



HAL
open science

Novel thermo-responsive micelles prepared from amphiphilic hydroxypropyl methyl cellulose-block-JEFFAMINE copolymers

Aijing Lu, Eddy Petit, S.M. Li, Yuandou Wang, Feng Su, Sophie Monge

► **To cite this version:**

Aijing Lu, Eddy Petit, S.M. Li, Yuandou Wang, Feng Su, et al.. Novel thermo-responsive micelles prepared from amphiphilic hydroxypropyl methyl cellulose-block-JEFFAMINE copolymers. International Journal of Biological Macromolecules, 2019, 135, pp.38-45. 10.1016/j.ijbiomac.2019.05.087 . hal-02141702

HAL Id: hal-02141702

<https://hal.science/hal-02141702v1>

Submitted on 25 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Novel Thermo-responsive Micelles Prepared from Amphiphilic Hydroxypropyl Methyl
Cellulose-*block*-JEFFAMINE Copolymers

Aijing Lu,^a Eddy Petit,^a Suming Li,^{a*} Yuandou Wang,^b Feng Su,^b Sophie Monge^c

^a*Institut Européen des Membranes, UMR 5635 CNRS-UM-ENSCM, Université de Montpellier, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France*

^b*College of Chemical Engineering, Qingdao University of Science and Technology, 266042 Qingdao, China*

^c*Institut Charles Gerhardt, UMR 5253 CNRS-UM-ENSCM, Université de Montpellier, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France.*

*Corresponding author: suming.li@umontpellier.fr (S. Li)

Abstract: A series of amphiphilic and thermo-responsive block copolymers were synthesized by reductive amination between the aldehyde endgroup of hydrophilic HPMC and the amine group of monoamine, diamine, or triamine JEFFAMINE as hydrophobic block. The resulting diblock, triblock and three-armed copolymers with different hydrophilic/hydrophobic ratios and block lengths were characterized by NMR, FT-IR, DOSY-NMR and SEC. The cloud point (CP) of copolymers was determined by UV–visible spectrometer. Data show that both the geometrical structure and the molar mass of HPMC affect the CP of HPMC-JEF copolymers. The higher the hydrophilic/hydrophobic ratio, the higher the CP of copolymers which is lower than that of HPMC homopolymers. The self-assembly behavior of the copolymers was investigated from dynamic light scattering, transmission electron microscope, and critical micelle concentration (CMC) measurements. Spherical nano-micelles are obtained by self-assembly of copolymers in aqueous solution, and the micelle size can be tailored by varying the block length of HPMC and the geometrical structure. Three-armed HPMC-JEF copolymers present lower CMC and smaller micelle size as compared to linear diblock and triblock ones. MTT assay evidenced the cytocompatibility of HPMC-JEF copolymers, indicating that they could be promising as drug carrier in drug delivery systems.

Key words: Hydroxypropyl methyl cellulose; JEFFAMINE; Self-assembly; Micelle; Topology; Thermo-responsive

1. Introduction

Amphiphilic block copolymers are able to self-assemble in aqueous solution yielding various aggregates such as spherical micelles, rod-like micelles, filomicelles, polymersomes, nanotubes, etc. These aggregates have received ever-increasing interest for biomedical and pharmacological applications in drug delivery,[1] imaging,[2] sensing,[3] etc. Micelles constitute a dynamic system with permanent exchanges between micelle-forming molecules and free molecules in solution, continuously breaking and re-forming. Some of the micellar systems are responsive to external stimuli such as pH, temperature, ionic strength and light irradiation.[4-9] Thermo-responsivity is one of the most interesting properties of stimuli-responsive polymers. Poly(*N*-isopropylacrylamide) (PNIPAAm) and poly(*N*-vinyl caprolactam) (PVCL) are most extensively investigated thermo-responsive polymers which exhibit a lower critical solution temperature (LCST) of approximately 32 °C in aqueous solution.[10] Interestingly, PNIPAAm can be used as hydrophilic shell-forming segments below the LCST and as hydrophobic core-forming segments above the LCST.[11]

Polysaccharides such as cellulose, pullulan, dextran, chitosan, and hyaluronan have attracted growing attention in recent years for uses as biomaterials because of their biodegradability, biocompatibility and inherent bioactivity which can help in improving drug targeting and diminishing inflammatory response. Cellulose is the most abundant biopolymer consisting of linear chains of numerous $\beta(1\rightarrow4)$ linked D-glucose units. It has been widely used for applications in fiber, paper, painting and pharmaceutical industries. Nevertheless, cellulose is poorly soluble in water due to strong hydrogen bonding between hydroxyl groups on the glucose from one chain with oxygen atoms on the same or on a neighbor chain.[12] Thus, soluble cellulose derivatives have been obtained by chemical modification and used as a hydrophilic building block to prepare amphiphilic copolymers, such as ethyl cellulose [13-15], hydroxyl propyl cellulose (HPC) [16], hydroxyl propyl methyl cellulose (HPMC) [16], hydroxyl ethyl cellulose[17], etc. Some derivatives such as HPC and HPMC exhibit thermo-responsive behaviors. Most modifications exploit the hydroxyl groups on the glucose moieties to prepare graft copolymers. But such reactions are hardly controllable because of

the presence of numerous hydroxyl groups along polymer chains. On the other hand, each cellulose chain has a hemiacetal end group which is easily transformed to aldehyde. The latter can serve as a reducing endgroup to build amphiphilic block copolymers by coupling or initiating polymerization. Nevertheless, such reactions have not been widely exploited despite their potential interest due to the limited availability of the reducing-end, the difficulty of finding a common solvent for both blocks, and the eventual necessity to protect the lateral hydroxyl groups.[18] Meanwhile, HPMC oligomers with molar masses between 5K and 20K have been rarely employed as hydrophilic segment to synthesize amphiphilic block copolymers. In our previous work, HPMC-poly lactide (HPMC-PLA) block copolymers were synthesized by using a three step procedure: ring-opening polymerization of L-lactide initiated by allyl alcohol, amination reduction of the aldehyde endgroup of low molar mass HPMC, and UV-initiated thiol-ene click reaction.[19]

JEFFAMINE is a family of polyetheramine composed of poly(propylene oxide) (PPO), poly(ethylene oxide) (PEO), or mixed PPO/PEO blocks with primary amino groups attached to the polyether chain ends. JEFFAMINE M2005 with a PPO/PEO ratio of 29/6 exhibits a LCST of around 30 °C in water [20]. JEFFAMINE exists in the form of monoamines, diamines, and triamines. Thus, amphiphilic block copolymers with different geometrical structures can be obtained combining JEFFAMINE and a hydrophilic block. It has been shown that the geometrical structure of copolymers affects the morphology, stability and size of self-assembled micelles because the hydrophobic and hydrophilic segments lead to complex spatial arrangements during the micellization process [21, 22]. In this work, HPMC-JEF amphiphilic block copolymers were synthesized from HPMC with different molar masses and JEFFAMINE monoamine, diamine, and triamine by reductive amination. The self-assembly properties of the resulting diblock, triblock and tri-armed copolymers were investigated from the morphology, size and size distribution, cloud point (CP) and critical micelle concentration (CMC) measurements. MTT assay was performed to evaluate the biocompatibility of HPMC-JEF copolymers as potential drug carrier.

2. Materials and Methods

2.1 Materials

HPMC was kindly provided by Dow Colorcon Limited France, and depolymerized to yield HPMC oligomers with weight average molar mass (M_w) of 7000 ($\mathcal{D}=2.3$) and 18000 ($\mathcal{D}=3.3$) Daltons, as reported in a previous work.[19] JEFFAMINE monoamine M-2005, diamine D-2000, and triamine T-5000 were purchased from Huntsman. Sodium triacetoxyborohydride ($\text{NaBH}(\text{OAc})_3$) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich, and used as received.

2.2 Synthesis of HPMC-JEF copolymers

HPMC-JEF copolymers were synthesized by reductive amination as reported in literature. Typically, 0.314 g (0.157 mmol) JEFFAMINE M-2005, 1.0 g (0.143 mmol) HPMC with $M_w=7000$, and 0.042 g (0.198 mmol) $\text{NaBH}(\text{OAc})_3$ were dissolved in 20 mL DMSO under stirring. The reaction proceeded at room temperature for 24 h. The product was obtained by dialysis against deionized water for 72 h at room temperature. The dialysis medium was renewed every 12 h. A white flocculent solid was obtained after 48 h freeze-drying. The yield of the reaction was 69.4%.

2.3 Characterization

^1H NMR spectra were recorded on Bruker spectrometer operating at 300 MHz (AMX300) using D_2O and $\text{DMSO}-d_6$ as solvent. Diffusion ordered spectroscopy (DOSY) NMR was performed on Bruker Avance (AQS600) NMR spectrometer, operating at 600 MHz and equipped with a Bruker multinuclear z-gradient inverse probe head which is able to produce gradients in the z direction with a strength of 55 Gcm^{-1} . The DOSY NMR spectra were acquired from Bruker topspin software with the ledbpgp2s pulse program. The strength of the pulsed field gradients with respect to maximum 32 increments on a quadratic scale was logarithmically incremented from 2 to 95%. The diffusion sensitive period (Δ) of 200 ms and the gradient duration (δ) of 5 ms were optimized to allow the signals of interest to decrease by a factor of 10-20, in order to keep the relaxation contribution to the signal attenuation constant and ensure full signal attenuation for all samples. MestRe Nova software was used for the treatment of all NMR spectra.

Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet NEXUS spectrometer with a DTGS detector at 4 cm^{-1} resolution. Sixty-four scans were taken for each analysis 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 molar mass and polydispersity of polymers were determined by size exclusion chromatography (SEC), equipped with multi-angle laser light scattering (MALLS) in combination with refractive index (RI) detector. 5 mg sample was added in the mobile phase (10×10^{-3} M NaNO₃ containing 0.02% NaN₃), and stirred for 24 h at room temperature. The solution was filtered using 0.45 μ m polytetrafluoroethylene (PTFE) filter (Millipore). The filtrate was injected through a 100 μ L loop (Rheodyne injector 7725), and eluted in a TSK-GEL GMPWXL 7.8 \times 300 mm column (TosoHaas Bioseparation Specialists, Stuttgart, Germany) at a flow rate of 0.5 mL min⁻¹ (Waters pump 515). MALLS detector (Dawn DSP, Wyatt Technology Co, Santa Barbara, CA, USA) and RI detector (Optilab Wyatt Technology Co) were used to online determine the absolute molar mass for each elution fraction of 0.01 mL, which enables to calculate the Mw and dispersity (\mathcal{D}). The refractive index increment (dn/dc) used for calculation was 0.129 mL g⁻¹ for HPMC-JEF copolymers, and 0.137 for HPMC homopolymers. Data analysis was realized using Astra 4 (Wyatt Technology Co.).

The cloud point (CP) of the copolymers was estimated from transmittance changes of 5.0 mg mL⁻¹ solution in the temperature range from 30 to 90 °C. The solution was stirred for 24 h and kept at 4 °C overnight before analysis. Measurements were made at a wavelength of 500 nm with a Perkin Elmer Lambda 35 UV–visible spectrometer equipped with a Peltier temperature programmer PTP-1+1. The temperature ramp was 0.2 °C min⁻¹. Temp Lab software was used for data treatment. The critical micelle concentration (CMC) was determined by fluorescence spectroscopy using pyrene as fluorescent probe. The concentration of polymers varied from 1.0×10^{-3} to 2.0 mg/ml, and the concentration of pyrene was fixed at 6×10^{-7} g/L. The excitation spectra of the micellar solutions were recorded between 300 to 360 nm at an excitation wavenumber of 395. The intensity ratio at 336 nm and 334 nm was plotted *versus* copolymer concentration. The CMC value was obtained from the cross-over point of the two regression lines.

The size and zeta potential of micelles were determined by dynamic light scattering (DLS) using Anton Paar Litesizer 500 at 25°C. The aqueous solutions of copolymers at 1.0 mg mL⁻¹ were filtered through a 0.45 μ m PTFE microfilter. Micelle size changes as a

function of temperature from 4 to 60 °C were followed using Zano-ZS (Malvern Instrument) equipped with a He–Ne laser ($\lambda=632.8$ nm). Measurements were made after 60 min equilibrium of copolymer solutions at 1.0 mg mL⁻¹.

Transmission electron microscopy (TEM) was performed on JEOL 1200 EXII instrument, operating at an acceleration voltage of 120 kV. 5 μ L micellar solution at 1.0 mg mL⁻¹ was dropped onto a copper grid, stained with phosphotungstic acid, and air dried before measurement.

2.4 MTT assay

L-929 cells in logarithmic growth phase were seeded in 96-well plates (Corning Costar, U.S.A.) with a density of 1×10^4 cells/mL per well in 100 μ L DMEM medium (10% calf serum, 100 μ g/mL Penicillin, 100 μ g/mL streptomycin). The cells were then placed in a CO₂ incubator (NU-4850; NuAire, U.S.A.) at 37 °C under humidified atmosphere containing 5% CO₂. After 24 h, the medium was replaced by 100 μ L of fresh medium or HPMC-JEF micellar solution at various concentrations (0.25, 0.5, 1.0, 2.0, 2.5 and 5.0 mg/mL) containing 10.0 % calf serum. 100 μ L fresh medium was used as the negative control, and 100 μ L solution of phenol in water as the positive control [23]. After 24, 48 and 72 h, 20 μ L 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, 5 mg/mL) was added. The medium was removed after 6 h incubation, and then 150 μ L dimethylsulfoxide (DMSO) was added. The plates were shaken for 10 min. The optical density (OD) was measured by using microplate reader (Elx800; BioTek, U.S.A.) at 570 nm. All experiments were carried out in triplicate. The cell viability was calculated from the OD values of the test sample and the negative control using the following equation.

$$\text{Relative activity (\%)} = (\text{OD}_{\text{test sample}} / \text{OD}_{\text{negative control}}) \times 100$$

The data were expressed as the mean \pm SD.

3. Results and discussion

3.1 Synthesis of HPMC-JEF

HPMC-JEF amphiphilic block polymers were synthesized by reductive amination of amine groups of JEFFAMINE with the reducing end group of HPMC using NaBH(OAc)₃ as

reducing agent, as shown in Scheme 1. HPMC samples with Mw of 7000 and 18 000 and JEFFAMINE samples M-2005 (monoamine), D-2000 (diamine), and T-5000 (triamine) were used to synthesize diblock, triblock and three-armed amphiphilic copolymers, respectively. The PO/EO molar ratios and molar masses of the three JEFFAMINE samples are shown in Table S1.

Scheme 1.

Figure 1.

The ^1H NMR spectra of HPMC7K-JEF M2K are shown in Fig. 1. Signals of both HPMC ($\delta=2.75\text{--}4.75$ and 1.05) and JEFFAMINE ($\delta=3.40\text{--}3.90$, 2.93 and 1.11) are observed. However, the various signals of both blocks are overlapped in almost the whole range. DOSY-NMR was performed to further confirm the block copolymer structure. In fact, DOSY is an excellent tool allowing virtual separation in multicomponent systems, and thus to evidence if the NMR signals belong to a same molecule or a mixture. DOSY-NMR data are presented in the form of a two-dimensional (2D) pattern: one dimension is related to the chemical shift information, while the other represents the diffusion coefficient which reflects the molecular effective sizes.[24] As shown in Fig. 2, the signals of both JEFFAMINE and HPMC present the same diffusion coefficient, which indicates that the two components are combined in one molecule. In other words, a block copolymer was effectively synthesized. In contrast, the DOSY NMR spectrum of a mixture of HPMC7K and JEF M2K exhibits two different diffusion coefficients, as shown in Fig. S1.

Figure 2.

Figure 3.

FT-IR was also employed to analyze the chemical structure of polymers. As shown in Fig. 3, JEFFAMINE presents characteristic absorption bands at 2935 , 1100 , 1030 and 900 cm^{-1} belonging to aliphatic C-H stretching, C-O stretching, C-N stretching, and N-H external deformation, respectively. On the other hand, HPMC presents characteristic absorption bands

at 3460, 2890, and 1060 cm^{-1} assigned to O-H, C-H and C-O bonds, respectively. In the case of the HPMC-JEF copolymer, all the characteristic bands from the two components are observed, in agreement with the successful coupling of JEFFAMINE and HPMC blocks.

Table 1.

The molar mass of copolymers was determined by using aqueous SEC in conjunction with online MALLS and RI detectors. RI detector is most commonly used as concentration detector whose response is proportional to the total solute concentration in the detector cell, whereas the response of MALLS detector depends on the molar mass of the solute.[25] The molar mass of copolymers was calculated combining with the MALLS and RI curves. Table 1 presents the M_w and dispersity ($D=M_w/M_n$) data of the various copolymers obtained by reaction of HPMC7K and HPMC18K with JEFFAMINE samples M2K, D2K and T5K. The M_w of HPMC7K derived copolymers increases from 6 500 for the diblock copolymer HPMC7K-JEF M2K, to 14 000 for the triblock copolymer (HPMC7K)₂-JEF D2K, and to 19 000 for the three-armed copolymer (HPMC7K)₃-JEF T5K. Similarly, the M_w of HPMC18K derived copolymers increases from 25 000 for HPMC18K-JEF M2K, to 43 000 and 56 000 for (HPMC18K)₂-JEF D2K and (HPMC18K)₃-JEF T5K, respectively. These results globally corroborate with the initially designed structure of copolymers, indicating the successful synthesis of copolymers by reductive amination. Nevertheless, there is some discrepancy between the M_w values obtained by SEC and those by theoretical calculation. In fact, GPC is a chromatographic method in which molecules in solution are separated by their size. Thus, HPMC-JEF copolymers with different architectures could have various error range for GPC analysis. Meanwhile, when an RI detector is combined with light scattering, the exact concentration of the sample at each data slice needs to be determined in order to calculate the absolute molecular weight. The key parameter to get exact concentration is the refractive index increment (dn/dc) which is a unique value for a sample-solvent combination. The dn/dc used for calculation was 0.129 mL g^{-1} for HPMC-JEF copolymers, and 0.137 for HPMC homopolymers. Copolymers with different architectures and different molar masses may have slight difference in dn/dc , thereby cause some error in SEC results. In addition,

HPMC oligomers were obtained by degradation from high molar mass HPMC and have a large molar mass distribution. The dispersity (\mathcal{D}) is 3.3 for HPMC 18K and 2.3 for HPMC 7K. And it is known that high-molar-mass species strongly influence the value of M_w [26]. In the case of the HPMC-JEF copolymers, the dispersity varies from 1.38 for HPMC18K-JEF M2K to 2.30 for (HPMC18K)₃-JEF T5K, which is narrower than the HPMC homopolymers due to dialysis.

3.2 Cloud point

HPMC is a thermo-responsive polymer which precipitates out of the solution when the temperature reaches the cloud point due to loss of water or dehydration.[27] The phase separation leads to a polymer rich phase and a polymer depleted phase. Thus the cloud point can be determined as the polymer rich phase is capable of scattering light.[28]

Figure 4.

The CP of the HPMC-JEF aqueous solutions was determined by transmittance measurements at 500 nm, as shown in Fig. 4. HPMC18K exhibits a CP around 78.0 °C, whereas HPMC7K has no obvious change in the tested temperature range. Thus, the CP of HPMC homopolymers decreases with the increase of molecule weight. Similar result has been reported in literature in the case of narrow-disperse PNIPAMs. As the $M_{n,NMR}$ increases from 2.8 to 26.5 KDa, the CP drops from 43.0 to 33.3 °C. [29]

The CP of HPMC-JEF copolymers is lower than that of HPMC homopolymers in all cases. Meanwhile, the CP of linear diblock and triblock HPMC18K-JEF copolymers was higher than that of HPMC7K-JEF ones. Therefore, the CP of linear JEF-HPMC copolymers is strongly dependent on the hydrophilic/hydrophobic ratio. The higher the hydrophilic/hydrophobic ratio, the higher the CP of copolymers. Similar finding has been reported for P(EO₂MA-*co*-OEGMA) copolymers whose CP could be tuned from 28 °C to 90°C by adjusting the copolymer composition.[30-32]. It also has been reported that PLLA-PNIPAAm copolymers exhibit a CP between 32.1 and 32.8 °C with the [NIPAAm]/[LA] ratio varying from 2.4 to 6.9.[33]

The situation of the three-armed (HPMC)₃-JEF T5K copolymers is more complicated. Compared with the linear copolymers, the three-armed (HPMC7K)₃-JEF T5K copolymer presents a higher CP (82.9°C) in spite of its higher JEFFAMINE fraction. This finding could be assigned to the topological structures which make the interactions between polymer and solvent different from those of linear copolymers. Honda et al. reported that the CP of amphiphilic poly(butyl acrylate)-*block*-poly(ethylene oxide) copolymers is strongly affected by the topological structure.[34] An increase by more than 40 °C was observed through the linear-to-cyclic topological conversion. Data for (HPMC18K)₃-JEF T5K are not available because it is not fully soluble at 5 g/L. It should be noted that different from the JEF D2K and JEF T5K composed exclusively of PO units, JEF M2K is a thermos-responsive block composed of hydrophobic PO moieties and hydrophilic EO ones with a PO/EO molar ratio of 29/6 (Table S1). In fact, JEF M2K exhibits a CP of 22.6°C at a concentration of 5 g/L (Fig. S2). Two transitions of transmittance corresponding to both components are observed for the diblock copolymers HPMC7K-JEF M2K and HPMC18K-JEF M2K (Fig. 4, Table 1).

3.3 Self-assembly of HPMC-JEF copolymers

Micelles of HPMC-JEF copolymers were prepared by direct dissolution method. 10 mg copolymer was dissolved in 10 mL deionized water under stirring overnight. The self-assembled HPMC-JEF micelles consisted of a JEF core surrounded by a HPMC corona.

Figure 5.

The critical micelle concentration was determined by fluorescence excitation spectroscopy using pyrene as fluorescence probe. Pyrene is highly hydrophobic and tends to migrate into the lipophilic micellar core in the aqueous solution. As shown in Fig. 5, the shift of the fluorescence excitation spectra indicates the process of micellar forming. The CMC value was determined from the two regression lines of the curves of the intensity ratio of I₃₃₆/I₃₃₄ versus polymer concentration. As shown in Table 1, no major difference is observed between the CMC values of the diblock and triblock copolymers which are in the range of 0.13 to 0.24 g/L. In contrast, the CMC of the three-armed copolymers is much lower.

(HPMC7K)₃-JEF T5K and (HPMC18K)₃-JEF T5K exhibit a CMC of 0.03 and 0.07 g/L, respectively. In other words, the CMC of three-armed copolymers increases with increasing hydrophilic HPMC block length, which is consistent with literature data.[35] In fact, Guo et al. reported that cellulose-g-PCL graft copolymers with higher degree of substitution (DS) of PCL present lower CMC value than those with lower DS. The fact that three-armed copolymers exhibit lower CMC than linear diblock and triblock copolymers could be assigned to the difference of the topological structures since star-shaped block copolymers facilitate micellization. Similar results have been found for star-shaped PEG-*b*-PCL copolymers [36] and PCL-*b*-PDEAS copolymers [21].

Figure 6.

The size, size distribution, zeta potential and morphology of the micelle were determined by DLS and TEM, and the results are displayed in Table 1 and in Fig. 6. The average size of the micelle with different HPMC and JEF block lengths are in the range from 180 to 320 nm. The three armed copolymers exhibited the smallest micelle size, whereas the triblock ones exhibited the largest micelle size. These findings could be assigned to the difference of topological structures. It has been reported that linear and star-shaped block copolymers with 3, 4 or 6 arms have significant difference in micelle size.[21, 36] On the other hand, HPMC18K derived copolymers exhibit larger micelle size than HPMC7K derived ones, indicating that the micelle size increases with increasing hydrophilic block length.[35] The polydispersity ranges from 0.18 to 0.25, in agreement with narrow distribution of micelle sizes. The morphology of micelles is shown in Fig. 6B. Spherical micelles are observed in all cases. It is also noted that the zeta potential of all micelles is close to zero, in agreement with the neutral nature of copolymers (Table 1).

Figure 7.

It is also of interest to follow the micelle size changes of doubly thermo-responsive diblock copolymers at different temperatures. As shown in Fig. 7, three phases of micelle size

changes are distinguished for HPMC7K-JEF M2K and HPMC18K-JEF M2K diblock copolymers. In the first phase, the micelle size of HPMC7K-JEF M2K increases from 162 nm at 4°C to 340 nm at 20°C. In the second phase, the micelle size decreases to reach 190 nm at 30 °C. Beyond, the micelle size increases again to reach 380 nm at 60°C. Similar changes are observed for HPMC18K-JEF M2K. The micelle size increase observed in the first and third phases can be assigned to the chain extension of HPMC blocks as the temperature increases. Similar result has been reported in literature. Yang et al. observed a micelle size increase for PLA-PEG copolymers with increasing temperature from 15°C to 35°C.[37] In the second phase from 20 to 30°C, the size decreases because of the phase transition of JEF M2K blocks. It has been reported that the JEF core size of JEF M2K-*b*-poly(L-glutamic acid) copolymer micelles decreases with increasing temperature as a result of dehydration.[20] Above 60°C, the micelles become unstable and aggregated as both blocks become hydrophobic.

3.3 MTT assay

Figure 8.

In vitro cytotoxicity of biomaterials is generally evaluated by MTT assay. This method is based on the reaction between MTT and mitochondrial succinate dehydrogenases in living cells to form a purple formazan which is soluble in DMSO but insoluble in water. The OD value of formazan-DMSO solution is considered to be proportional to the number of living cells [38, 39]. L-929 cells are a commonly used standard cell line in cyto-compatibility evaluation. The effect of micelle solutions on the growth of L-929 cells is shown in Fig. 8. The viability of cells is very high in micelle solutions, the relative activity being well above 80% for all samples. With the increase of micelle concentration from 0.25 to 5.0 mg/mL, the cell viability remained almost unchanged. Meanwhile, incubation up to 72 h does not affect the cell viability for all groups. According to the US Pharmacopeia Standards, it can be concluded that HPMC-JEF micelles are not toxic to L-929 cells. It is also noticed that the viability is very low in the positive control.

4. Conclusion

Amphiphilic and thermo-responsive block copolymers were synthesized by reductive amination between the aldehyde group of hydrophilic HPMC and the amine group of monoamine, diamine, or triamine JEFFAMINE as hydrophobic block. The resulting diblock, triblock and three-armed copolymers were characterized by NMR, FT-IR, DOSY-NMR and SEC. The various copolymers with different hydrophilic/hydrophobic ratios and block lengths self-assembled in water to yield spherical micelles. The geometrical structure of copolymers strongly affects the micelle size of micelles as well as the cloud point. Compared to the diblock and triblock copolymers, three-armed copolymers exhibit lower CMC, smaller size and lower cloud point. Interestingly, HPMC-JEF M2K diblock copolymers present two CP since both components are thermo-responsive. Moreover, the micelle size significantly changes with increasing temperature due to chain extension and/or dehydration. Last but not least, MTT assay showed that the HPMC-JEF micelles present outstanding cytocompatibility, suggesting that these novel copolymers can be safely used as potential drug carrier.

Acknowledgements

This work is supported in part by the scholarship from China Scholarship Council (Grant CSC N°201606240124).

References

- [1] P. Singh, N. Verma, A review on impact of nanomicelle for ocular drug delivery system, *International Journal of Pharmaceutical Sciences and Research* 9(4) (2018) 1397-1404.
- [2] S.R. Mane, I.L. Hsiao, M. Takamiya, D. Le, U. Straehle, C. Barner-Kowollik, C. Weiss, G. Delaittre, Intrinsically fluorescent, stealth polypyrazoline nanoparticles with large stokes shift for in vivo imaging, *Small* 14(36) (2018) e1801571.
- [3] M. Saleem, L. Wang, H. Yu, A. Zain ul, M. Akram, R.S. Ullah, Synthesis of amphiphilic block

copolymers containing ferrocene–boronic acid and their micellization, redox-responsive properties and glucose sensing, *Colloid and Polymer Science* 295(6) (2017) 995-1006.

[4] Z. Ge, S. Liu, Supramolecular self-assembly of nonlinear amphiphilic and double hydrophilic block copolymers in aqueous solutions, *Macromol Rapid Commun* 30(18) (2009) 1523-32.

[5] J. Xu, S. Liu, Polymeric nanocarriers possessing thermoresponsive coronas, *Soft Matter* 4(9) (2008) 1745.

[6] H. Cölfen, Double-hydrophilic block copolymers: synthesis and application as novel surfactants and crystal growth modifiers, *Macromolecular Rapid Communication* 22 (2001) 219-225.

[7] H.-i. Lee, W. Wu, J.K. Oh, L. Mueller, G. Sherwood, L. Peteanu, T. Kowalewski, K. Matyjaszewski, Light-induced reversible formation of polymeric micelles, *Angewandte Chemie International Edition* 46(14) (2007) 2453-2457.

[8] Y. Zhao, Rational design of light-controllable polymer micelles, *Chem Rec* 7(5) (2007) 286-94.

[9] D. Wang, T. Wu, X. Wan, X. Wang, S. Liu, Purely salt-responsive micelle formation and inversion based on a novel schizophrenic sulfobetaine block copolymer: structure and kinetics of micellization, *Langmuir* 23(23) (2007) 11866-11874.

[10] J. Ramos, A. Imaz, J. Forcada, Temperature-sensitive nanogels: poly(N-vinylcaprolactam) versus poly(N-isopropylacrylamide), *Polym. Chem.* 3(4) (2012) 852-856.

[11] H. Wei, S.-X. Cheng, X.-Z. Zhang, R.-X. Zhuo, Thermo-sensitive polymeric micelles based on poly(N-isopropylacrylamide) as drug carriers, *Prog Polym Sci* 34(9) (2009) 893-910.

[12] V.L. Finkenstadt, R.P. Millane, Crystal structure of valonia cellulose I β , *Macromolecules* 31(22) (1998) 7776-7783.

[13] H. Dou, M. Jiang, H. Peng, D. Chen, Y. Hong, pH-dependent self-assembly: micellization and

micelle-hollow-sphere transition of cellulose-based copolymers, *Angew Chem Int Ed Engl* 42(13) (2003) 1516-9.

[14] Y. Uraki, T. Imura, T. Kishimoto, M. Ubukata, Body temperature-responsive gels derived from hydroxypropylcellulose bearing lignin, *Carbohydrate Polymers* 58(2) (2004) 123-130.

[15] W. Yuan, J. Zhang, H. Zou, T. Shen, J. Ren, Amphiphilic ethyl cellulose brush polymers with mono and dual side chains: Facile synthesis, self-assembly, and tunable temperature-pH responsivities, *Polymer* 53(4) (2012) 956-966.

[16] E. Ostmark, D. Nystrom, E. Malmstrom, Unimolecular nanocontainers prepared by ROP and subsequent ATRP from hydroxypropylcellulose, *Macromolecules* 41(12) (2008) 4405-4415.

[17] M.-F. Hsieh, N. Van Cuong, C.-H. Chen, Y.T. Chen, J.-M. Yeh, Nano-sized micelles of block copolymers of methoxy poly(ethylene glycol)-poly(ϵ -caprolactone)-graft-2-hydroxyethyl cellulose for doxorubicin delivery, *Journal of Nanoscience and Nanotechnology* 8(5) (2008) 2362-2368.

[18] C. Schatz, S. Lecommandoux, Polysaccharide-containing block copolymers: synthesis, properties and applications of an emerging family of glycoconjugates, *Macromol Rapid Commun* 31(19) (2010) 1664-84.

[19] J. Wang, M. Caceres, S. Li, A. Deratani, Synthesis and self-assembly of amphiphilic block copolymers from biobased hydroxypropyl methyl cellulose and poly(L-lactide), *Macromol Chem Phys* 218(10) (2017) 1600558.

[20] W. Agut, A. Brulet, D. Taton, S. Lecommandoux, Thermoresponsive micelles from Jeffamine-b-poly(L-glutamic acid) double hydrophilic block copolymers, *Langmuir* 23(23) (2007) 11526-33.

[21] J. Cao, A. Lu, C. Li, M. Cai, Y. Chen, S. Li, X. Luo, Effect of architecture on the micellar properties of

poly (varepsilon-caprolactone) containing sulfobetaines, *Colloids Surf B Biointerfaces* 112 (2013) 35-41.

[22] Z. Li, E. Kesselman, Y. Talmon, M.A. Hillmyer, T.P. Lodge, Multicompartment micelles from ABC miktoarm stars in water, *Science* 306(5693) (2004) 98-101.

[23] W. Zhang, M. Torabinejad, Y. Li, Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method, *Journal of Endodontics* 29(10) (2003) 654-657.

[24] G. Pages, V. Gilard, R. Martino, M. Malet-Martino, Pulsed-field gradient nuclear magnetic resonance measurements (PFG NMR) for diffusion ordered spectroscopy (DOSY) mapping, *Analyst* 142(20) (2017) 3771-3796.

[25] L.K. Kostanski, D.M. Keller, A.E. Hamielec, Size-exclusion chromatography-a review of calibration methodologies, *J Biochem Biophys Methods* 58(2) (2004) 159-86.

[26] A. Striegel, W.W. Yau, J.J. Kirkland, D.D. Bly, *Modern size exclusion chromatography*, 2nd ed., Wiley, Hoboken, 2009.

[27] K. Mitchell, J.L. Ford, D.J. Armstrong, P.N.C. Elliott, C. Rostron, J.E. Hogan, The influence of additives on the cloud point, disintegration and dissolution of hydroxypropylmethylcellulose gels and matrix tablets, *Int J Pharm* 66(1-3) (1990) 233-242.

[28] M. Mohammadpour, B. Kerins, M.E. Crowley, G. Walker, A. Crean, HPMC cloud point: exploring hydroxypropylmethyl cellulose behavior in pharmaceutical formulations, *Proceedings of the 2016 AIChE Annual Meeting*, San Francisco, 13-18 November. <http://hdl.handle.net/10468/5000>.

[29] Y. Xia, X.C. Yin, N.A.D. Burke, H.D.H. Stover, Thermal response of narrow-disperse poly(N-isopropylacrylamide) prepared by atom transfer radical polymerization, *Macromolecules* 38(14) (2005) 5937-5943.

- [30] J.L. Zhang, R.S. Srivastava, R.D. Misra, Core-shell magnetite nanoparticles surface encapsulated with smart stimuli-responsive polymer: synthesis, characterization, and LCST of viable drug-targeting delivery system, *Langmuir* 23(11) (2007) 6342-51.
- [31] J.F. Lutz, O. Akdemir, A. Hoth, Point by point comparison of two thermosensitive polymers exhibiting a similar LCST: Is the age of poly(NIPAM) over?, *J Am Chem Soc* 128(40) (2006) 13046-13047.
- [32] J.-F. Lutz, A. Hoth, Preparation of ideal PEG analogues with a tunable thermosensitivity by controlled radical copolymerization of 2-(2-methoxyethoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate, *Macromolecules* 39(2) (2006) 893-896.
- [33] Y. Hu, V. Darcos, S. Monge, S. Li, Synthesis and self-assembling of poly(N-isopropylacrylamide-block-poly(L-lactide)-block-poly(N-isopropylacrylamide) triblock copolymers prepared by combination of ring-opening polymerization and atom transfer radical polymerization, *Journal of Polymer Science Part A: Polymer Chemistry* 51(15) (2013) 3274-3283.
- [34] S. Honda, T. Yamamoto, Y. Tezuka, Topology-directed control on thermal stability: micelles formed from linear and cyclized amphiphilic block copolymers, *J Am Chem Soc* 132(30) (2010) 10251-3.
- [35] Y. Guo, X. Wang, Z. Shen, X. Shu, R. Sun, Preparation of cellulose-graft-poly(varepsilon-caprolactone) nanomicelles by homogeneous ROP in ionic liquid, *Carbohydr Polym* 92(1) (2013) 77-83.
- [36] K.H. Kim, G.H. Cui, H.J. Lim, J. Huh, C.-H. Ahn, W.H. Jo, Synthesis and micellization of star-shaped poly(ethylene glycol)-block-poly(ϵ -caprolactone), *Macromol Chem Phys* 205(12) (2004) 1684-1692.
- [37] L. Yang, X. Qi, P. Liu, A. El Ghzaoui, S. Li, Aggregation behavior of self-assembling

polylactide/poly(ethylene glycol) micelles for sustained drug delivery, *Int J Pharm* 394(1-2) (2010) 43-9.

[38] X. Shen, X. Liu, R. Li, P. Yun, C. Li, F. Su, S. Li, Biocompatibility of filomicelles prepared from poly(ethylene glycol)-polylactide diblock copolymers as potential drug carrier, *J Biomater Sci Polym Ed* 28(15) (2017) 1677-1694.

[39] H. Li, S. Tao, Y. Yan, G. Lv, Y. Gu, X. Luo, L. Yang, J. Wei, Degradability and cytocompatibility of tricalcium phosphate/poly(amino acid) composite as bone tissue implants in orthopaedic surgery, *Journal of Biomaterials Science, Polymer Edition* 25(11) (2014) 1194-1210.

Table 1. Characterization of HPMC-JEF block copolymers

Sample	Mw ^a	\bar{D} ^a	CP ^b (°C)	Size ^c (nm)	PDI ^c	Zeta ^c (mV)	CMC ^d (g/L)
HPMC7K-JEF M2K	6500	1.73	42.1/69.0	231.9	0.22	-1.8±0.1	0.24
(HPMC7K) ₂ -JEF D2K	14000	1.95	78.5	261.1	0.19	-4.21±0.3	0.24
(HPMC7K) ₃ -JEF T5K	19000	2.0	82.9	183.7	0.24	-3.7±0.2	0.03
HPMC18K-JEF M2K	25000	1.38	35.1/60.1	298.4	0.18	-7.0±0.3	0.13
(HPMC18K) ₂ -JEF D2K	43000	2.01	55.7	328.2	0.25	-2.0±0.2	0.24
(HPMC18K) ₃ -JEF T5K	56000	2.30	-	270.7	0.18	-6.7±0.3	0.07

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

b) Determined from turbidimetry measurement in water at 5.0 mg mL⁻¹

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

d) Determined at 25°C by fluorescence spectrometer

Captions

Scheme 1. Synthesis of HPMC-JEF M2K diblock copolymer by reductive amination

Figure 1. ^1H NMR spectra of HPMC7K-JEF M2K in different solvents

Figure 2. DOSY NMR spectrum of HPMC7K-JEF M2K copolymer in $\text{DMSO-}d_6$.

Figure 3. FT-IR spectra of JEFFAMINE (A), HPMC (B) and HPMC-JEF M2K copolymer (C)

Figure 4. Plots of light transmittance as a function of temperature for aqueous solutions of HPMC7K-JEF (A) and HPMC18K-JEF (B) copolymers

Figure 5. Fluorescence excitation spectra (A) and I336/I334 vs. LogC plots (B) of HPMC7K-JEF M2K diblock copolymer solutions at different concentrations

Figure 6. Size distribution (A) and TEM image (B) of HPMC7K-JEF M2K diblock copolymer micelles

Figure 7. Micelle size changes of HPMC7K-JEF M2K (A) and HPMC18K-JEF M2K (B) as a function of temperature

Figure 8. Relative activity of L-929 cells after 24, 48 and 72 h culture with micelle solutions of HPMC7K-JEF M2K (A), HPMC18K-JEF M2K (B), $(\text{HPMC7K})_2$ -JEF D2K (C), and $(\text{HPMC7K})_3$ -JEF T5K (D) at different concentrations in comparison with the positive control.

Data are presented as the mean \pm sd ($n = 3$)















