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## **Synthesis of methylcellulose model copolymers with heterogeneous distribution and their solution properties**

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In order to elucidate the characteristic features of commercial methylcellulose precisely, *O*-methylcellulose model copolymers consisting of 2,3,6-tri-*O*-methylhydroglucose unit (236MeAGU) and 2-*O*-methylhydroglucose unit (2MeAGU) with various composition ratios were synthesized via cationic ring-opening copolymerization of the corresponding glucose orthoester derivatives, subsequent removal of pivaloyl and allyl groups, and methylation. The structure of the obtained copolymers was confirmed by <sup>1</sup>H-, <sup>13</sup>C-NMR, and FT-IR. Temperature-dependent turbidity measurement verified their thermoresponsive behavior in aqueous solution. The lower critical solution temperature (LCST) was tuned from 63 to 45 °C above 47 mol-% 236MeAGU content. The hydrophobicity along the cellulose chain was dominant to determine their physical properties. However, the aqueous properties of the MC model copolymers were strongly affected by the slight difference of the composition ratio. The present method would provide further details of the structure-property relationship of *O*-methylcellulose.

*Keywords: cationic ring-opening copolymerization; methylcellulose; model study; phase separation; structure-property relationship*

Abbreviations:

AGU anhydroglucose unit

DP degree of polymerization

DS degree of substitution

GPC gel permeation chromatography

LCST lower critical solution temperature

MC *O*-methylcellulose

2MeAGU 2-*O*-methylanhydroglucose unit

236MeAGU 2,3,6-tri-*O*-methylanhydroglucose unit

$M_n$  number-averaged molecular weight

$M_w$  weight-averaged molecular weight

2Piv36AllAGU 3,6-di-*O*-allyl-2-*O*-pivaloylanhydroglucose unit

2Piv36MeAGU 3,6-di-*O*-methyl-2-*O*-pivaloylanhydroglucose unit

$T_{CP}$  cloud point on heating

$T_{RCP}$  cloud point on cooling

## **Introduction**

*O*-Methylcellulose (MC) is the simplest type of cellulose ether derivatives, but nevertheless is one of the most attractive biomaterials for many industrial applications: foods, cosmetics, pharmaceuticals, adhesive agents, binders, and so on. The aqueous solution of commercial MC with the degree of substitution (DS) from 1.6 to 1.9 undergoes thermoreversible gelation in water at an elevated temperature, typically above 60 °C, known as the lower critical solution temperature (LCST) (Heymann 1935; Savage 1957). So far, many studies have been carried out to the elucidation of the phase separation and the sol-gel transition mechanism as well as the water solubility (Kato et al. 1978; Takahashi et al. 1987; Haque et al. 1993; Sarkar et al. 1995; Hirrien et al. 1996, 1998; Nishinari et al. 1997; Desbrières et al. 1998; Kobayashi et al. 1999; Takahashi et al. 2001; Li 2002; Li et al. 2002; Wang et al. 2005; Fitzpatrick et al. 2006; Schupper et al. 2008).

Most of the previous authors have ascribed the gelation to the hydrophobic association of methyl group or hydrophobic segments when water becomes a poorer solvent for them at higher temperatures. Despite a lot of studies concerning the physical properties of MC, it is still difficult to describe the phenomena quantitatively, reveal the molecular structure of the gelation point, and predict or control the LCST for desired applications. This is because MC can be regarded as a random copolymer consisting of one unsubstituted and seven substituted (2-*O*-, 3-*O*-, 6-*O*-, 2,3-di-*O*-, 2,6-di-*O*-, 3,6-di-*O*-, 2,3,6-tri-*O*-methyl) anhydroglucose units (AGU), and the distribution of methyl group along the cellulose chain is not uniform, which causes a barrier to gain detailed and quantitative information. The transition of MC is therefore relatively slow and involve hysteresis between the heating and cooling states stemming from their ill-defined structure, which is quite different from synthetic polymers including poly(*N*-isopropylacrylamide) (Fujishige et al. 1989). Whereas many efforts have been made to control the LCST by adding salts or surfactants to MC aqueous solutions (Hirrien et al. 1998; Kundu et al. 2001; Xu et al. 2004; Li et al. 2007) and/or introducing another substituents such as hydroxypropyl group to the remaining hydroxyl groups of MC (Haque et al. 1993; Sarkar et al. 1995; Viridén et al. 2009), it is also important to enhance the capabilities of unmodified MC by studying the influence of the structure on property in detail.

To date, regioselectively substituted MCs have been synthesized and studied as a model of commercial MC in order to resolve the remaining issues about the structure-property relationship (Kondo et al. 1991; Kondo 1997; Liu et al. 1997; Liu et al. 1998; Kern et al. 2000; Sekiguchi et al. 2003; Kamitakahara et al. 2006, 2008, 2010; Karakawa et al. 2007a, b). For example, Kondo (1997) has reported the synthesis of region-regular MCs, 2,3-di-*O*-MC with DS of 2.0 and 6-*O*-MC with DS of 1.0, and showed that these two samples are insoluble in water. Karakawa et al. (2002a,b; 2007a) have reported the syntheses of six highly regioselectively substituted MCs with DS of 1.0 (2-*O*-, 3-*O*-, 6-*O*-MC) and DS of 2.0 (2,3-di-*O*-, 2,6-di-*O*-, 3,6-di-*O*-MC) via cationic ring-opening polymerization and showed that all these completely regio-regular MCs are also insoluble in water. These results suggest that the homogeneous distribution of methyl group along the cellulose chain is undesirable for their water solubility of MC.

As a part of our continuous efforts to investigate the structure-property relationship, we have recently reported that the random copolymer of 2,3,6-tri-*O*-methylAGU (236MeAGU) and 2-*O*-methylAGU (2MeAGU) in a ratio of 1:1, P(236MeAGU<sub>50-co</sub>-2MeAGU<sub>50</sub>), is soluble in water, although that of 2,6-di-*O*-methylAGU (26MeAGU) and 2MeAGU in a ratio of 1:1,

P(26MeAGU<sub>50-co</sub>-2MeAGU<sub>50</sub>), is insoluble in water (Karakawa et al. 2007b). These results demonstrate that the discontinuance of intramolecular hydrogen bonding by 3-OH along a cellulose molecule is indispensable for the water solubility; the successive intramolecular hydrogen bonding makes MC insoluble, even if methyl groups were heterogeneously distributed along the cellulose backbone. This conclusion can be supported by the recent report of Rosenau et al. (2009) showing that 3-OH of AGU plays an essential role in cellulose swelling and dissolution at a molecular level in *N*-methylmorpholine oxide (NMMO). However, these reports have focused primarily on solubility while many aspects of their physical properties still have to be explored.

To examine the characteristic features of MC in more detail, it is necessary to define the distribution of substituent precisely, which can be only controlled by cationic ring-opening copolymerization of glucose orthoester derivatives with predetermined proportions. By this method, one can control and define the average distribution of methyl groups along the cellulose chain. Consequently, several MC model copolymers with various composition ratios can be prepared from copolymerizations of methylated and/or non-methylated glucose orthoester derivatives as comonomers.

In this study, we report on the synthesis of MC model copolymers consisting of 236MeAGU and 2MeAGU with various composition ratios, P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>) (Figure 1), and their thermoresponsive behavior in aqueous solutions. The nature of the phase separation of these copolymers in dilute aqueous solution was investigated using turbidimetry measurement. Some random copolymers here with various ratios of hydrophilic and hydrophobic units were found to show LCST in water by selecting appropriate composition.

## Experimental section

### *Materials.*

3,6-Di-*O*-methyl- $\alpha$ -D-glucopyranose 1,2,4-orthopivalate (comonomer **1**) and 3,6-di-*O*-allyl- $\alpha$ -D-glucopyranose 1,2,4-orthopivalate (comonomer **2**) were prepared according to the procedures reported previously (Karakawa et al. 2002a). Other reagents and solvents were purchased from Nakalai Tesque, Inc. (Kyoto, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), or TCI (Tokyo, Japan).

### *Instrumentation.*

$^1\text{H}$  (300 MHz) and  $^{13}\text{C}$  NMR (75 MHz) spectra were recorded on a Varian Inova 300 FT-NMR spectrometer in chloroform-*d* with tetramethylsilane as an internal standard or deuterium oxide with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as an external standard. Proton and carbon signals were assigned by two-dimensional NMR experiments. Chemical shifts ( $\delta$ ) and coupling constants ( $J$ ) are given in value (ppm) and Hz, respectively.

Fourier transform infrared (FT-IR) spectra were recorded on a Shimadzu FTIR-4000 spectrophotometer. Samples were mixed with KBr and pressed into disks. These pellets were dried in vacuo at 80 °C overnight before the measurement.

Molecular weight distribution of the copolymers was measured by gel permeation chromatography (GPC) in chloroform at 40 °C. A Shimadzu liquid chromatography injector (LC-10ATvp), a Shimadzu column oven (CTO-10Avp), a Shimadzu ultraviolet-visible detector (SPD-10Avp), a Shimadzu refractive-index detector (RID-10A), a Shimadzu communication bus module (CBM-10A), a Shimadzu LC workstation (CLASS-LC10), and Shodex columns (KF802, KF802.5, and KF805) were used. The flow rate was 1.0 mL·min<sup>-1</sup>. The calibration curves were obtained with polystyrene standards (Shodex). GPC based on calibration with polystyrene is known to produce errors and overestimation since flexible and relatively compact polystyrene elute earlier than the stiff and extended cellulose derivatives. Therefore, the molecular weights obtained should be apparent values.

The optical transmittance of the aqueous solution of P(236MeAGU<sub>m</sub>-*co*-2MeAGU<sub>n</sub>) at a wavelength of 600 nm was recorded on a Jasco Model V-560 UV-vis spectrophotometer equipped with a peltier type thermostatic cell holder coupled with a controller (EHC-477S). The temperature-dependent turbidity of the samples (1 wt.-% = 10 g·L<sup>-1</sup>) was examined with the heating and cooling rates of 1.0 °C·min<sup>-1</sup> between 10 and 70 °C. The light transmittance through the aqueous solutions was normalized to 100 % at the starting temperature. The cloud points on heating ( $T_{\text{CP}}$ ) and cooling ( $T_{\text{RCP}}$ ) were defined as the temperature corresponding to 50 % decreases of transmittance.

### *Polymerization Procedures.*

Polymerizations were carried out under a high vacuum line capable of maintaining a vacuum of  $1 \times 10^{-3}$  Torr (Nakatsubo et al. 1996). Comonomers **1** and **2** at a prescribed molar ratio were dried in a polymerization ampule equipped with a magnetic stirring rod by evacuating approximately for a

day. Dichloromethane (2 mL per 1 g of the combined weights of comonomers **1** and **2**) was dried over CaH<sub>2</sub>, distilled, and degassed by freeze-evacuate-thaw cycle three times in a high vacuum line. The solvent was transferred to the ampule containing the comonomers under high vacuum. The reaction apparatus was then separated by melting off and placed in a cooling bath at – 30 °C. BF<sub>3</sub>-Et<sub>2</sub>O (5 mol-%) was added into the reaction ampule by a syringe. After 48 hr at – 30 °C with stirring, the polymerization was terminated by adding a small amount of cold methanol-pyridine (1:1, v/v) to neutralize. The reaction mixture was precipitated in a large excess amount of *n*-hexane and collected by centrifuge separation. The precipitated copolymer was washed with *n*-hexane twice and dried in vacuo to yield P(2Piv36MeAGU<sub>m</sub>-co-2Piv36AllAGU<sub>n</sub>) as a white powder. The yields and molecular weights of the copolymers are shown in Table 1. The expanded <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of the copolymers with different composition ratios are shown in Figure 3.

The average composition (2Piv36MeAGU : 2Piv36AllAGU) in the copolymers was determined using <sup>1</sup>H-NMR spectroscopy by a comparison of peaks associated with the two comonomers. The multiplet peak at 5.7 – 6.0 ppm is attributed to the allyl methine protons of the 2Piv36AllAGU at the C-3 and 6 positions, whereas the peaks at 1.2 ppm correspond to the pivaloyl methyl protons of both units at the C-2 position. Thus, the comonomer composition can be calculated using equation 1,

$$\frac{2x}{9} = \frac{\text{integral at 5.7 – 6.0 ppm}}{\text{integral at 1.2 ppm}} \quad (1)$$

where *x* is the fraction of 2Piv36AllAGU in the random copolymer.

*Conversion of P(2Piv36MeAGU<sub>m</sub>-co-2Piv36AllAGU<sub>n</sub>) to P(236MeAGU<sub>m</sub>-co-2Me36AcAGU<sub>n</sub>).*

To a solution of P(2Piv36MeAGU<sub>m</sub>-co-2Piv36AllAGU<sub>n</sub>) in THF-methanol (4:1, v/v), 28 % sodium methoxide in methanol (2 equivalents per one pivaloyl group) was added. The reaction mixture was kept under reflux overnight. The reaction mixture was treated with 1*N* HCl for neutralization, concentrated, and dried in vacuo to yield the crude depivaloylated copolymer, P(36MeAGU<sub>m</sub>-co-36AllAGU<sub>n</sub>).

Powdered sodium hydroxide (10 equivalents per one hydroxyl group) and methyl iodide (10 equivalents per one hydroxyl group) was added to a solution of the depivalated copolymer in anhydrous *N,N*-dimethylformamide at 0 °C. The reaction was stirred at room temperature for 24 hr. After addition of a second portion of sodium hydroxide (5 equivalents of one hydroxyl group) and

methyl iodide (5 equivalents of one hydroxyl group), the mixture was stirred at room temperature for another 48 hr. The reaction mixture was neutralized with 1N HCl, diluted with dichloromethane, washed with distilled water and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to yield the methylated copolymer, P(236MeAGU<sub>m-co</sub>-2Me36AllAGU<sub>n</sub>).

To a solution of P(236MeAGU<sub>m-co</sub>-2Me36AllAGU<sub>n</sub>) in chloroform-methanol (1:1, v/v), palladium dichloride (0.3 equivalents per one allyl group) was added. The reaction mixture was kept at 60 °C for 4 hr. The reaction mixture was filtered and concentrated to dryness. The dried product was treated with acetic anhydride and pyridine at 60 °C overnight. The reaction mixture was coevapulated with ethanol to dryness. The crude acetylated copolymer was purified by gel chromatography (Sephadex LH-20, Pharmacia) with dichloromethane as an eluent to yield the acetylated copolymer, P(236MeAGU<sub>m-co</sub>-2Me36AcAGU<sub>n</sub>). The yields of P(236MeAGU<sub>m-co</sub>-2Me36AcAGU<sub>n</sub>) via the series of reaction from P(2Piv36MeAGU<sub>m-co</sub>-2Me36AllAGU<sub>n</sub>) were 67 to 88 %.

*P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>).*

To a solution of P(236MeAGU<sub>m-co</sub>-2Me36AcAGU<sub>n</sub>) in 1,4-dioxane-methanol (4:1, v/v), 28 % sodium methoxide (10 equivalents per one acetyl group) was added. The reaction mixture was stirred at room temperature overnight and then at 60 °C for 2 hr. The reaction mixture was treated with 1N HCl for neutralization, concentrated, and dried in vacuo. The product was dialyzed against water by a dialysis membrane (Spectra/Por, regenerated cellulose, MWCO=1000) and lyophilized to yield P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>) as a powder with the yields of 66 to 81 %.

The aqueous solutions were prepared by dispersing P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>) into hot distilled water to avoid coagulation and then stored in a refrigerator (ca. 4 °C) for at least 24 hr prior to measurement.

## Results and Discussion

*Synthesis of P(2Piv36MeAGU<sub>m-co</sub>-2Piv36AllAGU<sub>n</sub>) via Cationic Ring-Opening Random Copolymerization.*

Copolymerization of comonomers **1** and **2** was carried out with BF<sub>3</sub>-Et<sub>2</sub>O (5 mol-%) in dichloromethane at – 30 °C for 48 hr in a high vacuum system (Figure 2). Although the cationic copolymerization (**1:2** = 50:50 molar feed ratios) has been shown to be feasible to get a clear



information concerning the difference of comonomer reactivities (Karakawa et al. 2007b), that on the different comonomer composition remains untested. Then, the five different compositions were investigated in the present work: mol-% feed ratios of comonomers **1** and **2** = 25:75, 32.5:67.5, 50:50, 67.5:32.5, and 75:25. The global concentration of the comonomers was kept at 0.5 g·mL<sup>-1</sup>. The results of the copolymerization are listed in Table 1. In every case, the copolymers, P(2Piv36MeAGU<sub>m</sub>-*co*-2Piv36AllAGU<sub>n</sub>) (m = mol-% of 2Piv36MeAGU; n = mol-% of 2Piv36AllAGU), were obtained as precipitates in good yields. Since both comonomers were consumed after 48 hr from the TLC analysis of the reaction mixture, the conversions were assumed to be 100 %. The low-molecular-weight products could be removed in the process of precipitation. The range of number-averaged molecular weights ( $M_n$ ) and polydispersity values determined by GPC were  $8.5 \times 10^3$  to  $1.4 \times 10^4$  and 1.6 to 2.5, respectively, and that of degree of polymerizations ( $DP_n$ ) were 29 to 46 (Table 1).

Figure 3a shows the <sup>1</sup>H-NMR spectra of P(2Piv36MeAGU<sub>m</sub>-*co*-2Piv36AllAGU<sub>n</sub>) in CDCl<sub>3</sub>. The characteristic peaks at 5.7 – 6.0 ppm (m, -CH<sub>2</sub>CH=CH<sub>2</sub>), 5.0 – 5.3 ppm (m, -CH<sub>2</sub>CH=CH<sub>2</sub>), 4.0 and 4.5 ppm (m, -CH<sub>2</sub>CH=CH), and 3.37 and 3.43 ppm (2×s, -OMe) are clearly seen in all spectra, which attribute to the substituents attached to the C-3 and C-6 positions of the copolymers. In addition, the peaks of the backbone are clearly visible at 4.8 ppm (C<sub>2</sub>-H), 3.9 – 4.0 ppm (C<sub>4</sub>-H), 3.5 – 3.8 ppm (C<sub>6</sub>-H), 3.3 ppm (C<sub>3</sub>-H), and 3.1 – 3.2 ppm (C<sub>5</sub>-H). Two anomeric protons of 2Piv36MeAGU and 2Piv36AllAGU appear at 4.4 and 4.1 ppm as broad doublets, respectively. The molar fractions of the copolymers estimated from equation (1) were in reasonable agreement with the comonomer feed ratios. Additionally, the integrals of the allyl and methyl groups are well compatible with the composition ratios (Figure 3a). This behavior can be also seen in the expanded <sup>13</sup>C-NMR spectra (Figure 3b). The integrals of the peaks at 59.2, 60.4 ppm (OCH<sub>3</sub>) and 71.9, 72.2 ppm (OCH<sub>2</sub>-CH=CH<sub>2</sub>) increase with an increase in the percentage of 2Piv36MeAGU and 2Piv36AllAGU, respectively. The C-3 and C-6 resonances of 2Piv36MeAGU and 2Piv36AllAGU can be clearly assigned and are also well compatible with the composition ratios: the C-3 and C-6 peaks of 2Piv36MeAGU were assigned to be 82.6 and 69.8 ppm and those of 2Piv36AllAGU were 80.9 and 67.5 ppm, respectively. It should be mentioned that all P(2Piv36MeAGU<sub>m</sub>-*co*-2Piv36AllAGU<sub>n</sub>) were stereoregular β-1,4-glucopyranans consisting of two types of AGU because each C-1 carbon signal appeared only at 99.5 ppm (C-1 of 2Piv36AllAGU) and 99.6 ppm (C-1 of 2Piv36MeAGU). The anomeric peaks of the copolymers were distinctly

different from those of the corresponding homopolymers: 103.2 and 100.5 ppm of P(2Piv36MeAGU) and P(2Piv36AllAGU), respectively (Karakawa et al. 2007b). Because random distribution in fact includes homopolymeric blocks in statistical proportions where the chemical shifts of anomeric carbons should be equal to the corresponding homopolymers, the obtained copolymers have irregular structure along the cellulose chain.

*Conversion to P(236MeAGU<sub>m</sub>-co-2MeAGU<sub>n</sub>).*

The obtained copolymers were converted to P(236MeAGU<sub>m</sub>-co-2Me36AcAGU<sub>n</sub>) via deprotection, methylation, deallylation, and acetylation. The yields of P(236MeAGU<sub>m</sub>-co-2Me36AcAGU<sub>n</sub>) via the series of the reaction after the copolymerization were 67 to 88 %. From GPC analysis of the acetylated copolymers, the DP<sub>n</sub> values were found to be almost unchanged during deprotection (Table 2), indicating that the decomposition did not occur.

Finally, the target MC model copolymers, P(236MeAGU<sub>m</sub>-co-2MeAGU<sub>n</sub>), were prepared by deacetylation with sodium methoxide in 1,4-dioxane/ methanol in the yield of 66 to 81 %. Figure S1 shows the FT-IR spectra of P(236MeAGU<sub>m</sub>-co-2MeAGU<sub>n</sub>). The stretching vibration of carbonyl was not observed in the FT-IR spectra, indicating the protecting acetyl groups were successfully removed. In addition, the intensity of the OH stretching band around 3460 cm<sup>-1</sup> decreased with increasing DS of methyl group. <sup>13</sup>C-NMR spectra of the five copolymers in D<sub>2</sub>O are shown in Figure 4. The assignments of the peaks were confirmed by two-dimensional NMR experiments and according to the report of Kern et al (2000). The peaks C-2<sub>s</sub> and C-2<sub>u</sub> were assigned to C-2 carbons bearing a substituted (methylated) and an unsubstituted (unmethylated) hydroxyl group, respectively. Because C-1 and C-4 carbons have no hydroxyl group, the peaks C-1<sub>(2s)</sub> and C-1<sub>(2u)</sub> are assigned to be the C-1 carbons adjacent to C-2 carbons bearing a substituted and an unsubstituted hydroxyl group, respectively, while the peaks C-4<sub>(3s)</sub> and C-4<sub>(3u)</sub> to C-4 carbons adjacent to C-3 carbons bearing a substituted and an unsubstituted hydroxyl group, respectively. The peak heights of C-2<sub>s</sub> and C-3<sub>s</sub> increase with an increase of the 236MeAGU ratio. However, the peaks C-2<sub>s</sub> and C-3<sub>s</sub> in P(236MeAGU<sub>41</sub>-co-2MeAGU<sub>59</sub>) and P(236MeAGU<sub>47</sub>-co-2MeAGU<sub>53</sub>) are ambiguous, suggesting that these peaks would become broader than those in the biased copolymers such as P(236MeAGU<sub>20</sub>-co-2MeAGU<sub>80</sub>) and P(236MeAGU<sub>74</sub>-co-2MeAGU<sub>26</sub>). X-ray diffraction analyses of P(236MeAGU<sub>m</sub>-co-2MeAGU<sub>n</sub>) reveal that these copolymers were essentially amorphous (data not shown).

It should be mentioned that the weight-averaged molecular weights of the copolymers obtained could be estimated to be around  $10^4$  after the deprotection, indicating that the copolymers showed rather lower molecular weight than that of actual MC. As described in the introduction section, however, it is difficult to gain insight into MC because of their ill-defined molecular structure. Thus, we think our approach, model studies using random copolymers, is advantageous to come close to the real MC. Although the molecular weight is certainly of a problem, these copolymers are suitable models to reveal the relationship between the substitution pattern and the phase separation behavior in aqueous solution. In fact, some experimental data will show similar tendency of commercial MC, as described in the next section.

#### **Thermally-Induced Phase Separation of P(236MeAGU<sub>m</sub>-*co*-2MeAGU<sub>n</sub>) in water.**

Aqueous solution of the five copolymers, P(236MeAGU<sub>m</sub>-*co*-2MeAGU<sub>n</sub>) (m:n = 20:80, 41:59, 47:53, 63:37, 74:26), with concentration of 1.0 wt.-% was prepared by dispersed the weighed powders into hot distilled water, shook for 30 min, then kept in a refrigerator (4 °C) for at least 24 hr.

The temperature-dependent turbidity of the aqueous solution of P(236MeAGU<sub>m</sub>-*co*-2MeAGU<sub>n</sub>) was examined using UV spectroscopy ( $\lambda = 600$  nm) at a constant scanning rate of  $1.0$  °C·min<sup>-1</sup> on heating and cooling in order to observe their thermal properties (Figure 5). The solution of P(236MeAGU<sub>20</sub>-*co*-2MeAGU<sub>80</sub>) exhibited a small hysteresis and low turbidity (Figure 5a), whereas that of P(236MeAGU<sub>41</sub>-*co*-2MeAGU<sub>59</sub>) did not change over the experimental temperature range (Figure 5b). However, in the case of other copolymers (m:n = 47:53, 63:37, and 74:26), the transmittance decreased on heating, indicative of a LCST-type phase separation, and reversibly increased at the subsequent lower temperature on cooling with hysteresis (Figures 5c-e). As an example, the images of the phase separation of P(236MeAGU<sub>74</sub>-*co*-2MeAGU<sub>26</sub>) before and after heating are shown in Figure 5f. On heating these copolymers (m:n = 47:53, 63:37, 74:26) up to 70 °C, no precipitation was observed, whereas the solution remained opaque.

The composition ratio was found to determine the temperature of phase separation, varying from 63 °C at m:n = 47:53 to 45 °C at m:n = 74:26. Here, the cloud points on heating ( $T_{CP}$ ) and cooling (return cloud point;  $T_{RCP}$ ) are defined as the temperature corresponding to 50 % transmittance (Table 2). The LCST-type phase separation occurred with a similar sensitivity in all cases with appreciable hysteresis ( $\Delta =$  ca. 20 °C), and both  $T_{CP}$  and  $T_{RCP}$  decreased linearly with increasing 236MeAGU content (Figure 6). It was demonstrated that the phase separation could be

manipulated in the range of 45 – 63 °C by controlling the composition ratios of 236MeAGU and 2MeAGU. The aqueous solution properties of P(236MeAGU<sub>41-co</sub>-2MeAGU<sub>59</sub>) and P(236MeAGU<sub>47-co</sub>-2MeAGU<sub>53</sub>) were quite different in spite of the small variation, which would be due to the probability of the existence of 236MeAGU block parts along the chain. Moreover, it should be pointed out the phase separation of P(236MeAGU<sub>74-co</sub>-2MeAGU<sub>26</sub>) aqueous solution occurred gradually and weakly, presumably because of very weak intermolecular hydrogen bonding through hydroxyl groups in 2MeAGU.

It is said that thermally-induced phase separation of MC is dependent mainly on molecular structure (Takahashi et al. 1987; Sarkar et al. 1995; Fitzpatrick et al. 2006), although there are another factors such as molecular weights (Nishinari et al. 1997; Takahashi et al. 2001; Wang et al. 2005), concentration in water (Kobayashi et al. 1999), rate of heating (Sarkar et al. 1995), and so on. In this study, difference of hydrophilic and hydrophobic compositions should be related to the thermal behavior of P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>), because other experimental factors are kept almost constant. The copolymer with the higher DS value showed a similar phase separation behavior to that of commercial MCs: Li et al (2002) reported that the transmittance of commercial MC (SM4000, DS = 1.8, MW = 3.8 × 10<sup>5</sup>) aqueous solution begins to decrease at about 50 °C, falls to 50 % at 62 °C ( $T_{CP}$ ), and becomes completely turbid at about 74 °C on heating, and the 50 % transmittance is achieved at 33 °C ( $T_{RCP}$ ) on subsequent cooling. In the present case, hydrogen bonding between hydrophilic 2MeAGU and water leads the cellulose copolymers to be soluble in water, whereas hydrophobic interaction between 236MeAGU is enhanced with increasing temperature, as illustrated in Figure 7. Apparently, the hydrophobicity along the cellulose chains is dominant to determine the LCST and physical properties of MC.

Kato et al. (1978) and Kamitakahara et al. (2006) have previously suggested that the existence of block parts of hydrophobic 236MeAGU plays an essential role in the phase transition on temperature. In this study, the LCST was found to be tuned by the composition ratio, that is, the hydrophobicity along the cellulose chain. Furthermore, the aqueous properties of the MC model copolymers were strongly affected by the slight difference of the composition ratio, which are not fully understood at present but it seems to be more complicated and concerted mechanism. Future work should be directed to reveal the structure-property relationship of MC model copolymers synthesized by this method.

## Conclusion

*O*-Methylcellulose model copolymers, P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>), were synthesized via cationic ring-opening random copolymerization of 3,6-di-*O*-methyl- and 3,6-di-*O*-allyl- $\alpha$ -D-glucopyranose 1,2,4-orthopivalate as comonomers with different molar compositions. The chemical structures were fully confirmed by <sup>1</sup>H-, <sup>13</sup>C-NMR, and FT-IR spectroscopy. The aqueous solution of P(236MeAGU<sub>20-co</sub>-2MeAGU<sub>80</sub>) exhibited a small hysteresis and low turbidity, whereas that of P(236MeAGU<sub>41-co</sub>-2MeAGU<sub>59</sub>) did not change over the experimental temperature range. However, in the case of other copolymers (m:n = 47:53, 63:37, and 74:26), the transmittance decreased on heating, indicative of a LCST-type phase separation, and reversibly increased at the subsequent lower temperature on cooling with hysteresis. From the temperature-dependent turbidity, the LCST was found to be determined by the hydrophobicity along the cellulose chain. Furthermore, the aqueous properties of the MC model copolymers were strongly affected by the slight difference of the composition ratio. The findings obtained through this study would increase the understanding of commercial MC and expand their range of application.

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Supporting Information Available: FT-IR data (Figure S1).

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## Legends of Figures

**Fig. 1** Structure of P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>)

**Fig. 2** Synthetic route for P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>)

**Fig. 3** (a) <sup>1</sup>H- and (b) <sup>13</sup>C-NMR spectra of P(2Piv36MeAGU<sub>m-co</sub>-2Piv36AllAGU<sub>n</sub>) in CDCl<sub>3</sub>

**Fig. 4** <sup>13</sup>C-NMR spectra of P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>) in D<sub>2</sub>O

**Fig. 5** Temperature dependence of the transmittance at 600 nm of P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>) aqueous solution: (a) m:n = 20:80; (b) 41:59; (c) 47:53; (d) 63:37; (e) 74:26; concentration: 1.0 wt.-%; rate 1.0 °C·min<sup>-1</sup>; heating (solid curve) and cooling (dashed curve). (f) Photographs of 1.0 wt.-% aqueous solution of P(236MeAGU<sub>74-co</sub>-2MeAGU<sub>26</sub>) at room temperature (left) and 60 °C (right)

**Fig. 6** Relationship between % composition of 236MeAGU and temperature of the 50 % transmittance on heating ( $T_{CP}$ ) (●) and cooling ( $T_{RCP}$ ) (○) for 1.0 wt.-% aqueous solution of P(236MeAGU<sub>m-co</sub>-2MeAGU<sub>n</sub>)

**Fig. 7** Schematic representation for relationship between the polymer composition and their phase separation in aqueous solutions. (Left) Schematic polymer chain consisting of black and white circles as 236MeAGU and 2MeAGU, respectively. (Right) Schematic illustration of polymer aggregation structure in aqueous solution; black and gray lines as hydrophobic 236MeAGU and hydrophilic 2MeAGU parts, respectively. Models of (a) P(236MeAGU<sub>20-co</sub>-2MeAGU<sub>80</sub>), (b) P(236MeAGU<sub>41-co</sub>-2MeAGU<sub>59</sub>), and (c) P(236MeAGU<sub>47-co</sub>-2MeAGU<sub>53</sub>), P(236MeAGU<sub>63-co</sub>-2MeAGU<sub>37</sub>), and P(236MeAGU<sub>74-co</sub>-2MeAGU<sub>26</sub>)

## Table

### Table 1 Results of copolymerization of comonomers 1 and 2 <sup>a</sup>

<sup>a</sup> Copolymerization conditions: BF<sub>3</sub>-Et<sub>2</sub>O (5 mol-%), CH<sub>2</sub>Cl<sub>2</sub>, -30 °C, 48 hr, concentration = 0.5 g·mL<sup>-1</sup>, total conversion ≈ 100 % (TLC);

<sup>b</sup> Yield of insoluble fraction in chloroform/ *n*-hexane (1/15 , v/v);

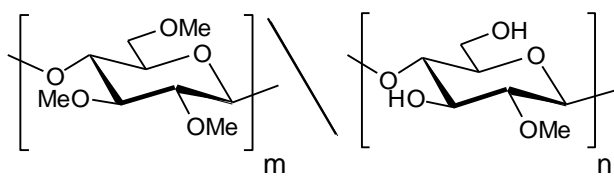
<sup>c</sup> Comonomer composition (m = 2Piv36MeAGU, n = 2Piv36AllAGU) determined by <sup>1</sup>H-NMR;

<sup>d</sup> Determined by GPC in chloroform with polystyrene calibration; these are apparent values because GPC based on calibration with polystyrene produces errors and overestimation

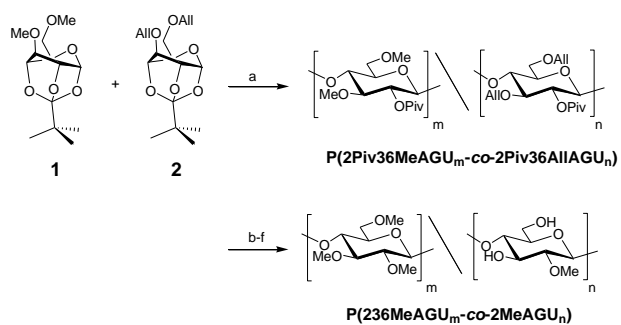
### Table 2. Characterization of P(236MeAGU<sub>m</sub>-*co*-2MeAGU<sub>n</sub>) and their aqueous solution properties

<sup>a</sup> Number-average molecular weight and polydispersity of the corresponding acetate determined by GPC in chloroform with polystyrene calibration; these are apparent values because GPC based on calibration with polystyrene produces errors and overestimation;

<sup>b</sup> Cloud points on heating ( $T_{CP}$ ) and cooling ( $T_{RCP}$ ) from turbidity measurements (polymer concentration: 1.0 wt.-%);

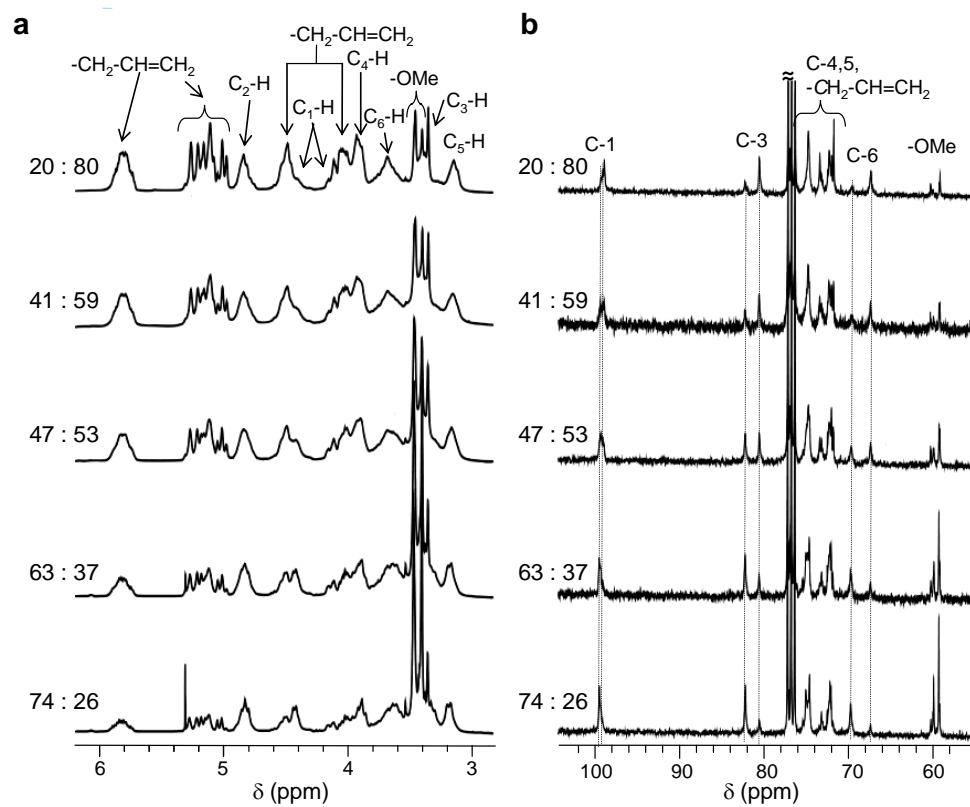


**Fig. 1**

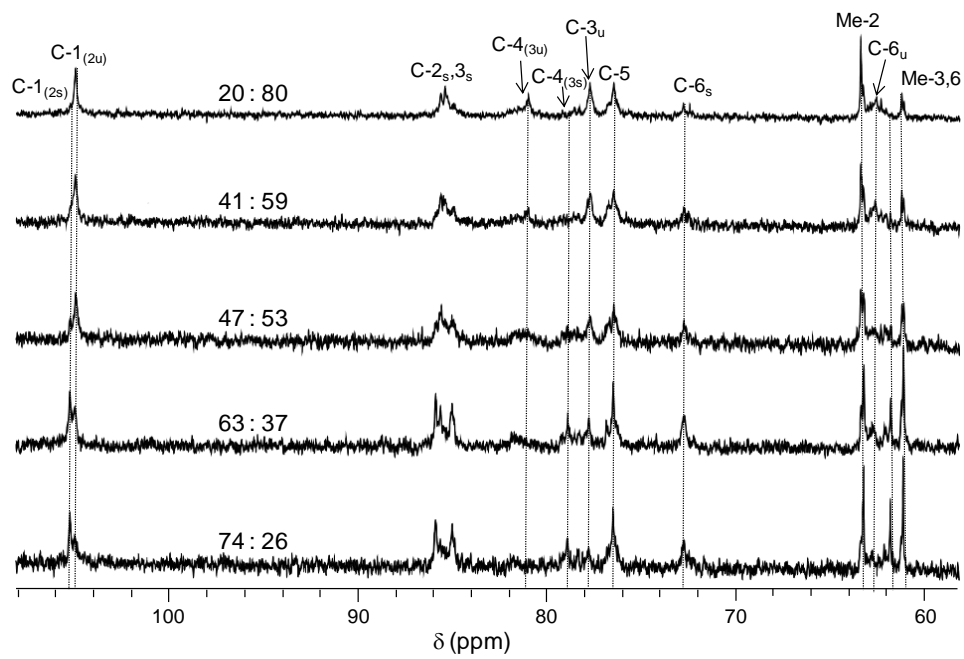


Conditions: <sup>a</sup> BF<sub>3</sub>-Et<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub>, -30 °C, 48 h.; <sup>b</sup> NaOMe/dioxane:MeOH (4:1, v/v), reflux, overnight.; <sup>c</sup> CH<sub>3</sub>I/NaOH/DMF, r.t., 3 d.; <sup>d</sup> PdCl<sub>2</sub>/CHCl<sub>3</sub>:MeOH (1:1, v/v), 60 °C, 4 h.; <sup>e</sup> Ac<sub>2</sub>O/Pyridine, 50 °C, overnight.; <sup>f</sup> NaOMe/dioxane : MeOH (4:1, v/v), r.t.→60 °C, overnight → 2 h.

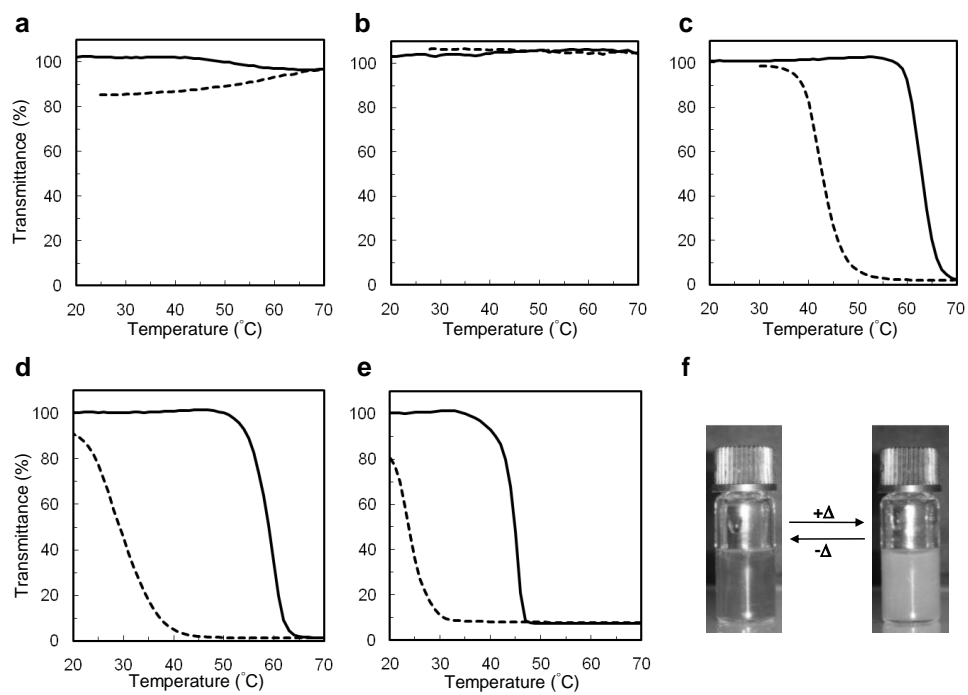
**Fig. 2**



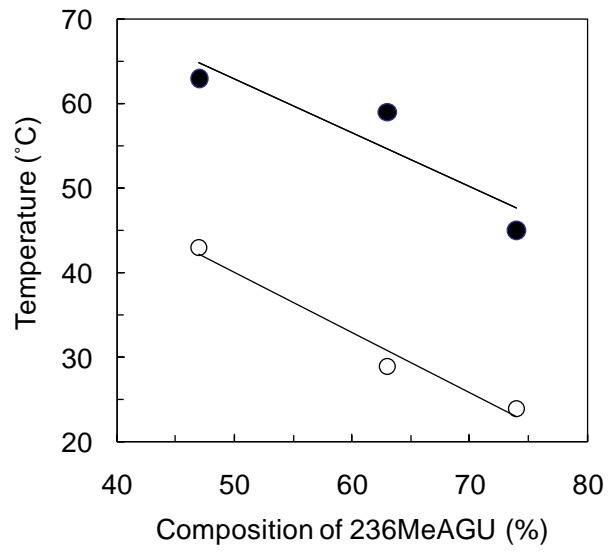
**Fig. 3**



**Fig. 4**

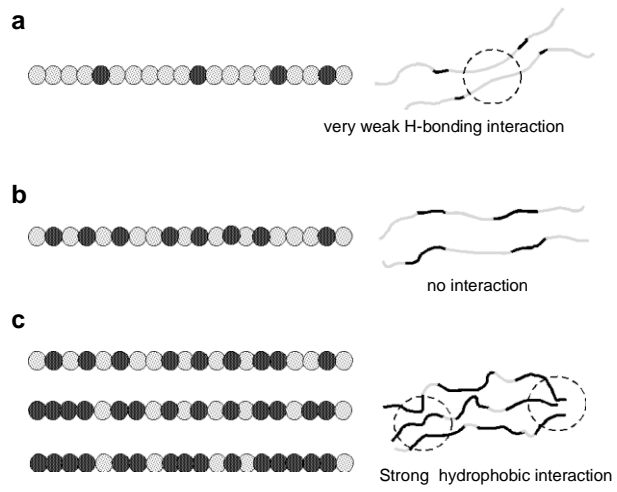


**Fig. 5**



**Fig. 6**





**Fig.**

**Table 1** Results of copolymerization of comonomers **1** and **2**<sup>a</sup>

entry	feed ratio	yield <sup>b</sup>	m : n <sup>c</sup>	10 <sup>-4</sup> M <sub>n</sub> <sup>d</sup>	M <sub>w</sub> /M <sub>n</sub> <sup>d</sup>	DP <sub>n</sub>
	<b>1</b> : <b>2</b>	%				
1	25 : 75	86	20 : 80	0.96	2.5	30
2	32.5 : 67.5	88	41 : 59	1.2	1.8	38
3	50 : 50	71	47 : 53	1.4	1.9	46
4	67.5 : 32.5	88	63 : 37	0.85	1.6	29
5	75 : 25	82	74 : 26	1.0	1.8	36

<sup>a</sup> Copolymerization conditions: BF<sub>3</sub>-Et<sub>2</sub>O (5 mol-%), CH<sub>2</sub>Cl<sub>2</sub>, -30 °C, 48 hr, concentration = 0.5 g·mL<sup>-1</sup>, total conversion ≈ 100 % (TLC);

<sup>b</sup> Yield of insoluble fraction in chloroform/ *n*-hexane (1/15, v/v);

<sup>c</sup> Comonomer composition (m = 2Piv36MeAGU, n = 2Piv36AllAGU) determined by <sup>1</sup>H-NMR;

<sup>d</sup> Determined by GPC in chloroform with polystyrene calibration; these are apparent values because GPC based on calibration with polystyrene produces errors and overestimation

**Table 2.** Characterization of P(236MeAGU<sub>m</sub>-co-2MeAGU<sub>n</sub>) and their aqueous solution properties

m : n	Total DS <sub>Me</sub>	DS <sub>Me</sub>			10 <sup>-4</sup> M <sub>n</sub> <sup>a</sup>	M <sub>w</sub> /M <sub>n</sub> <sup>a</sup>	DP <sub>n</sub>	T <sub>CP</sub> <sup>b</sup> (°C)	T <sub>RCP</sub> <sup>b</sup> (°C)
		C-2	C-3	C-6					
20 : 80	1.40	1.0	0.20	0.20	1.0	1.4	40	–	–
41 : 59	1.82	1.0	0.41	0.41	0.94	1.5	40	–	–
47 : 53	1.94	1.0	0.47	0.47	0.99	1.5	42	63	43
63 : 37	2.26	1.0	0.63	0.63	0.71	1.4	31	59	29
74 : 26	2.48	1.0	0.74	0.74	0.85	1.4	39	45	24

<sup>a</sup>Number-average molecular weight and polydispersity of the corresponding acetate determined by GPC in chloroform with polystyrene calibration; these are apparent values because GPC based on calibration with polystyrene produces errors and overestimation;

<sup>b</sup>Cloud points on heating ( $T_{CP}$ ) and cooling ( $T_{RCP}$ ) from turbidity measurements (polymer concentration: 1.0 wt.-%);

**Synthesis of methylcellulose model copolymers with heterogeneous distribution and their solution properties**

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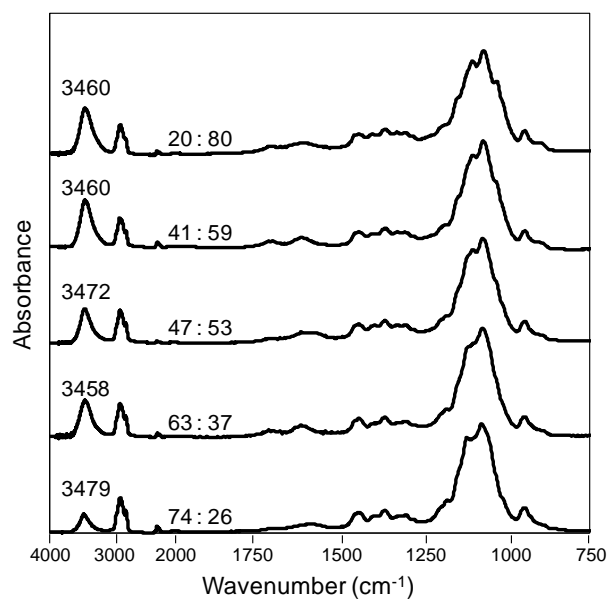
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**CONTENTS**

**Fig. S1** FT-IR spectra of P(236MeAGU<sub>m</sub>-co-2MeAGU<sub>n</sub>). All spectra were normalized at 1110 cm<sup>-1</sup>

<sup>1</sup> derived from C-O-C stretching band within anhydroglucose ring



**Fig. S1** FT-IR spectra of P(236MeAGU<sub>m</sub>-co-2MeAGU<sub>n</sub>). All spectra were normalized at 1110 cm<sup>-1</sup> derived from C-O-C stretching band within anhydroglucose ring