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Synthesis of hydroxyl-capped comb-like poly(ethylene glycol) to develop shell cross-linkable micelles

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Abstract

A novel hydroxyl-capped comb-like poly[poly(ethylene glycol) methacrylate] (PPEGMA) was prepared via atom transfer radical polymerization (ATRP) of α-methylacryloyl-ω-hydroxyl-poly(ethylene glycol) at ambient temperature. The polymerization kinetics of the block copolymer was studied by gel permeation chromatography (GPC) and ^{1}H NMR. It is of interest to find the well-defined comb-like PEG can associate into micelles, which have hydrophilic PEG shell end-capped by hydroxyl groups. The hydroxyl in the shell were further cross-linked by divinyl sulfone (DVS), which could couple with two capped-end hydroxyl groups. The XPS, TEM, AFM and laser scattering particle size distribution analyzer results revealed that reactive micelles could be cross-linked by DVS. The reactive, cross-linkable micelles with PEG shell may have great potential as new drug carrier and nanoreactor, etc.

Keywords: Micelle; Poly(ethylene glycol); Comb-like

1. Introduction

Block copolymers are with increasing interest because of their unique solution and associative properties as a consequence of their molecular structure. In particular, because of their segmental incompatibility, their surfactant and self-associative characteristics lead to polymeric micellar systems [1]. The segregation of insoluble blocks in block copolymer goes into the core, which is surrounded by hydrophilic shell composed of hydrophilic blocks. The micelles formed through the multi-molecular assembly of block copolymer are novel core–shell typed colloidal carriers in the biomedical field such as drug and gene targeting [2–7]. However, in micelles with different structures the shell cross-linked (SCL) micelles are of more potential application because they combine the properties of micelles, microgels, nanoparticles, and dendrimers. The formation of shell cross-links in the polymer micelles offers sufficient stability to the nanostructured assemblies so as to allow them to withstand dramatic alterations in the environment. Cross-linked shell will also serve as a membrane-like layer to gate transport of guests into and out of the core domain, while the lack of covalent bonds between the core chains will offer a great degree of variability in mobility and density for the core [8]. Recent efforts have focused on the synthesis of SCL micelles that have either hollow cores or tunabely hydrophilic cores. In principle, hollow SCL micelles offer larger loading capacities, but tunable hydrophilic cores are more attractive since no core removal step is required and there is the potential for the triggered release of encapsulated actives via various chemical and physical stimuli (pH, temperature, ionic strength, etc.) [6,7,9].

Poly(ethylene glycol) (PEG) is a highly investigated polymer because it is water-soluble, lack of toxicity, weak immunogenicity and ready clearance from the body by a combination of renal and hepatic pathways [10–12]. PEG chains forming the shell of nanometer-scaled micelle is particularly useful for preventing the adsorption of proteins and adhesion of cells [2]. Furthermore, the PEG shell prevents recognition by the reticuloendothelial system. Therefore, it will prevent preliminary elimination of the micelles from the bloodstream. Thus, the so-called 'stealth' property of the PEG shell results in increased blood circulation times and allows drugs to be administered over prolonged periods of time. In this regard, these PEG-based block copolymers should be of a substantial importance for the development of cell and drug delivery systems (DDS) [4,13].

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Despite the widely application of linear PEG, PEG chains with a multi-arm architecture may provide better performance than their linear counterparts depending on the biomedical and pharmaceutical applications considered [14]. The comb-like PEO chains [15] and dendron–PEG conjugate [16] have been regarded as the most effective structures to reduce the non-specific interaction. However, the well-defined high-branched PEG is difficult to be developed by traditional polymerization, such as radical polymerization. ATRP is a recently developed polymerization technology and allows the effective synthesis of various copolymers with well-defined molecular weights, low polydispersity, and many different types of architectures and controlled topology [17–19]. Recently, a large number of monomers were polymerized in a well-controlled fashion with several PEG-based derivatives as either macroinitiators or macromonomers in ATRP [20–24]. A novel highly branched 'comb-like' PEG with well-defined molecular weights, low polydispersity has been synthesized by ATRP of α-methylacryloyl-ω-methoxyl-poly(ethylene glycol) [24]. But the methyl-capped comb-like PEG was limited for its further biomedical applications due to the lack of functional groups for end-tethered ligand or further modifications.

In this study, the hydroxyl-capped comb-like poly(ethylene glycol) was prepared by ATRP of α-methylacryloyl-ω-hydroxyl-poly(ethylene glycol) in water/THF mixed solvent at ambient temperature. Subsequently, the self-assembly of the comb-like PEG dissolved in water was studied and the critic micelle concentration was determined by the pyrene fluorescence probe. The reactive micelles were further cross-linked by DVS, which could couple with two end-capping hydroxyl groups. The TEM, AFM, XPS and laser scattering particle size distribution analyzer results revealed that reactive micelles might form and be cross-linked.

2. Experiment and characterization

2.1. Materials

Poly(ethylene glycol) methyl ether (M_n = 2000, Aldrich), α-methylacryloyl-ω-hydroxyl-poly(ethylene glycol) (M_n = 360, 526 Aldrich), 2-bromoisobutyl bromide (Aldrich, 98%). CuCl (AR, Shanghai No. 1 Chemical Reagent Factory) was purified according to the procedure of Keller and Wycoff [25]. Pyrene was recrystallized twice in acetone. Dichloromethane (Hangzhou Chemical Reagent Factory) and triethylamine was refluxed with calcium hydride for 24 h. D860 chelate resin (Zhengguang Resin Co., Ltd) was activated by acid–base–acid in turn and washed up to neutrality. Divinyl sulfone (AR, Shanghai Chemical Reagent Factory), 2,2′-bipyridyl (bpy) (AR, Hangzhou Chemical Reagent Factory) was used as received. The redistilled deionized water was self-made in our lab. Unless otherwise specified, all other reagents were purchased from commercial sources and used without further purification.

2.2. Synthesis of macro-initiator PEG_{2000}–Br

The synthesis of PEG_{2000}–Br referred to the reference [26]. ^{1}H NMR: 1.94 (m, 6H), 3.38(s, 3H), 3.56–3.74 (s, 189H), 4.33 (t, 2H). $M_{n,NMR}$ = 2200.

2.3. Polymerization procedure of PEGMA

A scheme of typical synthesis is showed in Fig. 1, the ATRP of α-methylacryloyl-ω-hydroxyl-poly(ethylene glycol) was carried out in H_2O/THF (10/1) under an argon atmosphere. The deactivator $CuBr_2$/2bpy ([CuCl/2bpy]/[$CuBr_2$/2bpy] = 1/0.8, mol%), monomer α-methylacryloyl-ω-hydroxyl-poly(ethylene glycol), and H_2O/THF (10:1, vol%) were added to a reaction flask. The solution was degassed through three freeze–pump–thaw cycles. And the reaction flask was immersed into the water at ambient temperature, then the copper catalyst complex CuCl/2bpy was added to start the polymerization. The reaction solution became brown first. After a certain time termination occurred rapidly on exposure to air, as indicated by the color change from brown to blue (from oxidation of Cu(I) to Cu(II)). Tetrahydrofuran was added into the solution then anhydrous sodium sulfate was added to dry. After filtered the chelate resin were added into the reaction solution in order to remove the blue copper catalyst and yield a colorless polymer. Then the solution was filtered and solvent was vaporized. The polymer was dried rigorously under vacuum. The dilute solution of PPEGMA in THF was precipitated in a large amount of isopropyl ether for three times to remove the unreacted monomers [27] until the peak of the double bond was not visible in ^{1}H NMR.

2.4. Preparation and characterization of micelles

The molecular weight and polydispersity index of the comb-like PEG used in the study of solution property was M_n = 16,000, M_w/M_n = 1.25 determined by GPC. The molecular

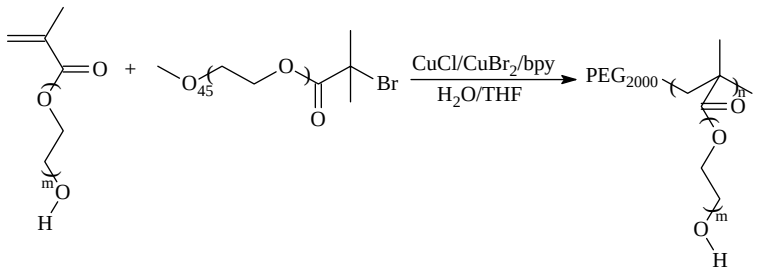

Fig. 1. Scheme of atom transfer radical polymerization of poly(ethylene glycol) methylacrylate initiated by PEG_{2000}–Br in H_2O/THF at room temperature (m = 6 or 11).

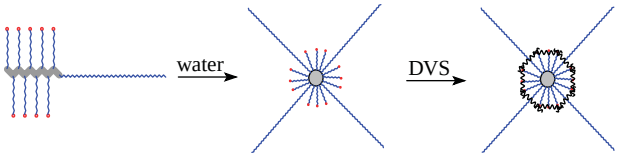

Fig. 2. Schematic illustration of shell cross-linked micelles from comb-like PPEGMA.

weight of polymer was further characterized by ^{1}H NMR method, which is PEG_{2000}-*b*-$P(PEGMA)_{28}$ with $M_{n,NMR}$ = 12,133 [28,29]. The micelle solution was prepared by the followed procedure. Stock solutions were prepared as follows: the THF solution of polymer was filtered by membrane with 0.22 μm aperture. The solvent in the filtered solution was evaporated. The refined block copolymer (0.2–0.3 g) first dissolved in redistilled de-ionized water in a 10 ml volumetric test tube. To get sample solutions, a known amount of pyrene in acetone was added to each of a series of 10.0 ml volumetric test tubes and the acetone was evaporated. The amount of pyrene was chosen to give a pyrene concentration in the final solution of 6.0×10^{-7} M [30]. To each flask was then added a measured amount of a stock solution, followed by redistilled deionized water. The samples were sonicated to homogenize the solution. A series of polymer solution samples with concentrations from 5.62×10^{-5} to 1.12 g/l were prepared and studied.

2.5. Cross-linking of polymer micelles

Shell cross-linking was achieved by adding DVS drop by drop under stirring, and the reaction between the divinyl sulfone (DVS) and hydroxyl group were kept stirring for 12 h at ambient temperature (Fig. 2). The target degree of cross-linking is 2×[DVS]/[hydroxyl group]×100% [31]. Some fraction of the DVS may react with hydroxyl groups on the same polymer molecule and will not, therefore, contribute to shell cross-linking. Furthermore, the DVS is prone to hydrolysis under the cross-linking conditions [31], which is likely to reduce the actual degree of cross-link achieved. So the excess amount of DVS was added into the certain polymer solution. In addition, the cross-link condition was not optimized. For the cross-linking of micelles the study focused on polymer solution of 8.43×10^{-3} g/l.

2.6. Characterization and preparation of samples

Molecular weight and molecular weight distribution were measured by GPC with Waters 515 HPLC pump equipped with Waters 2410 RI detector using tetrahydrofuran as eluent solvent and the flow rate was 1.5 ml/min at 40 °C. Narrow molecular weight distribution polystyrene standards were used for calibration in the GPC test.

Copolymer compositions were analyzed using a Bruker Avance 400/500 DMX NMR spectrometer. $CDCl_3$ and D_2O were used as solvent. The absolute molecular weight of polymer was characterized by ^{1}H NMR method.

The size distribution of micelles was measured using the laser scattering particle size distribution analyzer (Brookhaven 90 plus, Brookhaven Instruments Corporation) at 25 °C.

For fluorescence measurements, approximate 5 ml of solution was placed in the quartz tubes. All spectra were run on air-saturated solutions using a Shimadzu RF-5301pc spectrometer using bandwidth of 3 nm. For fluorescence spectra, λ_{em} = 390 nm for excitation spectra with scan scope ranging from 300 to 450 nm.

Utilizing the transmission electron microscopy (TEM, JEM-1230) morphology of micelles with difference concentration was studied. A drop of the micelle solution was deposited on the carbon hole-coated grid and then stained by a drop of 2% uranyl acetate solution. The samples were dried under the infrared lamp prior to test. As for the diameters of micelles, at least 2000 micelles' diameters were measured.

An atomic force microscopy (SPI3800N, Seiko Instruments Inc.) sample was made as follows: a certain concentration micelle solution was dip-coated on the fresh cleaved mica uniformly. Then the sample was transferred into a desiccator and kept for at least 24 h. The topographic feature of the micelle was characterized by AFM in the taping mode.

For X-ray photoelectron spectroscopy (XPS) analysis the sample was tested with PES-STM, OMICRON. Cross-linked polymer solution was dialyzed for a week before test in order to remove the free divinyl sulfone or other byproducts. Then the solution was dropped onto the surface of fresh stainless steel to keep in the clean room to be dried. The sample was again cleaned by ethanol and dried before the test. The take-off angles was 35°. The lowest energy peak in the high-resolution C_{1s} scans was referenced to 285 eV. A portion of this SCL micellar solution was dried under vacuum prior to XPS analysis.

3. Results and discussion

3.1. ATRP of poly(ethylene glycol) methylacrylate (PEGMA)

The ATRP of macromonomer, α-methylacryloyl-ω-hydroxyl-poly(ethylene glycol) (PEGMA M_n = 360) was conducted under a homogeneous condition using 2,2′-bipyridine (bpy) as the ligand, which can coordinate Cu(I) to lead to form the soluble complex in the aqueous medium [22,24,26,32]. The polymerization was carried out using PEG_{2000}–Br as the macro-initiator in conjunction with CuCl in water/THF (= 10/1, vol%) solution at ambient temperature with $[initiator]_0/[bpy]_0/[catalyst\ system]$ = 1/2/1 (mol%) (catalyst system: $[CuCl]_0/[CuBr_2]_0$ = 1/0.8, mol%) and $n_{monomer}/n_{initiator}$ = 50.

As the polymerization proceeds, in the ^{1}H NMR the peaks of the signal of the double bond hydrogen (δ = 5.6 and 6.1) disappear, and the methylacrylate backbone signal arises at δ = 0.8–2.0. The small peak at δ = 3.75 is contributed by the terminal hydroxyl group of PEO side chain. In Fig. 3, one peak at δ = 4.3 indicates the polymerization of PEGMA and produces the final polymer PPEGMA [32]. Fig. 4 shows the time-conversion curves for the polymerization of PEGMA. A semilogarithmic plot of monomer conversion vs. reaction time

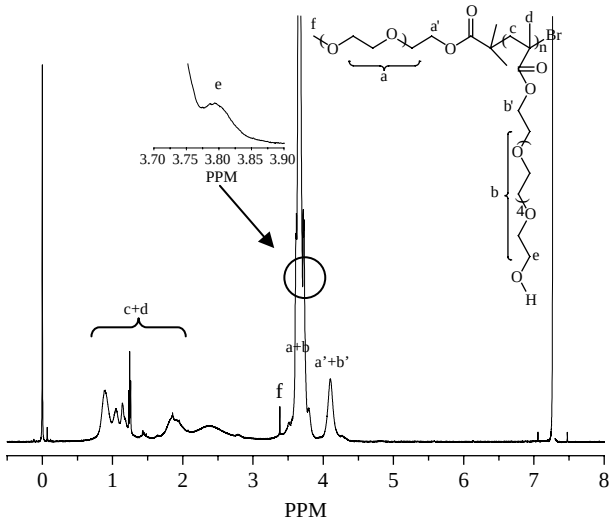

Fig. 3. The ^{1}H NMR profile of PPEGMA in $CDCl_3$. The polymer was dissolved in $CDCl_3$ with $C=0.02$ g/ml.

is linear up to 74%, which indicates that the polymerization kinetics is first order with respect to monomer and the concentration of active centers (polymer radical) remains constant during the polymerization process. By prolonging the polymerization time, the 100% conversion (no signal of the double bond hydrogen in ^{1}H NMR) can be available. As shown in Table 1 and Fig. 5, the number-average molecular weights ($M_{n,GPC}$) by GPC of the obtained PPEGMA increases linearly with increase of the monomer conversion, while the polydispersity index (PDI) stayed fairly low with $M_w/M_n<1.2$. The trend of PDI evolution only increases slightly.

The above results indicate that a novel hydroxyl-capped comb-like poly[poly(ethylene glycol) methacrylate] (PPEGMA) was synthesized with 'living'/controllable ATRP of α-methylacryloyl-ω-hydroxyl-poly(ethylene glycol). The self-aggregation of the hydroxyl-capped comb-like PEG copolymer in aqueous was then investigated to assess the possibility of developing the micelles with the hydroxyl groups in PEG shell. Subsequently, the micelles were further cross-liked by DVS to develop stable PEG based micelles, which are supposed to be able to resist non-specific interaction and enhance the steric stability.

Table 1
Experimental conditions and properties of the polymers synthesized by the ATRP method

Sample	Reaction (time/min)	Conversion (%)[a]	$M_{n,th}$[b]	$M_{n,GPC}$[c]	PDI[c]
Initiator			2200	3600	1.05
PPEGMA-1[d]	40	65	13,900	10,500	1.13
PPEGMA-2[d]	50	74	15,600	11,000	1.16
PPEGMA-3[d]	240	>99	20,300	14,600	1.19
PPEGMA-4[e]	20	–[f]	–[f]	11,000	1.09
PPEGMA-5[e]	30	–[f]	–[f]	14,700	1.17

[a] Determination by ^{1}H NMR. Conversion ratio (%)$=(I_{c+d})/(I_{doublebond}+I_{c+d})\times100\%$.
[b] Calculated according to $M_{n,th}=2200+[M]_0/[initiator]_0\times conversion\times 360$, where 2200 and 360 are molecular weight of initiator and monomer.
[c] Determination by GPC using polystyrene as standards.
[d] Polymerization with PEGMA ($M_n=360$) and $n_{monomer}/n_{initiator}=50$.
[e] Polymerization with PEGMA ($M_n=526$).
[f] No characterization about these properties.

3.2. Characterization of micelles and shell cross-linked micelles

Pyrene as fluorescence probe is of importance in studies of multimolecular aggregates such as micelles and membranes [33]. Fluorescence spectrum of pyrene is effected by environment and sensitive to the polarity of the surrounding environment [34]. When the pyrene experiences a change from polar environment to non-polar environment, a red shift of the (0,0) band in the excitation spectra is observed. Thus, transformation of the I_{338}/I_{333} ratio, where I_{338} and I_{333} are the pyrene fluorescence intensities excited at 338 and 333 nm, respectively, reflects change of environmental polarity [33]. Fig. 6 shows the excitation spectra of pyrene as a function of polymer concentration in water. It is every evident that peak of (0,0) band moved from 333 to 338 nm with the increase of concentration. The ratio of intensities (I_{338}/I_{333}) vs. log(c) is plotted in Fig. 7. It is believed that the major change in the slope indicates the onset of micellization [33]. At lower concentrations this ratio takes the value characteristic of pyrene in water, and at higher concentrations it takes the value of pyrene entirely in the hydrophobic environment afforded by the micellar core. The intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low

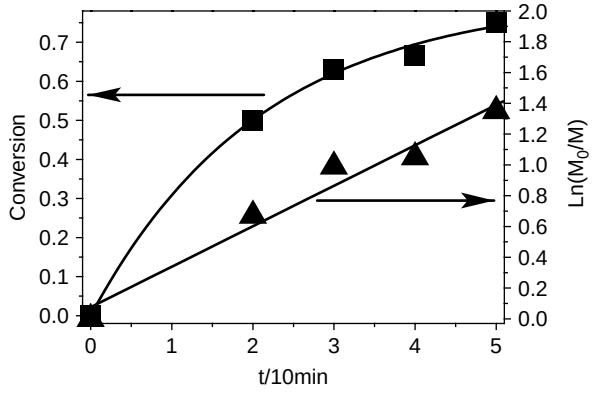

Fig. 4. Plots of conversion and $\ln[M]_0/[M]$ vs. time for the polymerization.

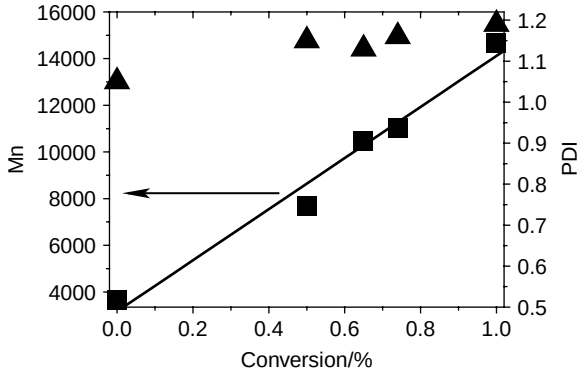

Fig. 5. M_n and PDI vs. conversion curves of PPEGMA with PEGMA.

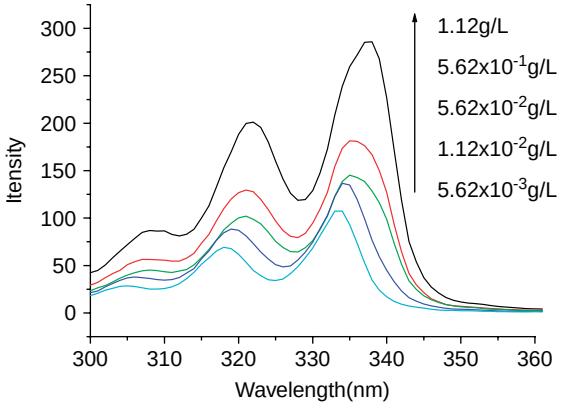

Fig. 6. Excitation intensity of pyrene as a function of CPEG concentrations in water, monitored at $\lambda_{em}=390$ nm.

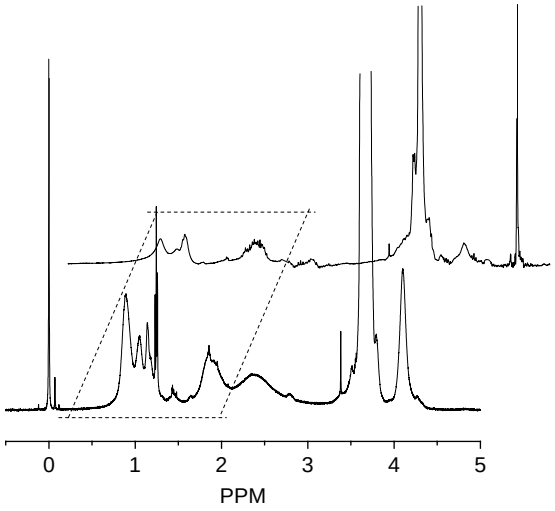

Fig. 8. The ^{1}H NMR spectrum of CPEG in $CDCl_3$ and D_2O. The polymer was dissolved in $CDCl_3$ or D_2O with $C=0.02$ g/ml.

polymer concentration is determined as the CMC that is the apparent CMC value [30]. By this ways the CMC is determined and equal to 5.76×10^{-3} g/l that far lower than that of the PEG macromonomer (~1 g/l) [35]. To understand further the assembly of polymer micelles in water, ^{1}H NMR studies were performed. Through ^{1}H NMR, resonances associated with the core and shell-forming blocks were study. The polymer solution with 0.02 g/ml was studied here. In $CDCl_3$, the methyl protons of backbone appears in the range of 1.3–2 ppm, as shown in Fig. 8. But in D_2O, the intensity of them becomes weak. This suggests again that the backbone in polymer might associate in water as the core of the polymer micelle, with hydroxyl-capped PEG shell.

The polymer micelles with dense PEG in the shell present a large number of reactive hydroxyl groups in PEG, which can be further end-tethered with ligand or cross-linked to prepare shell cross-linked (SCL) micelles. DVS has been widely used to cross-link hydroxyl-containing polymers to prepare stable gels, microgels, and nanoparticle [31,36–39]. In this study, SCL micelles were prepared by directly dropping DVS to the polymer micelles solution with 8.43×10^{-3} g/ml at ambient temperature and reacting for 12 h under stirring. Then the

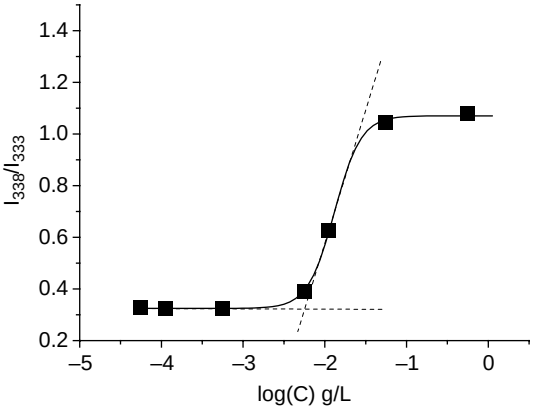

Fig. 7. The plot of the ratio of intensities (I_{338}/I_{333}) of the vibrational bands in the pyrene fluorescence excitation spectrum as a function of polymer concentration. $\lambda_{em}=390$ nm. The dash line was the tangent line to the plot.

cross-linked polymer solution was dialyzed for a week to remove the free DVS or byproducts.

XPS is a surface-sensitive analysis method, which can report detailed information on near-surface elemental compositions, i.e. the shell of micelles [40]. There were many reports of using XPS to verify the cross-linking or characterize the core–shell structure of SCL micelles [39,41,42]. In this study, the sulfur is a characteristic elemental marker because it only originates from the DVS reagent, which was used to cross-link the micelles. So the sulfur signal can be used to track the cross-linking. The XPS spectra of the cross-linked polymer micelles show the peak of S_{2p} (Fig. 9(a)), while there is no peak of S_{2p} in uncross-linked micelles. It indicates that the DVS reacted with the hydroxyl groups and was incorporated in micelles. By comparing the spectrum of substrate with the spectrum of cross-linked micelles, a new fitting peak at 285.48 eV is found (Fig. 9(b) and (c)). Based on the analysis of the chemical situation of the carbon element, it is believed the peak at 285.48 eV is C_{1s} of –C–S– in cross-linked micelles. In addition, in Fig. 9(c), the intensity of –C–O– increases evidently, which arises from the contribution of PEG in the shell of micelles and the intensity of –C–C–/–C–H or –COO– decreases relatively after cross-linked, which means that the shell of micelles were detected by XPS. The above results indicate the –C–S– exists in the shell of micelles with the same location as the PEG. So the reaction between the hydroxyl groups and DVS happened indeed and was in the shell of micelles.

The laser scattering particle size distribution analyzer is often used to measure the size distribution of colloid particles relatively [43–45]. The size distribution results of micelles of 8.43×10^{-3} g/ml including the uncross-linked and cross-linked micelles are shown in Fig. 10. The peak of the size distribution of uncross-linking micelles is at 169.32 nm, but that of cross-linked micelles is at 131.51 nm. The cross-linking decreases the micelles' diameter and leads to the more compact

aggregate of micelles. When the cross-linked micelles were swelled by THF for 12 h, the micellar structure was kept but the peak of the micelles' diameter steeply moved to 265.90 nm. However, no particles were detected after the uncross-linked micelles were swelled for 12 h. All indicates the shell cross-linking of micelles prevents from the dissociation due to the swell of solvent. The change of the size distribution of micelles proves again that the DVS cross-linked the hydroxyl groups in the shell of micelles and enhanced their tolerance to solvent.

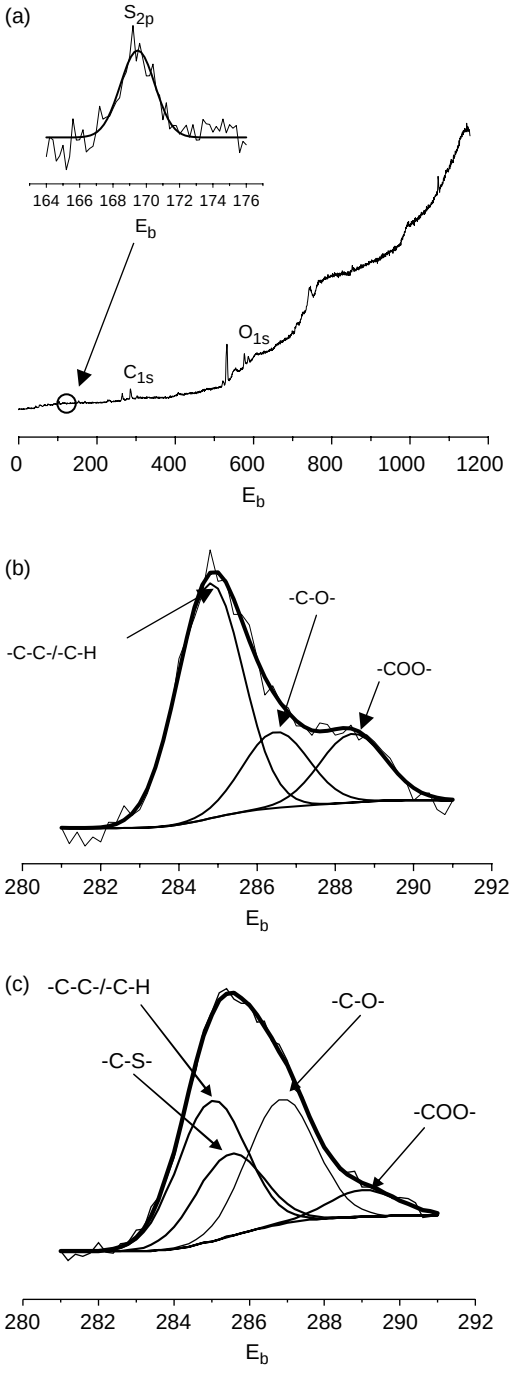

Fig. 9. The results of XPS spectra. (a) Full spectrum and the inserted spectrum of S_{2p}; (b) C_{1s} spectrum of the substrate; (c) C_{1s} spectrum of the cross-linking micelles. The sample prepared from the polymer solution with $C = 8.43\times10^{-3}$ g/ml.

The morphology of uncross-linked and cross-linked micelles in 8.43×10^{-3} g/l solution was characterized by TEM and AFM. In Fig. 11(a) and (b), representative figures of TEM of uncross-linked and cross-linked micelles are shown. The average diameter of uncross-linked micelles is mean 28.23 nm (Fig. 11(c)) by the statistic measurement of more than two thousands micelles in different pictures. However, the average diameter of cross-linked micelles is 25.34 nm (Fig. 11(d)) which is a little less than that of the uncross-linked micelles. This confirmed the expected intramicellar cross-linking mechanism. Since, the micelles would shrink a lot when it was dried for SEM and AFM investigation, it is not surprised to find the diameter determined by SEM is much less than the diameter determined by the laser scattering particle size distribution analyzer. In addition, the TEM investigation indicated that the uncross-linked micelles in Fig. 11(a) seem to be very flat, but the cross-linked micelles in Fig. 11(b) present evident steric structure which can be approved by the contrast and shadow from the TEM. Presumably, the cross-linked micelles could sustain the three-dimensional structure during the significant shrink [46,47], while the uncross-linked micelles would collapse to be the flat structure. It can be further approved by the AFM investigation [48,49] in the Fig. 12. The uncross-linked micelles are 0.5 nm maximum height in Fig. 12(a) but the height of cross-linked micelles was 10 nm maximum height in Fig. 12(b). The change of height before and after cross-link can prove the cross-linking reaction successful again.

The prolate morphology was observed not only from TEM figure but also from AFM figure. The prolate morphology was similar with the micelle architecture found in polystyrene-*b*-poly(4-vinylpyridine) block copolymers in dilute solution [50]. It was also believed that their prolate morphology originated from the same reason.

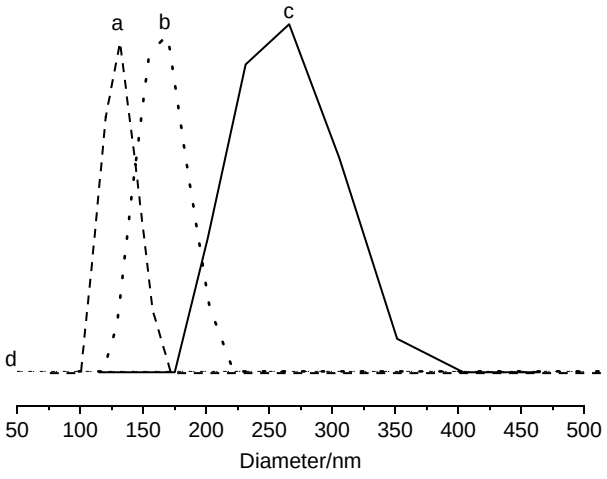

Fig. 10. The results of the size distribution of the micelles by particle size distribution analyzer. (a) Cross-linked micelles in water; (b) uncross-linked micelles in water; (c) cross-linked micelles in THF; (d) uncross-linked micelles in THF. The uncross-linked or cross-linked polymer solution with $C = 8.43\times10^{-3}$ g/ml was studied here.

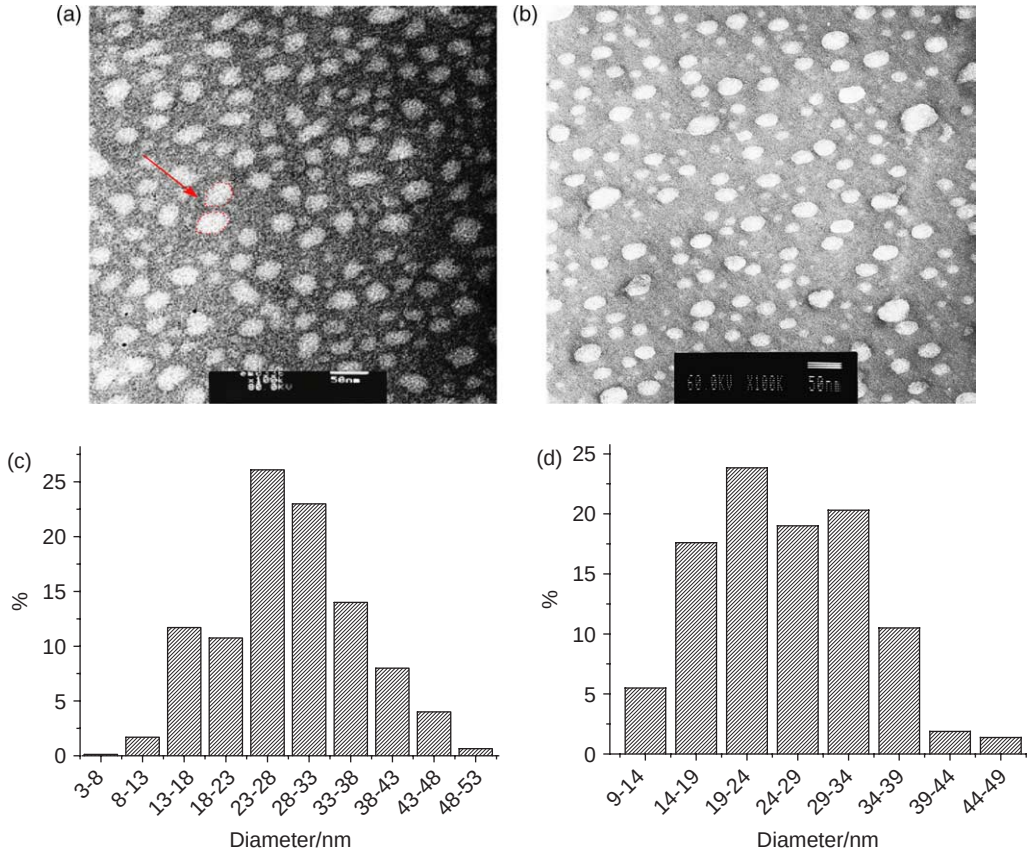

Fig. 11. TEM images obtained by negatively staining with uranyl acetate solution: (a) before cross-link; (b) after cross-link; red arrow indicated the irregular spheral shape. The statistic size distribution of CPEG micelles by TEM: (c) before cross-link, average diameter was 28.23 nm; (d) after cross-link, average diameter was 25.34 nm. The bars were 50 nm. The polymer solution with $C=8.43\times10^{-3}$ g/ml was dropped onto the carbon hole-coated grid for TEM characterization.

4. Conclusion

A novel hydroxyl-capped comb-like PPEGMA was firstly synthesized via aqueous ATRP at ambient temperature. The kinetic study of ATRP of PEGMA shows the curve of $\ln[M_0]/[M]$) vs. time is linear, which indicates the polymerization is first order and the concentration of active centers (polymer radicals) remains constant. The PDI of PPEGMA is lower, PDI <1.2. The hydroxyl groups at the side chains in PPEGMA are confirmed by ^{1}H NMR. It is of interest to find the hydroxyl-capped comb-like PEG can associate into micelles whose CMC is 5.76×10^{-3} g/l as determined by fluorescence spectrometer. The reactive micelles were further cross-linked by DVS. The XPS, TEM, AFM and laser scattering particle size analyzer results approve the occurrence of shell cross-linking and the expected intramicellar cross-link. The reactive, cross-linkable micelles with PEG shell will have great potential application in biomedical and nanomaterial such as drug delivery and nanoreactor, etc.

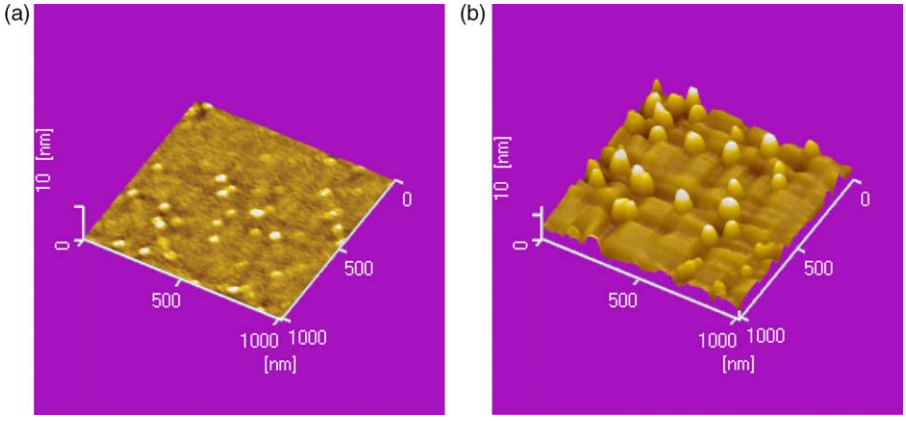

Fig. 12. Three-dimensional AFM images of polymer micelles: (a) uncross-linking micelles; (b) cross-linking micelles. The polymer solution with $C=8.43\times10^{-3}$ g/ml was dropped on the fresh cleaved mica for characterization.

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