# Effects of Molecular Weight Distribution of Amphiphilic Block Copolymers on Their Solubility, Micellization, and Temperature-Induced Sol−Gel Transition in Water

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ABSTRACT: Poly(D,L-lactide-co-glycolide)-b-poly(ethylene glycol)-b-poly(D,L-lactide-co-glycolide) (PLGA−PEG−PLGA) was synthesized via ring-opening polymerization of D,L-lactide and glycolide in the presence of poly(ethylene glycol) (PEG), and then a series of triblock copolymers with different molar-mass dispersities ($Đ_M$s) under similar weight-average molecular weight (M_w) or number-average molecular weight (M_n) were obtained via step precipitation and mixing of synthesized copolymers with a given PEG length and varied PLGA lengths. The effects of molecular weight distribution (MWD) on thermogelling and related behaviors of the copolymer aqueous solutions were investigated. The increase of $Đ_M$, namely M_w/M_n, was found to enhance the solubility of the amphiphilic block copolymer in water and accelerate the solubilization process significantly. The critical micelle concentration (CMC) determined via a hydrophobic dye incorporation method also increased with $Đ_M$, and the corresponding copolymer micelles became less hydrophobic as reflected by the fluorescence of 10-hydroxycamptothecin as a hydrophobic probe. Some of the copolymers with appropriate balance of hydrophilicity and hydrophobicity underwent sol−gel transition upon heating if the copolymer concentration was over its critical gel concentration (CGC). The CGC and sol−gel transition temperature T_{gel} increased with $Đ_M$ under both a given M_w and a given M_n. So, different components in polymers with broad MWD might cooperate with each other. The present study illustrates that $Đ_M$ serves as a regulator of the condensed state of amphiphilic block copolymers in a selective solvent.
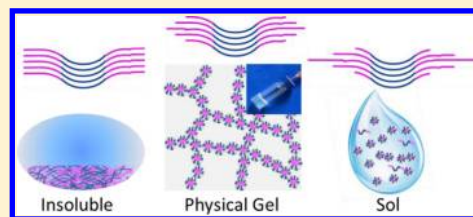
INTRODUCTION

Concentrated aqueous solutions of some biodegradable amphiphilic block copolymers such as poly(D,L-lactide-co-glycolide)-b-poly(ethylene glycol)-b-poly(D,L-lactide-co- glycolide) (PLGA−PEG−PLGA) triblock copolymer exhibit reversible sol−gel transition upon increasing temperature.[1,2] The thermogelling materials show great potential as injectable hydrogels in biomedical fields, such as sustained release of drugs,[3−8] tissue engineering,[9,10] prevention of postoperative adhesion,[11] and some other medical applications in surgery.[12]

While most of amphiphilic block copolymers can form micelles in water, only a small part of copolymers under appropriate conditions can spontaneously gel upon heating. It is thus meaningful to reveal various factors that influence the gelation ability. It is well-known that the sol−gel transition of the copolymer aqueous solution can be adjusted by the molecular structural parameters, such as block length,[13,14] the ratio of D,L-lactide (LA) and glycolide (GA),[15] the sequence of LA and GA in the hydrophobic block,[16] and even the modification of the end groups of polymers;[17,18] some addition agents[19] can also modulate the sol−gel transition behaviors. Although various factors to influence the thermogelling ability of PLGA−PEG−PLGA have been reported, a very common parameter for a polymeric system, molar-mass dispersity ($Đ_M$) to characterize molecular weight distribution (MWD), has not yet been examined. (Note: we use the term "molar-mass dispersity" ($Đ_M$) to replace "polydispersity index" (PDI) as

suggested by IUPAC in 2009.[20]) The present study is aimed at investigating the MWD effect of the block copolymer PLGA−PEG−PLGA on solubility, micellization in water, and thermogelling ability of the concentrated aqueous solutions.

Amphiphilic block copolymers afford an excellent model in studies of self-assembly of amphiphiles in a selective solvent.[21−25] Hillmyer et al.[26,27] extensively examined the influence of MWD on self-assembly of block copolymers and proposed the potential use of dispersity to tune the morphology of block copolymers. A group led by An made analysis of statistical thermodynamics of nonuniform polymer systems in the framework of a lattice fluid model to examine the effect of molecular weight (MW) and its distribution on the spinodal in polymer solution.[28] The Eisenberg group studied the effect of the block length distribution of poly(acrylic acid) on vesicle formation of polystyrene-b-poly(acrylic acid) in solution and illustrated that the size of the vesicles decreased with increased poly(acrylic acid) dispersity.[29,30] The effects of dispersity on micelle formation of amphiphilic copolymers were examined also by some other groups.[31−33]

In this work, we used ring-opening polymerization to synthesize PLGA−PEG−PLGA copolymers with different block lengths of PLGA under a fixed length of PEG. Then,

we introduced a multistep precipitation method to collect copolymers with narrow MWD. The samples with a series of $Đ_M$ under a given number-average or weight-average MW were obtained by adjusting feeding ratios in synthesis and mixing ratios after synthesis. The solubility and thermogelability were then examined, and the MWD effects on the biodegradable thermogelling systems were revealed for the first time.

Although the effect of MWD on thermogelling of amphiphilic block copolymers in water has not yet been reported to the best of our knowledge, its effects on micellization of poly(ethylene glycol)-*b*-poly(propylene glycol)-*b*-poly(ethylene glycol) (PEG−PPG−PEG, commercially called Pluronic) in water and other amphiphilic block copolymers in selective solvents have been theoretically predicted in the early 1990s,[34,35] and "the single most important result appears to be that the polydispersity strongly facilitates micellization".[35] Since our sample of PLGA−PEG−PLGA can definitely form micelles in water under appropriate conditions, we will, by the way, also check this classic prediction about the effect of $Đ_M$, one of the most basic parameters of macromolecules.

■ MATERIALS AND METHODS

Materials. The monomers LA and GA were purchased from Purac and used as received without any further purification. The initiator poly(ethylene glycol) (PEG, with number-average molecular weight M_n 1500 Da) was purchased from Sigma-Aldrich Corporation. Stannous octoate (95%) was bought from Sigma and dissolved in anhydrous toluene (30 mg/mL). The other chemicals, such as methanol and methylene dichloride, were chemical regent grade and bought from Shanghai Chemical Reagents Co., Ltd., China.

Copolymer Synthesis. The PLGA−PEG−PLGA triblock copolymers were synthesized by ring-opening polymerization. PEG as the initiator was dehydrated at 120 °C under vacuum for 3 h, and then LA and GA with molar ratio 8:1 were added and dehydrated under vacuum at 80 °C for 3 h. After stannous octoate (0.15 wt % of the total weight of LA and GA) was added as catalyst, the mixture was stirred at 140 °C for 12 h. The unreacted monomers and toluene were removed under vacuum at 120 °C for 3 h. The crude products were washed by 80 °C water for three times. After lyophilization, the products were stored at −20 °C.

Multistep Precipitation. The synthesized copolymer **P7** (39.38 g) was dissolved in dichloromethane at the concentration of 50 wt % and then dropped in 1000 mL of methanol under stirring. After 30 min, the copolymer was precipitated at −20 °C for 12 h. The supernatant and sediment were rotary evaporated under vacuum to obtain the copolymers with narrower MWD. Then the sediment was dissolved in methylene dichloride again, and the precipitation procedure was repeated. Six components (from **P1** to **P6**) were isolated.

Multiple-Component Mixing. Some specimens with wide MWD such as **P8** were obtained by mixing. The weight ratio of the mixing components was first calculated upon the assumption of the logarithm normal distribution written as

$$W(M) = \frac{1}{\beta \sqrt{\pi}} \frac{1}{M} \exp\left(-\frac{1}{\beta^2} \ln^2 \frac{M}{M_p}\right) \tag{1}$$

Here, $\beta = [2 \ln(M_w/M_n)]^{1/2}$ and $M_p = (M_w M_n)^{1/2}$. The mixing components were dissolved completely in dichloromethane to guarantee the molecular-level mixing. Then, rotary evaporation and lyophilizing were carried out to remove dichloromethane. The mixing ratios were further adjusted according to the premeasurement of gel permeation chromatography (GPC) in order to make the final MWD of the mixture close to the usual distribution.

GPC Measurement. MW and $Đ_M$ were measured by GPC with a differential refractometer (Agilent 1100). The copolymers were dissolved in tetrahydrofuran (THF) with 10 mg/mL. The flow rate was selected at 1.0 mL/min, and the temperature was 35 °C. MWs were calibrated with polystyrene standards.

Nuclear Magnetic Resonance (NMR). The ^{1}H NMR spectrum was recorded in a Bruker spectrometer (DMX 500). The sample was dissolved in CDCl$_3$ at concentration 5 mg/mL. Tetramethylsilane was used as the internal reference.

Phase Diagram or State Diagram. The sol−gel transition was examined by the vial-inverting method.[36−38] Each vial contained 0.6 mL of copolymer aqueous solution. The solutions were equilibrated at 4 °C for 12 h before measurement. The vials were immersed in a water bath. The samples were measured from 10 to 50 °C and equilibrated for 15 min at each temperature. If no visual flow was observed within 30 s after inverting the vial, the sample was regarded as a gel.

Dynamic Rheological Measurement. The sol−gel transition is always accompanied by a significant change of dynamic rheological properties.[39] In this study, the viscoelasticity of the copolymer aqueous solution was detected in a stress-controlled rheometer (Malvern Kinexus). First, 1.5 mL of copolymer solution was added onto a cone− plate (1°, 60 mm diameter, 0.3 mm gap); then, low-viscosity silicone oil was added as a cover to minimize the volatilization. To measure the viscosity and modulus change with temperature, the heating rate was set as 1 °C/min, with angular frequency ω as 10 rad/s.

Critical Micelle Concentration (CMC). CMC of copolymer in aqueous solution was determined with a hydrophobic probe 1,6-diphenyl-1,3,5-hexatriene (DPH).[40,41] Briefly, the DPH was added in all the copolymer solutions at the final concentration of 4 μM. The solution was equilibrated in dark at 25 °C for 4 h and then measured in a UV−vis spectrophotometer (Lambda 35, PerkinElmer). The absorbance from 320 to 400 nm was measured, when DPH was partitioned into the hydrophobic core upon micelle formation. The CMC value was estimated as the inflection point of absorbance difference between 377 and 400 nm against logarithm concentration of copolymer.

Dynamic Light Scattering (DLS). The micelle sizes in dilute copolymer aqueous solution were measured in a laser light scattering instrument (Zetasizer Nano ZS90. Malvern). The solutions were filtered through a 0.45 μm filter and equilibrated at each temperature for 15 min before measurement.

Transmission Electron Microscopy (TEM). The TEM experiments were carried out in a JEOL electron microscope (JEM-2100F). In sample preparation, an ultrathin carbon network was dipped in 1 wt % copolymer aqueous solution and then immediately transferred to liquid nitrogen. After volatilized under infrared lamp, the samples were observed at 200 kV.

Fluorescence Spectra. To investigate the MWD effect on local hydrophobicity of PLGA−PEG−PLGA micelles in dilute copolymer solutions, we employed a hydrophobic molecule 10-hydroxycamptothecin (HCPT), which can emit fluorescence.[42,43] Copolymers (0.5 wt %) were dissolved in the HCPT aqueous solution (10 μg/mL) at pH 4.0. The fluorescence spectra were recorded in the fluorescence spectrophotometer FLS920 (Edindurgh Instrument) at 25 °C. The excitation and emission wavelengths were set at 380 and 570 nm, respectively.

CMC Determination of Pluronics in Water. Three Pluronic products, L64 (EG$_{13}$PG$_{30}$EG$_{13}$), P105 (EG$_{37}$PG$_{56}$EG$_{37}$), and F127 (EG$_{106}$PG$_{69}$EG$_{106}$), were gifted by BASF Corporation. The CMC values of them and their mixtures were measured by the DPH dye solublization method, which was also used in determination of CMC of our PLGA−PEG−PLGA copolymer aqueous solution.

■ RESULTS

Synthesis and Preparation of Triblock Copolymers with Different $Đ_M$s. PLGA−PEG−PLGA copolymers were synthesized via ring-opening polymerization of LA and GA initiated by the hydroxy end groups of PEG. A typical ^{1}H NMR spectrum of the synthesized PLGA−PEG−PLGA copolymer is shown in Figure 1. The characteristic peaks at 5.1−5.3, 4.6−4.9, and 3.5−3.8 ppm are assigned to the methane protons of the
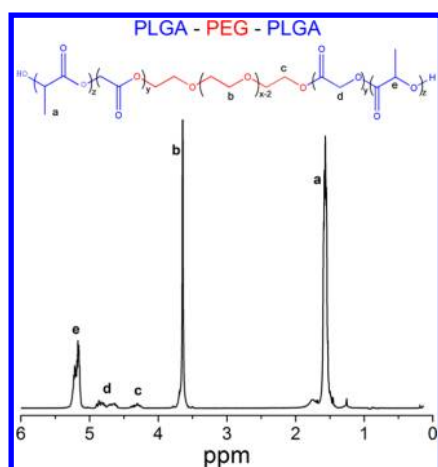
Figure 1. ^{1}H NMR spectrum of PLGA–PEG–PLGA triblock copolymer in CDCl$_3$.

LA units and the methylene protons of the GA units and PEG, respectively. From these peaks, the ratio LA/GA of the copolymers was calculated, resulting in about 8.0. It is also available to obtain the number-average molecular weight M_n of PLGA with a given M_n of PEG (1500 Da).

A series of the triblock copolymer PLGA–PEG–PLGA with different MWs and $Đ_M$s were obtained through synthesis, isolation via precipitation, and mixing. The samples examined in this study are summarized in Table 1. Samples **P1–P6**

Table 1. Molecular Parameters of Triblock Copolymers

	PLGA–PEG–PLGA		
sample	M_w[a]	M_n[b]	$Đ_M$[c]
P1	3845	1030–1500–1030	1.08
P2	4817	1420–1500–1420	1.11
P3	5243	1700–1500–1700	1.07
P4	5733	1880–1500–1880	1.09
P5	6890	2440–1500–2440	1.08
P6	8614	3130–1500–3130	1.11
P7	5832	1640–1500–1640	1.22
P8	5766	1520–1500–1520	1.27
P9	5866	1330–1500–1330	1.41
P10	6425	1820–1500–1820	1.25

[a]Weight-average molecular weight (M_w) calculated from M_n and $Đ_M$. [b]Number-average molecular weight (M_n) calculated from ^{1}H NMR spectroscopy. [c]Molar-mass dispersity ($Đ_M$) measured via GPC.

exhibit increased MWs under the similar narrow MWD with $Đ_M$ around 1.1. **P4**, **P7**, **P8**, and **P9** exhibit increased $Đ_M$s with a similar M_w. **P2** has a similar M_n to **P8** and **P9**; **P4** has a similar M_n to **P10**; they would be used to investigate the effect of MWD on the sol–gel transition of this copolymer solution under given M_n.

Effect of MW on Solubility and Thermogelling. Copolymers from **P1** to **P6** have different average MWs, yet similar $Đ_M$s close to 1.1. With the increase of MW, the dissolution rate and the eventual solubility decreased dramatically. As shown in Figure 2, **P1** was dissolved in water with solubility as high as 35 wt %, due to the short length of the hydrophobic block PLGA. **P2**, **P3**, and **P4** exhibited relatively lower solubilities, resulting in 30, 20, and 12.5 wt %, respectively. The aqueous system of **P5** with even the 10 wt % concentration in water was cloudy, exhibiting a partial
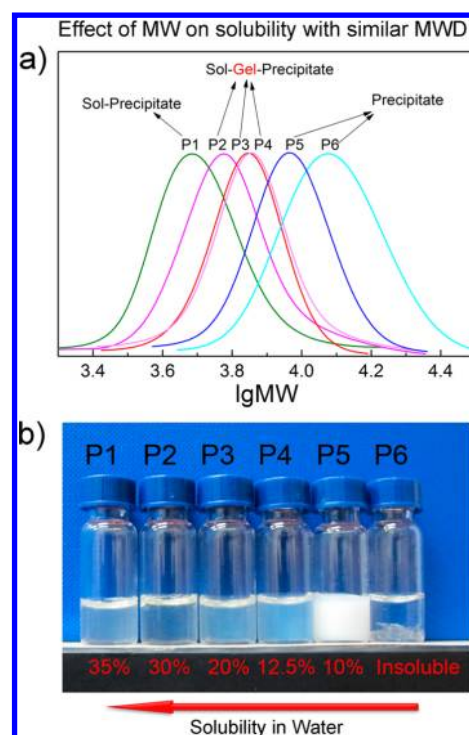

Figure 2. Properties of the series of copolymers with increased MW and similar $Đ_M$. (a) GPC profiles of copolymers. The legend describes the condensed states of these copolymers in water with concentration close to solubilities at room temperature. (b) Image of the copolymer aqueous systems at room temperature with indicated weight concentrations.

dissolution of this copolymer. **P6** was too hydrophobic to dissolve in water, and this sample was just a bit swollen even after stirred for 1 week.

Aqueous solution of **P2**, **P3**, and **P4** underwent a sol-to-gel transition upon heating. The **P2** solution physically gelled at high temperatures (above 40 °C) with a very high concentration 30 wt %. For **P3** and **P4** with higher MW of the hydrophobic block, the gelation temperature decreased to room temperature. **P5** and **P6** with relatively long hydrophobic blocks did not dissolve in water to form a sol, and thus the sol–gel transition is not available. On the other hand, the short PLGA length is beneficial for the dissolution of the copolymer in water but disfavors the formation of the thermogel: The **P1** solution did not form the physical hydrogel at any concentration and temperature; even at a very high concentration, only a sol–precipitate transition occurred upon heating.

Effect of MWD on Solubility of Copolymers with Similar M_w. **P4**, **P7**, **P8**, and **P9** constitute a series of samples under a similar M_w around 5800, with an increase of $Đ_M$ from 1.09 to 1.41. The MWDs of these four samples (Figure 3) are well fitted with the logarithm normal distribution. The corresponding R-square values were 0.972, 0.992, 0.943, and 0.986. With the increase of $Đ_M$, the solubility of copolymers in water increased from 12.5 to 35 wt %.

P4 is very stiff, and a scissor had to be used to separate it while weighing. It dissolved very slowly in water; an 80 °C water bath had to be used with stirring, until the polymer turned totally soft and white. After the water bath cooling down naturally to room temperature, we spent 3 days under stirring at 4 °C to make **P4** totally dissolved in water with the
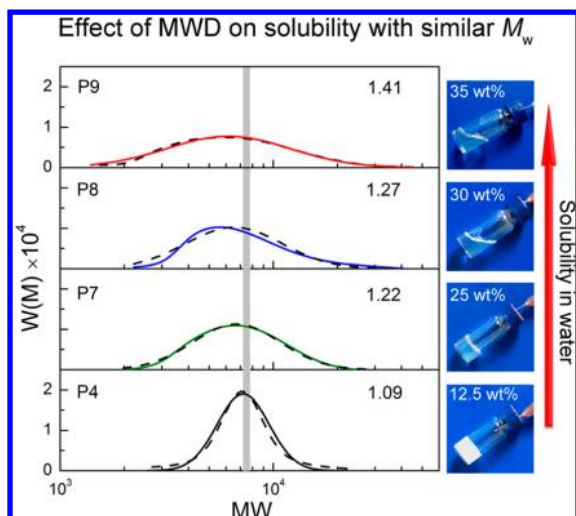
Figure 3. GPC profiles of copolymers **P4**, **P7**, **P8**, and **P9** and photographs of their aqueous systems at body temperature. In the left, the dashed curves came from fitting via logarithm normal distribution; the shadow region indicates the range of the M_w values of these four copolymers; marked in the legend are $Đ_M$s. The right photographs demonstrate the sol or gel states at 37 °C; the legends indicate weight concentrations of copolymers in water, which are near the corresponding solubilities.

concentrations at or below 12.5 wt %. Dissolving copolymer **P4** in water at a predesigned concentration 15 wt % was also tried, but even stirring for 1 week, the sample was still just swollen and could not flow.

P7, **P8**, and **P9** are sticky pastes. They were able to be handled by spoon during weighing and dissolved in water at 4 °C without pretreatment in a hot water bath. Their highest dissolving concentrations determined by flow ability read as 25, 30, and 35 wt %.

While the 12.5 wt % **P4** aqueous solution formed an opaque gel at room temperature, the 25 wt % **P7** aqueous solution physically gelled at body temperature (37 °C), and the 30 wt % **P8** aqueous solution gelled only above 45 °C. In contrast, even higher concentrated **P9** (35 wt %) did not gel at any temperature.

Comparison of the Effects of M_n, M_w, and $Đ_M$ on Thermogelling of PLGA−PEG−PLGA Copolymers in Water. The thermogelling behaviors of the PLGA−PEG−PLGA triblock copolymer aqueous solutions were further investigated. A significant increase of complex modulus G^* occurred upon heating along with the sol−gel transition, as shown in Figure 4a. With the increase of $Đ_M$, the shear modulus of polymer solution decreased and the sol−gel transition temperature T_{gel} increased.

The abrupt increase of shear modulus is not a definite indicator of a sol−gel transition because the formation of a physical hydrogel also depends upon the absolute value of system viscosity. So, a vial-inverting method was used to judge the sol or gel state, with the results shown in Figure 4b. For samples with broader MWD, the gelation windows moved to higher concentration and higher temperature, and the global gel window became narrower. For the aqueous solution of **P9** (20 wt %), the maximum modulus was still not sufficiently high, and the corresponding aqueous system did not form an apparent gel even at high temperatures and high concentrations; just a sol−precipitate transition was observed with temperature increased to 50 °C.
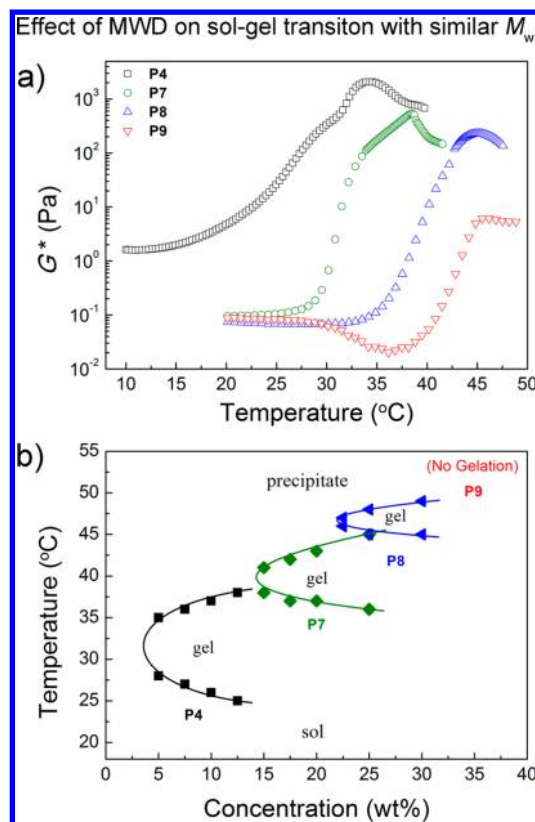

Figure 4. Sol−gel transition of copolymer aqueous solutions. (a) Complex shear modulus (G^*) of copolymer aqueous solution as a function of temperature. The concentrations were 20 wt % for **P7**, **P8**, and **P9** but 12.5 wt % for **P4** due to its low solubility. (b) State diagrams of the above four copolymers in water. The lowered data points in the state diagram for each sample indicate the sol−gel transition, and the upper data indicate the gel−precipitate transition.

We further examined thermogelling copolymers with different M_n, M_w, and $Đ_M$ values. The results are shown in Figure 5 with five copolymer samples included. **P10** and **P4** have similar M_n; **P7** and **P4** have similar M_w; **P10** and **P7** have broader MWD than **P4**. With the increase of $Đ_M$, the aqueous solutions of both **P10** and **P7** exhibited smaller modulus G^*, and higher sol−gel transition temperature T_{gel}, and thus the gel window shifted to higher concentrations and temperatures.

By contrast, **P2** and **P7** have narrower MWD than **P8**. Although MWs of **P2** and **P7** are smaller and larger than that of **P8**, respectively, both **P2** and **P7** solutions exhibited lower T_{gel} than **P8**. So, MWD plays a key role on thermogelling, and $Đ_M$ serves as another important parameter apart from MW.

Effect of MWD on Micellization of Copolymers in Dilute Aqueous Solutions. As it is known, the thermogel has an interior structure of percolated micelle network.[13,17,44] We then examined the micellization of the copolymers with different $Đ_M$s. The CMC was detected by using the hydrophobic probe DPH. The CMC values in Figure 6 are significantly lower than the corresponding CGC values in Figure 5. As illustrated in Figure 6a, with an increase of $Đ_M$, the CMC values increase with the sequence of **P4**, **P10**, and **P7**. The concentration region of stable micelles (Figure 6b) also complies to this sequence, which is consistent with the rheological measurements in Figure 5c and the phase diagram in Figure 5e.
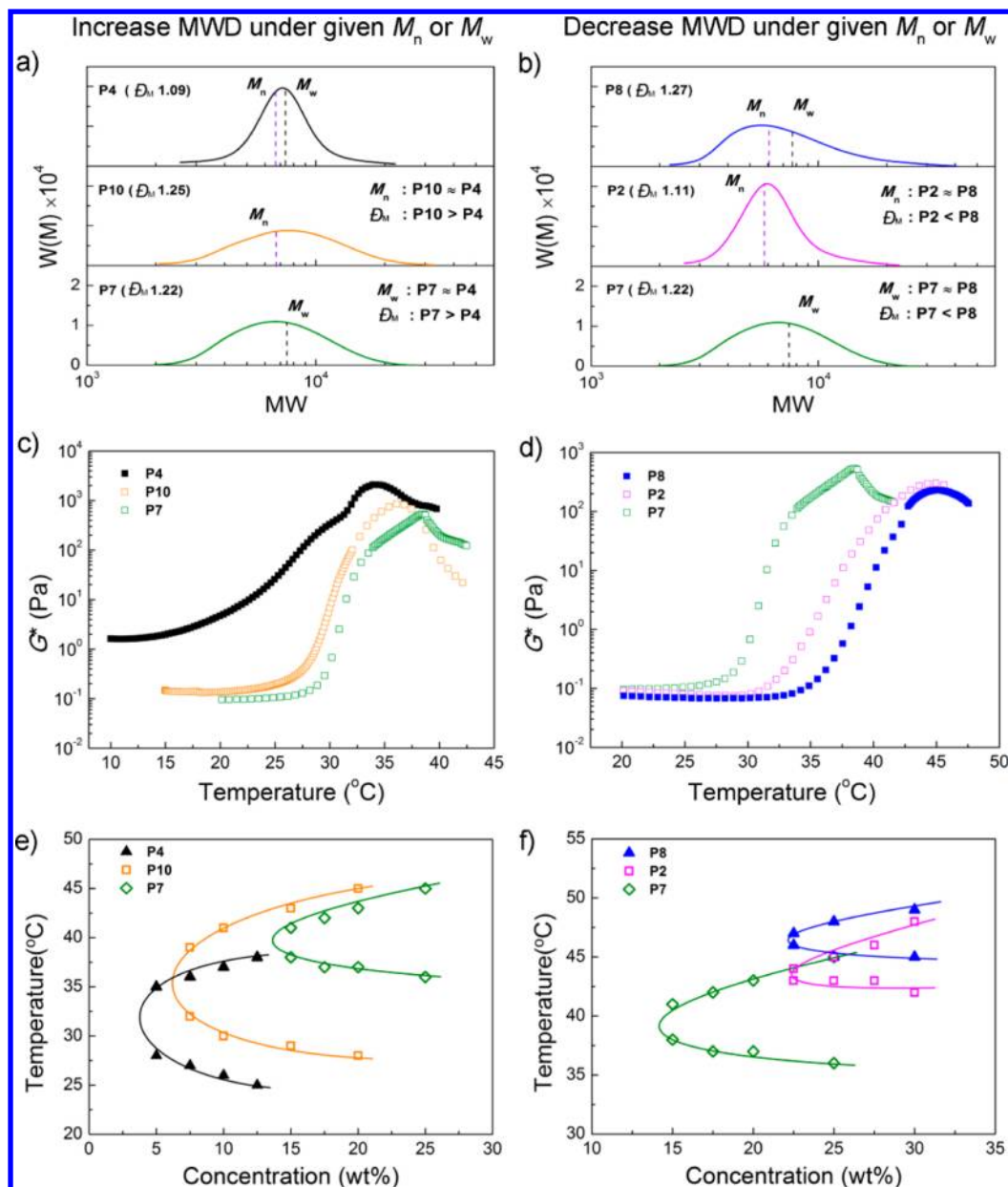
Figure 5. Effects of M_n, M_w, and $Đ_M$ on thermosensitivity of copolymer aqueous solutions. (a, b) GPC profiles of the indicated copolymers. (c, d) Complex shear modulus ($G*$) of copolymer aqueous solutions as a function of temperature. The concentration of copolymer solutions was 20 wt %, except **P4** (12.5 wt % due to its low solubility). (e f) State diagrams determined by the vial-inverting method.

The temperature dependence of the micelle size distribution was also examined. **P4** was stable in the examined temperature range from 15 to 55 °C. **P10** has a similar number-average MW to **P4**, but an increased $Đ_M$; and its micelle sizes increased at high temperature around 45 °C. **P7** has a similar weight-average MW to **P4**, but an increased $Đ_M$; and its micelle sizes increased obviously from 35 °C. As illustrated in the TEM images shown in Figure 6d, the micelles diminished obviously with the increase of $Đ_M$, regardless of similar M_n or M_w.

Effect of MWD on Local Hydrophobicity of Copolymer Micelles. The fluorescence method has been used to probe the assembling and disassembling of amphiphilic copolymers.[45,46] In our study, HCPT, a hydrophobic probe with fluorescence, was employed to detect the local hydrophobicity of micelles composed of PLGA−PEG−PLGA block copolymers with different $Đ_M$s of the hydrophobic block. As

demonstrated in Figure 7a, the fluorescence intensity of HCPT from 400 to 450 nm was enhanced with the increase of concentration of copolymer **P7**, reflecting the local hydrophobicity of micelles. The fluorescence intensity of HCTP at 425 nm was found to be decreased with the increase of $Đ_M$ under the same copolymer concentration.

Examination of $Đ_M$ Dependence of CMCs of Pluronics in Water. In preparing this manuscript, we realized that the trend of CMC change with $Đ_M$ of the block copolymer PLGA−PEG−PLGA in water was reversed to that of another block copolymer Pluronic theoretically predicted in the literature.[35] But no corresponding experimental data of the CMCs of Pluronics with a series of $Đ_M$ have been reported. So, the MWD effect on CMCs of Pluronic blends with the same mixing fraction as theoretically treated in the literature was examined by us, as shown in Figure 8.
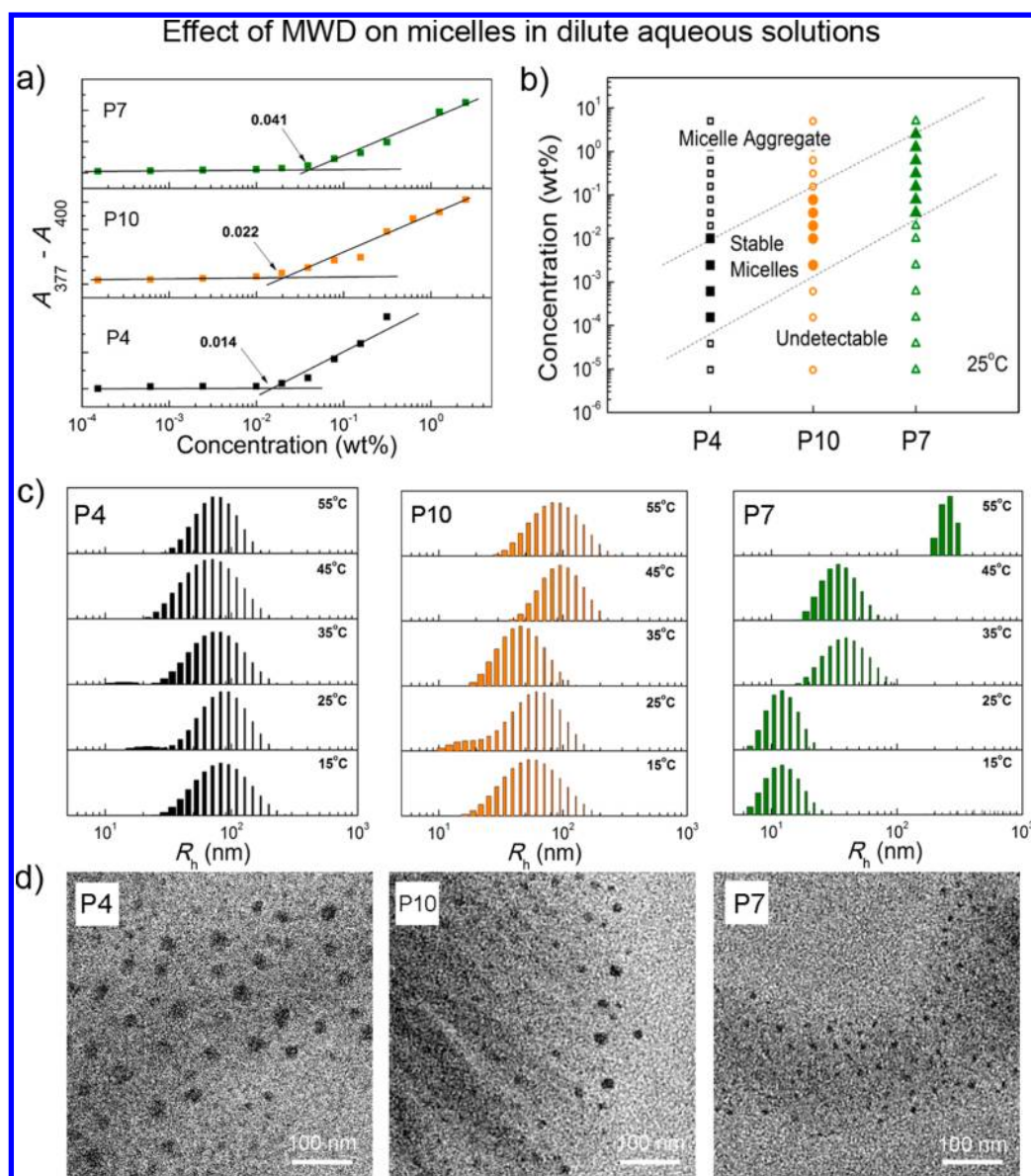
Figure 6. Micellization of the copolymers **P4**, **P7**, and **P10**. (a) Absorption difference as a function of copolymer concentration to determine CMC using the hydrophobic probe DPH. (b) Summary of stable region of micelles tested by DLS at 25 °C. At very low copolymer concentration, the light intensity was too fluctuant to be detected; at high concentration, the micelles aggregated. (c) The micelle size distribution (intensity-weighted) as a function of temperature; the concentration is 0.1 wt %. For **P4** the micelle size was very stable with the increase of temperature; for **P7** and **P10** the micelles aggregated as the temperature increased. (d) TEM images of **P4**, **P7**, and **P10** micelles. In micelle preparation, the copolymer concentration was 1 wt %, and the detection temperature was 25 °C.

The ethylene glycol (EG) contents in L64 ($EG_{13}PG_{30}EG_{13}$), P105 ($EG_{37}PG_{56}EG_{37}$), and F127 ($EG_{106}PG_{69}EG_{106}$) in the Pluronic series are 40%, 50%, and 70%, respectively. The CMC value increased with the EG content in the copolymer. These three copolymers had $Đ_M$ values of 1.03, 1.09, and 1.12, and that of the mixture was 1.19 according to the mixing fraction, leading to $Đ_M$ 1.2 as suggested in the literature.[35] While the theoretical calculation predicted a very significant CMC lowering of the mixture compared to CMC of the central copolymer P105,[35] the CMC of the mixture in our experiment was enlarged to those of the three Pluronics.

■ DISCUSSION

Experimentally, polymer samples inevitably have MWD. In polymer engineering, $Đ_M$ is an important factor to adjust the property of a polymer product. For instance, changing of the MWD of polyethylenes[47] and polypropylenes[48] has been used to facilitate easy processing and improve the product properties. The Eisenberg group examined the influence of dispersity of poly(acrylic acid) on vesicle formation in a selective solvent, and our group even made corresponding computer simulation to reproduce the asymmetric distribution of block copolymers with different block lengths in the outer and inner layers of the vesicles,[49] by using the dynamic Monte Carlo algorithm.[50,51] The Eisenberg group[34] and then Per Linse[35] have theoretically examined the dispersity dependence of CMC of amphiphilic block copolymers in selective solvents. Yet, the influences of $Đ_M$ on the local hydrophobicity of micelles, copolymer solubility, and thermogelling in a selective solvent have, to the best of our knowledge, not been reported. In this study, we experimentally investigate the effects of
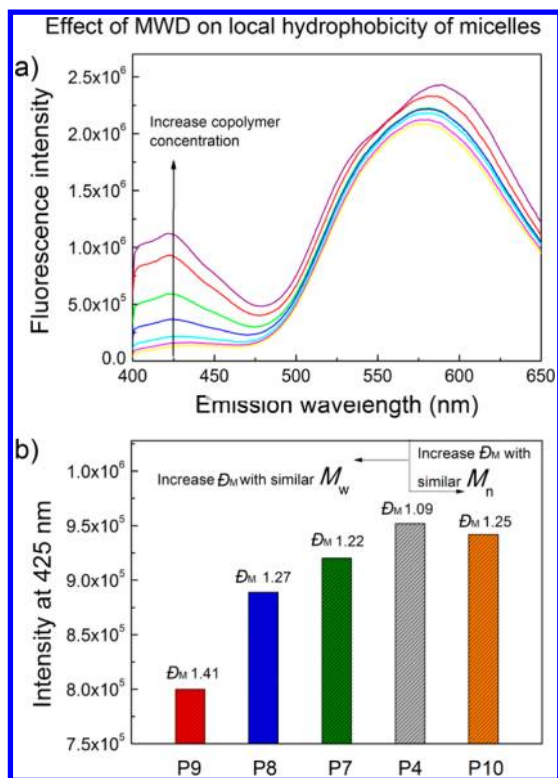
Figure 7. Fluorescence of HCPT in aqueous solutions of copolymers at 25 °C and pH 4.0. (a) Fluorescence spectra of HCPT in the copolymer solution. (b) Fluorescence intensity of HCTP in 425 nm in indicated samples with the polymer concentration 0.5 wt %.

Figure 8. (a) GPC profiles of the Pluronics L64, P105, and F127 and their mixture. The mixing mass fractions of L64, P105, and F127 were 0.0783, 0.7041, and 0.2176, respectively, according to the case of $Đ_M$ = 1.2, as theoretically treated in ref 35. (b) Data about CMC determination of Pluronics L64, P105, and F127 and their mixture.

MWDs on stability, micellization, and especially physical gelation of a thermogellable block copolymer in water for the first time.

Broad MWD Enhances the Solubility of Amphiphilic Block Copolymers in Water. With the increase of $Đ_M$, the copolymer PLGA−PEG−PLGA was found to dissolve in water more easily, resulting in higher solubility. We observed that the premixing degree played also an important role in solubility. Take **P9** ($Đ_M$ 1.41) as an example, which was obtained by mixing four components. When we mixed the four components just at the solid state, the larger molecular part in **P9** did not dissolve in water. So we tried to dissolve all the components in dichloromethane and achieved a molecular level premixing after rotary evaporation and freeze-drying. Then **P9** could be easily dissolved in water with high concentration. The higher solubility seems to be attributed to the enhancement of solubility of high-MW component by the relatively small-MW component or the synergetic effect of the components of different MWs, both thermodynamically and kinetically. We interpret this phenomenon from a pre-environment hypothesis, namely, the solution of small-MW copolymers affords a more "organic" pre-environment to dissolve high-MW copolymers. Li et al. ever examined the effect of solvent size on physical gelation in triblock copolymer solutions via dynamic Monte Carlo simulation.[52] While they dealt with just a series of uniform polymers, their elegant modeling of solvent size effects on physical gelation is very helpful for interpretation of our observation of nonuniform polymer if we regard the relatively short-chain components in our copolymer samples as large cosolvents. So, the above "organic pre-environment" interpretation could also be regarded as the cosolvent effect.
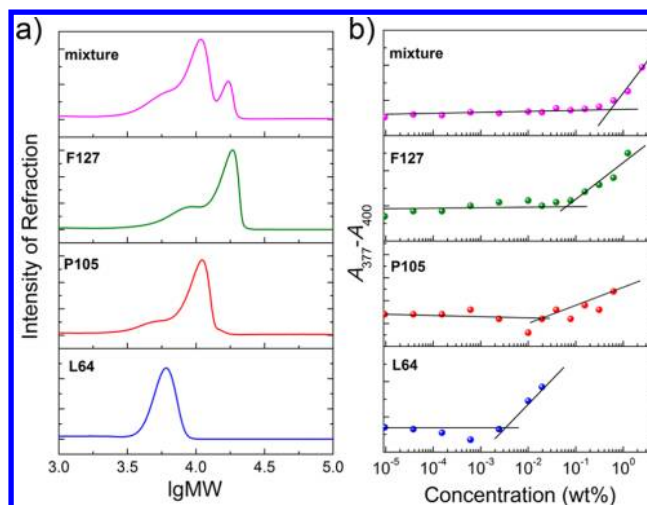
In our experiments, we found that for samples of narrower MWD a relatively dilute copolymer solution had to be achieved from a corresponding concentrated solution. For instance, a solution of 0.1 wt % **P4** ($Đ_M$ 1.09) could not be obtained by simply dissolving 10 mg of copolymer in 10 mL of water within the examined time period, but the stable solution was easily obtained by dilution of its concentrated solution. This phenomenon could also be interpreted by the pre-environment hypothesis or the cosolvent effect. A large amount of copolymer affords a sufficient amount of the small-MW component, which presolution affords a more organic environment and thus accelerates the further dissolution of high-MW components.

Increase of $Đ_M$ of Amphiphilic Block Copolymer Decreases the Local Hydrophobicity of the Corresponding Micelles in Water. As the effect of MWD on copolymer aqueous solution is concerned, the CMC was detected by the hydrophobic probes DPH and HCPT within micelles. Both dyes preferred partition into the hydrophobic cores of micelles. After being entrapped in the micelle cores, the dyes exhibited stronger UV absorption by DPH and fluorescence emission by HCPT. From our experiments, the lower $Đ_M$ led to the lower CMC under similar MW. This is consistent with another observation that a sample of lower $Đ_M$ was harder to be dissolved in water and thus globally more hydrophobic. Once formed, the micelles of copolymers with low $Đ_M$ have a stronger hydrophobic environment, which makes UV absorption higher, and makes fluorescence intensity stronger. So, the DPH and HCPT tests agree with each other very well.

P9 with a relatively broader MWD was very easy to dissolve in water at 0.1 wt % concentration. The micelles were relatively smaller, as seen from DLS and TEM observations in Figure 6. The notable detection of DPH and HCPT happened only at higher copolymer concentration. All these experiments illustrated that **P9** with higher $Đ_M$ exhibited a weaker local hydrophobicity within micelles.

Narrow MWD Promotes the Sol−Gel Transition of PLGA−PEG−PLGA Block Copolymers. The temperature-induced sol−gel transition of PLGA−PEG−PLGA block copolymers in water was adjusted by MWD significantly, and the effect could not simply be interpreted via MW. In this

study, we observed that MWD dramatically influenced solubility of copolymer in water, the local hydrophobicity of micelle, and also the sol−gel transition behavior. These phenomena are consistent with each other, for instance, a lower solubility favored the physical gelation driven by the hydrophobic association.

For our thermosensitive macromolecular system, we revealed that samples of narrower MWDs physically gelled not only at lower concentration but also at lower temperature, and the gel window was wider. This may be related to its stronger local hydrophobicity. The enhanced local hydrophobicity made the micelles more stable, so its gel state was kept in a wider temperature range. For samples of higher $Đ_M$ and thus of weaker hydrophobicity, the micelle aggregation only happened at higher temperature, and the gelation thus had to take place at higher temperature.

$Đ_M$ Dependence of CMCs of Amphiphilic Copolymers in Selective Solvents and the Open Question of the Theoretical Criterion of a "Micelle". To the best of our knowledge, the Eisenberg group made the first theoretical treatment of micellization of nonuniform block copolymers in selective solvents in 1993.[34] They calculated the single chain concentration versus the total block copolymer concentration, and the concentration deviating from a linear relation was defined as CMC. "The cmc (mix) was found to decrease as the polydispersity index increases".[34] This conclusion was confirmed in 1994 by the same group[53] via static light scattering of polystyrene-*b*-poly(sodium acrylate) in THF and polystyrene-*b*-poly(4-vinylpyridine) in toluene, by which the apparent weight-average molecular weight of aggregates $(M_w)_{app}$ were detected and the copolymer concentration with an abrupt decrease of the inverse of $(M_w)_{app}$ was determined as CMC.

In another classic publication, Per Linse treated the effect of molar-mass dispersity on micellization of PEG−PPG−PEG in water by a random-mixing (mean-field) theory of spherical lattices in 1994.[35] According to his analysis, the change of dispersity from 1.1 to 1.2 can lead to a reduction of CMC by orders of magnitude, and the theoretical phenomenon was interpreted as "it is the properties of the longest component(s) that determine the CMC". We have checked all of 66 following publications citing this 1994 paper and found that noone had really used the predicted system or any other samples to directly confirm or criticize that conclusion either experimentally or theoretically. We are herein arguing this classic conclusion about the MWD effect on micellization: if it be justified, a sample of amphiphiles with a continuous MWD must lead to a zero CMC, which seems unreasonable.

Our experimental data of PLGA−PEG−PLGA (Figure 6) did not support the classic prediction. Considering the different architectures of block copolymers (ABA or BAB type), we further did the experiments of Pluronic blends of L64, P105, and F127 with exactly the same mixing proportion of the three Pluronics as used in the theoretical calculation of Per Linse. Our experimental results of Pluronics (Figure 8) are consistent with those of PLGA−PEG−PLGA (Figure 6): first, the CMC did not change over orders of magnitude; second, a broader MWD led to an increase of CMC, instead of a decrease.

The discrepancy might origin from the definition of "micelles" and CMC. In theoretical treatments,[34,35] "CMC" is essentially a critical contact concentration between two copolymer chains, and then it is reasonable that such a critical concentration might be lowered with the increase of $Đ_M$: the size of a random coil R_g is scaled with chain length N as $R_g \sim$

$N^{0.6}$ under athermal condition, and the occupied density of a coil and thus the critical contact concentration C^* are scaled with chain length as $C^* \sim N^{-0.8}$; so, an infinitely longer chain leads to $C^* \rightarrow 0$. The experiment of static light scattering measures actually a critical aggregation concentration (CAC). However, can an aggregate with just two chains represent a typical "micelle"? In our experiment, the micelles were reflected by the enrichment of a hydrophobic dye, and CMC was obtained by intersect of two fitted lines with respect to several amphiphile concentrations, and thus the critical concentration might correspond to formation of a sufficiently large aggregates with a core−corona micelle structure. The discrepancy of theories and experiments revealed in this study illustrates that the theoretical criterion of a "micelle" is still open now. We are here strongly suggesting that the forthcoming theorists explicitly state their definition of "micelle" in their publications and try to improve the criterion.

■ CONCLUSIONS

This is the first report of the effects of molecular weight distribution on sol−gel transition of amphiphilic copolymer aqueous solutions and other pertinent properties. A series of PLGA−PEG−PLGA block copolymers with a given central hydrophilic block PEG and various lengths of the hydrophobic blocks PLGA were obtained. The increase of $Đ_M$ was found to significantly enhance the solubility of copolymers in water. The local hydrophobicity of the micelles decreased correspondingly, which led to higher CGC and T_{gel} and lower modulus of the physical hydrogel. The MWD effect cannot be interpreted by a simple addition of MW effects. The low-MW component seems more beneficial for the solubility of the amphiphilic copolymers and the high-MW component more beneficial for the hydrophobic aggregation; the cooperation of the different components in a broad-MWD sample tunes the balance of hydrophilicity and hydrophobicity, which is very subtle to regulate thermogelling. Hence, this work affords a new way to adjust the proper sol−gel transition concentration or temperature and has shed new insight into the effect of molecular weight distribution on copolymer assembly in a selective solvent. The increase of $Đ_M$ was found to increase CMC of amphiphilic copolymers, if the CMC was determined by the conventional DPH dye incorporation method. This study and corresponding analysis also call for further theoretical and experimental investigations of the criterion of a very popular term—"micelle".

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Notes
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