable yellow-colored product is formed 127 when DTNB reacts with sulfhydryl in solution. 128

129 2.5 Synthesis of (HPMC)₂-*b*-PLLA

Amphiphilic block copolymers were synthesized by thiol-yne click reaction connecting
 thiol terminated HPMC and alkynyl terminated PLLA. The reaction was initiated in a Dinics
 M3 UV chamber equipped with a PL-L 36 W/01/4P Hg Lamp. Typically, thiol terminated

HPMC (1.0 g), alkynyl terminated PLLA (0.15 g), and DMPA (0.013 g) were dissolved in 10
mL DMSO. The solution was degassed for 30 min using purified argon, and then subjected
to UV irradiation for 5 h. The crude product was collected by precipitation in acetonitrile,
followed by centrifugation at 4500 rpm for 15 min. The above dissolution–precipitation
cycle was repeated twice. Finally, (HPMC)₂-*b*-PLLA copolymers were obtained as a white
solid after vacuum drying.

139 2.6 Characterization

¹H NMR spectra were recorded on Bruker spectrometer operating at 300 MHz 140 (AMX300) using CDCl₃ and DMSO-*d*₆ as solvent. Diffusion ordered spectroscopy (DOSY) 141 NMR was performed on Bruker Avance (AQS600) NMR spectrometer, operating at 600 MHz 142 and equipped with a Bruker multinuclear z-gradient inverse probe head which is able to 143 produce gradients in the z direction with the strength of 55 Gcm⁻¹. The DOSY spectra were 144 acquired from Bruker topspin software (version 2.1) with the ledbpgp2s pulse program. The 145 strength of the pulsed field gradients with respect to maximum 32 increments on a quadratic 146 scale was logarithmically incremented from 2 to 95%. The diffusion sensitive period (Δ) of 147 200 ms and the gradient duration (δ) of 5 ms were optimized to allow the signals of interest to 148 decrease by a factor of 10-20, in order to keep the relaxation contribution to the signal 149 attenuation constant and ensure full signal attenuation for all samples. Data were processed 150 151 using the MestRe Nova software.

Size exclusion chromatography (SEC), equipped with MALLS in combination with refractive index (RI) detection allows to determine the absolute molar mass of polymers. 5 mg of samples were added in the mobile phase (0.01 M NaCl containing 0.02% NaN₃), and

stirred for 24 h at room temperature. The solution was filtered using 0.45 µm 155 polytetrafluoroethylene (PTFE) filter (Millipore). The filtrate was injected through a 100 µL 156 loop (Rheodyne injector 7725), and eluted on a TSK-GEL GMPWXL 7.8×300 mm column 157 158 (TosoHaas Bioseparation Specialists, Stuttgart, Germany) at a flow rate of 0.5 mL min⁻¹ (Waters pump 515). MALS detector (Dawn DSP, Wyatt Technology Co, Santa Barbara, CA, 159 USA) and RI detector (Optilab Wyatt Technology Co) were used to online determine the 160 absolute molar mass for each elution fraction of 0.01 mL, which enables to calculate the 161 weight average molar mass (M_w) and the dispersity $(D=M_w/M_n)$. The refractive index 162 increment (dn/dc) for calculation was 0.137 mL g⁻¹. Data analysis was realized using Astra 4 163 (Wyatt Technology Co). 164

Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet NEXUS spectrometer with a DTGS detector at 4 cm⁻¹ resolution. 64 scans were taken per sample in the frequency range from 400 to 4000 cm⁻¹. Sample pellets were prepared by mixing 2–2.5 mg of polymer with 200 mg of spectral grade potassium bromide, followed by compression using a press.

The cloud point (Cp) of the copolymers was estimated from transmittance changes of copolymer solutions in the temperature range from 30 to 90°C. The solution at 3.0 mg mL⁻¹ was stirred for 24 h and kept at 5 °C overnight before analysis. Measurements were made at a wavelength of 600 nm with a Perkin Elmer Lambda 35 UV–visible spectrometer equipped with a Peltier temperature programmer PTP-1+1. The temperature ramp was 0.1 °C min⁻¹. Temp Lab software was used for data treatment.

176 Dynamic light scattering (DLS) was performed at 25°C with 90° scattering angle using

2ano-ZS (Malvern Instrument) equipped with a He–Ne laser (λ =632.8 nm). The aqueous solutions of copolymers at 1.0 mg mL⁻¹ were filtered through a 0.45 µm PTFE microfilter before measurements. The correlation of the distribution of diffusion coefficient (D) was analyzed via the general purpose method (non-negative least squares). The apparent equivalent hydrodynamic radius (R_H) was obtained using the cumulate method from the Stoke–Einstein equation.

The critical micelle concentration (CMC) of copolymers was determined using an 183 autocorrelation function according to the methodology proposed by Muller et al., 184 2015). Aqueous solutions of copolymers were prepared in deionized water at concentrations 185 ranging from 0.006 to 1.0 g L^{-1} , and were incubated overnight. The scattering intensity of the 186 solutions was measured at 25 °C with a Malvern Instrument Nano-ZS. The scattering intensity 187 proportional to the size and number of objects present in the solution was recorded. The CMC 188 was taken as the intersection of regression lines from the plots of the scattering intensity 189 against the polymer concentration. 190

191 Transmission electron microscopy (TEM) was performed on JEOL 1200 EXII 192 instrument, operating at an acceleration voltage of 120 kV. 5 μ L of micellar solution at 1.0 mg 193 mL⁻¹ were dropped onto a carbon coated copper grid, and air dried before measurements.

194

195 3. Results and Discuss

196 3.1 Synthesis of amphiphilic block copolymers

HPMC was depolymerized by using cellulase to yield oligomers with various molar
 masses. The weight average molar mass (M_w) of the resulted HPMC oligomers was 5000,

199 7000 and 10000 Daltons, respectively, as determined by SEC. The NMR spectra of acetylated 200 HPMC were recorded in CDCl₃ (Figure S1). The DS_{Me} and MS_{HP} were calculated according 201 to the method reported by Fitzpatrik (Table S1). The DS_{Me} varies from 1.17 to 1.43, indicating 202 that HPMC samples are water soluble.

The synthesis of (HPMC)₂-*b*-PLA copolymers was achieved in three steps (Scheme 1): a) synthesis of alkynyl terminated PLLA, b) thiolation of HPMC, and c) UV-initiated thiolyne click reaction between thiolated HPMC and alkynyl terminated PLLA.

206





Scheme 1. Synthesis route of (HPMC)₂-*b*-PLA block copolymers

209

Alkynyl terminated PLLA was synthesized by ring-opening polymerization of L-lactide using propynol as initiator with a monomer/initiator molar ratio of 25/1. Figure 1 shows the ¹H NMR spectrum of the resulting alkynyl terminated PLLA. The signals at 2.4 (**a**) and 4.75 (**b**) ppm are assigned to the alkynyl group and the methylene groups adjacent to the alkynyl group, respectively. The signals at 1.62 (**d**) and 5.25 (**c**) ppm are assigned to the methyl and methyne protons of PLA main chain, and the signals at 1.50 (**f**) and 4.42 (**e**) ppm to methyl and methyne protons of the hydroxyl terminal unit. The polymerization degree (DP) of PLA calculated from the integration ratio of signals **c** to **e** was 28, corresponding to a number average molar mass ($M_{n,NMR}$) of about 2000 g/mol.





220

Figure 1. NMR spectrum of alkynyl-terminated PLA in CDCl₃

221

Thiol terminated HPMC was obtained by reductive amination of HPMC with 222 cysteamine. The molecular chain of cellulose has a non reducing group at one end, and a 223 hemiacetal group at the other end. The latter is a reducing group which can be easily 224 converted to aldehyde group (Schatz & Lecommandoux, 2010). The amine functional group 225 of cysteamine reacted with the unique aldehyde endgroup of HPMC through reductive 226 amination to yield HPMC-S-S-HPMC together with small amounts of unreacted HPMC, 227 HPMC-SH, and HPMC-S-S-(CH₂)₆-NH₂. HPMC-S-S-HPMC was then reduced using DTT to 228 yield HPMC-SH (Wang et al., 2017). The final product was characterized by using Ellman 229 assay and SEC measurement. Ellman's assay showed that the content of thiol terminated 230

HPMC in the final product was 88%. SEC confirmed that the final product is predominantly
composed of HPMC-SH because its M_w is almost the same as that of the initial HPMC. These
findings showed that thiol terminated HPMC was successful synthesized.

234 Finally amphiphilic block copolymers were obtained by UV-initiated thiol-yne click reaction between thiol terminated HPMC and alkynyl terminated PLLA. Three copolymers, 235 namely (HPMC5K)₂-PLA2K, (HPMC7K)₂-PLA2K and (HPMC10K)₂-PLA2K were 236 synthesized using the same hydrophobic PLA block with M_n of 2000 g mol⁻¹ and different 237 hydrophilic HPMC-SH blocks with M_w of 5000, 7000, and 10000 g mol⁻¹, respectively. 238 Figure 2 shows the ¹H NMR spectra of (HPMC7K)₂-PLA2K copolymer in D₂O and DMSO-239 d_6 . The spectrum in DMSO- d_6 shows the signal at 1.05 ppm (a) corresponding to the methyl 240 241 protons in the hydroxypropyl group, and those in the range of 2.75-4.75 ppm (b) to the methyl protons adjacent to the oxygen moieties of the ether linkages, inner methylene and 242 methine protons and HPMC backbone protons (Figure 2A). The signals at 1.49 (d) and 5.24 243 ppm (c) belong to the methyl and methine protons of PLLA blocks. Moreover, the signal 244 detected at 2.3 ppm is assigned to the methylene group of the connecting unit. These results 245 confirm the successful synthesis of copolymers by thiol-yne click reaction. 246





248

Figure 2. ¹H NMR spectra of (HPMC)₂-PLA in DMSO-d₆ (A) and in D₂O (B)

The structure of block copolymers was also confirmed by DOSY-NMR. DOSY is an 250 excellent tool commonly used for the analysis of complex mixtures as it allows virtual 251 separation of multicomponent systems. DOSY-NMR data are presented in a two-dimensional 252 (2D) pattern: one dimension is related to the chemical shift information, and the other 253 represents the diffusion coefficient which reflects the molecular effective sizes (Pages, Gilard, 254 Martino, & Malet-Martino, 2017). As shown in Figure 3, the signals of both PLA and HPMC 255 components present the same diffusion coefficient, which indicates that the two blocks are 256 attached in one molecule. In other words, the copolymer was effectively synthesized. 257



Figure 3. DOSY NMR spectrum of $(HPMC7K)_2$ -PLA2K copolymer in DMSO- d_6 at 298 K

FT-IR is also used to examine the structure of copolymers. Figure 4 presents the FT-IR 261 spectra of alkynyl terminated PLLA, thiol terminated HPMC, and (HPMC7K)₂-PLA2K 262 copolymer. Alkynyl terminated PLLA presents characteristic absorption bands at 1760, 2130 263 and 3300 cm⁻¹ belonging to the carbonyl group, and alkynyl group, respectively. Thiol 264 terminated HPMC presents an amide adsorption band at 1652 cm⁻¹, a strong C-O absorption 265 band at 1047 cm⁻¹, and a broad O-H stretching vibration band at 3480 cm⁻¹. (HPMC)₂-b-PLA 266 shows the characteristic bands from both components, in agreement with the successful 267 coupling of PLLA and HPMC blocks. 268



Figure 4. FT-IR spectra of (a) alkynyl terminated PLLA, (b) thiol terminated HPMC, and (c)
(HPMC7K)₂-PLA2K copolymer.

269

The molar mass of the copolymers was determined by SEC in conjunction with online light scattering and refractive index (RI) detectors, as shown in Figure 5. RI detector is most commonly used as concentration detector whose response is proportional to the total solute concentration in the detector cell. Meanwhile, the response of multi-angle laser light scattering (MALLS) detector depends on the molar mass of a polymer in the detector cell (Kostanski, Keller, & Hamielec, 2004). Combination of the two curves allows to determine the weight average molar mass of copolymers.



Figure 5. Refractive index and light scattering curves of (HPMC7K)₂-PLA2K (left), and SEC 281 curves of HPMC-SH and (HPMC7K)₂-PLA2K in water 282 283 The M_w and dispersity data of the three copolymers are summarized in Table 1. The M_w 284 ranges from 12000 for (HPMC5K)₂-PLA2K to 22 0000 g mol⁻¹ for (HPMC10K)₂-PLA2K. It 285 is noted that the M_w of the copolymers well agrees with the sum of the molar masses of both 286 components although the value of M_n is used for PLA. Meanwhile, the dispersity ($D=M_w/M_n$) 287 ranges from 1.13 to 1.26, in agreement with narrow molar mass distribution of copolymers. 288 289

- 290 291

Table 1. Characterization of (HPMC)₂-PLA copolymers

Sample	M _{w, SEC} a)	Đ a)	Cp ^{b)}	CMC ^{c)}	$R_h^{\overline{d})}$	PDI ^{d)}
1	(g/mol)		(°C)	(mg/mL)	(nm)	
(HPMC10K) ₂ -PLA2K	22000	1.26	65.2	0.16	120	0.21
(HPMC7K) ₂ -PLA2K	16000	1.24	64.8	0.15	90	0.12
(HPMC5K) ₂ -PLA2K	12000	1.13	64.1	0.14	66	0.19

292

a) Determined from SEC/MALS/RI measurement in water at 5.0mg mL⁻¹ 293

b) Determined from turbidimetry measurement in water at 20.0 mg mL⁻¹ 294

c) Determined at 25°C by dynamic light scattering 295

d) Determined at 25°C by dynamic light scattering at 1.0 mg mL⁻¹ 296

297

3.2. Cloud point of (HPMC)₂-PLA copolymers 298

The cloud point (Cp), also named lower critical solution temperature (LCST), is 299 considered as the solubility limit of amphiphiles due to phase-separation as the temperature 300 increases. In fact, the hydrogen bonding between polymer chains and surrounding water 301

molecules weakens when the temperature approaches the cloud point, leading to decrease of
the polymer solubility and phase separation. Thus the polymer precipitates out of solution as a
consequence of equal chemical potentials between the two phases: one is rich in polymer, and
the other rich in solvent (Sardar, Kamil, Kabir ud, & Sajid Ali, 2011).

Figure 6 shows the transmittance changes of (HPMC7K)₂-PLA2K solutions at 3.0 306 mg/mL, in comparison with HPMC7K homopolymer. The two solutions initially exhibit a 307 transmittance close to 100%. With increasing temperature, the transmittance of HPMC7K 308 remained almost unchanged till 90°C. This indicates that HPMC oligomers are not thermos-309 responsive in this temperature range, in contrast to high molar mass HPMC (Sardar et al., 310 2011). Interestingly, (HPMC7K)₂-PLA2K exhibits a decrease of light transmittance to nearly 311 312 20% from about 60°C up to 90 °C. The clouding at a given temperature results from the destruction of hydrogen bonding between water and HPMC molecules leading to phase 313 separation (Khan, Anjum, Koya, Qadeer, & Kabir ud, 2014). The Cp value was determined by 314 extrapolation of the linear region of 100% of transmittance and tangent line of the inflexion 315 point. A Cp value of 64.8°C is obtained for (HPMC7K)₂-PLA2K. Similarly, Cp values of 65.2 316 and 64.1°C are obtained for (HPMC10K)₂-PLA2K and (HPMC5K)₂-PLA2K (Table 1). 317 Therefore, the length of HPMC blocks has little effect on the Cp value of the copolymers. 318 Similar results have been previously reported in literature for poly(lactide-b-N-319 isopropylacrylamide-b-lactide) (PLA-b-PNIPAAm- b-PLA) triblock copolymers (You, Hong, 320 Wang, Lu, & Pan, 2004). In fact, a Cp value of about 31°C was obtained for copolymers with 321 PLA/PNIPAAm ratios of 1.0/1.9, 1.0/3.0 and 1.0/3.6. 322

323



Figure 6. Transmittance changes of HPMC7K (a) and (HPMC7K)₂-PLA2K (b) solutions at

3.0 mg/mL as a function of temperature.

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324

In our previous work, the phase separation behavior of linear HPMC-b-PLA diblock 328 copolymers with different HPMC block lengths (HPMC5K-PLA2K, HPMC8K-PLA2K, and 329 HPMC10K-PLA2K) was investigated (Wang et al., 2017). All copolymers exhibit a slow 330 decrease of light transmittance from 55 to 80 °C. Beyond, a sharper decrease of transmittance 331 down to 78% is detected. The three diblock copolymers exhibit almost the same Cp values of 332 *c.a.* 80°C. Therefore, the length of HPMC blocks has little effect on the Cp, but the copolymer 333 topology seems to significantly affect the phase separation behavior of HPMC-b-PLA 334 copolymers. It has been reported that the cloud point was elevated by more than 40 °C 335 through the linear-to-cyclic topological conversion of the polymer amphiphiles (Honda, 336 Yamamoto, & Tezuka, 2010). 337

338

339 3.3. Self-assembly of (HPMC)₂-PLA copolymers

340

0 The self-assembly properties of (HPMC)₂-PLA copolymers were investigated to evaluate

their potential as drug carrier. NMR provides a means to analyze the self-assembly behavior 341 of amphiphilic block copolymers in solvents which can dissolve one of the two blocks only. 342 Figure 2(B) shows the ¹H NMR spectrum of (HPMC7K)₂-PLA2K copolymer in deuterated 343 344 water (D₂O). Different from DMSO- d_6 which is a good solvent for both HPMC and PLLA, D₂O only solubilizes hydrophilic HPMC segments. Therefore, only signals belonging to 345 HPMC block are detected. Signals corresponding to PLLA block are not detected because of 346 the limited molecular motion in aqueous medium, which is consistent with the formation of 347 micelles with a hydrophilic HPMC shell and a hydrophobic PLLA core. 348

Similar to low molar mass surfactants, the self-assembly of the amphiphilic copolymers 349 occurs when the concentration reaches the CMC. The latter is an important parameter of 350 amphiphilic copolymers as it determines the stability of micelles. In the case of drug delivery 351 systems, drug loaded micelles should remain stable with dilution after intravenous injection. 352 In this work, the CMC was determined from scattering intensity changes of copolymer 353 solutions as a function of concentration. At low concentrations, the scattered intensity is very 354 low because of the absence of nano-objects. With increasing concentration, the intensity 355 strongly increases due to the formation of nano-micelles. The CMC value is obtained from the 356 crossover point of the two regression lines. As shown in Table 1, the CMC of (HPMC10K)₂-357 PLA2K, (HPMC7K)₂-PLA2K, and (HPMC5K)₂-PLA2K copolymers is 0.16, 0.15, and 0.14 g 358 L^{-1} , respectively. These values well agree with the reported CMC value 0.13 mg/mL for 359 cellulose-g-polylactide (Guo et al., 2012). Obviously the CMC increases with the hydrophilic 360 ratio or the HPMC block length as the self-assembly of neutral block copolymers is mainly 361 determined by the hydrophilic/hydrophobic balance (Larue et al., 2008). 362

363	The self-assembled (HPMC) ₂ -PLA micelles are composed of a PLA core surrounded by
364	a HPMC corona. The average diameter of (HPMC5K) ₂ -PLA2K, (HPMC7K) ₂ -PLA2K,
365	(HPMC10K) ₂ -PLA2K micelles was 66, 90 and 120 as determined by DLS, as shown in Table
366	1. All the micelles exhibited a unimodal size distribution, and the PDI was in the range of 0.12
367	to 0.21. Thus, the micelle size of copolymers increases with the increase of hydrophilic
368	HPMC chain length, in agreement with literature. In our previous work, the average micelle
369	size of HPMC5K-PLA2K, HPMC8K-PLA2K, HPMC10K-PLA2K diblock copolymers was
370	62, 96, 120 nm, respectively (Wang et al., 2017). Thus, micelles of (HPMC5K) ₂ -PLA2K had
371	a smaller size compared to HPMC10K-PLA2K with the same hydrophilic/hydrophobic
372	balance but a Y-type topology. It has been reported that the chain architecture affects the
373	micellization properties, including the aggregation number, size, polydispersity, and micelle
374	density (Liu et al., 2007). Generally, the micelle size of multiple-arm copolymers is smaller
375	than that of linear ones. Meanwhile, micelles with the same HPMC block length but different
376	topologies had almost the same size. This finding could be attributed to the fact that the core
377	is composed of the same PLA blocks, and the corona of similar HPMC chains although the
378	chain density should be higher for Y-type copolymers.



Figure 7. TEM images of (HPMC)₂-PLA micelles in water: (A) (HPMC5K)₂-PLA2K; (B)

(HPMC7K)₂-PLA2K; (C) (HPMC10K)₂-PLA2K.

The morphology of (HPMC)₂-PLA micelles was characterized by using TEM. Figure 7 shows that the micelles were spherical in shape and uniformly distributed. The observed micelle size increased with the increasing of the HPMC chain length, which well agreed with the results obtained by DLS.

388

389 4. Conclusion

AB₂-type amphiphilic block copolymers (HPMC)₂-PLA with three different HPMC 390 block lengths were synthesized by ring opening polymerization, reductive amination and click 391 reaction. The copolymers were characterized by using various methods, including NMR, 392 393 DOSY-NMR, SEC and FT-IR. The cloud point of AB₂-type (HPMC)₂-PLA are nearly 64 °C, which is obviously lower than HPMC oligomers and linear HPMC-b-PLA diblock 394 copolymers. The HPMC block length has little effect the Cp value. (HPMC)₂-PLA 395 copolymers can self-assemble to form spherical micelles with narrow distribution. The size of 396 copolymer micelles increases with the increase of HPMC fraction. So does the CMC of 397 (HPMC)₂-PLA. It is thus concluded that biobased and biodegradable HPMC-PLA copolymers 398 could be promising as nano-carrier of hydrophobic drugs. 399

400

401 Keywords: Hydroxypropyl methyl cellulose; Poly(L-lactide); Topology; Self-assembly;
402 Micelle

403

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