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pH-Responsive Vesicles with Tunable Membrane Permeabilityand Hydrodynamic Diameters from Self-Catalyzed Cross-Linkable Amphiphilic Block Copolymer

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ABSTRACT:A series of cross-linked pH responsive vesicles with tunable diameters were prepared by self-assembly of poly (ethyleneoxide)-b-poly [2-(diethylamino) ethyl methacrylate-co-glycidyl methacrylate] [PEO-b-P(DEA-co-GMA)] inTHF/water mixture. The copolymers were synthesized by random copolymerization of DEA and GMA initiated by PEO-Br.With epoxy group and tertiary amine in its structure, self-catalyzed cross-linking was triggered easily by mild heating. The vesicles showed pH responsiveness as characterized by DLS and tunable permeability can be understood accordingly. As the pH of the solvent changed from 9.8 to 7.4, the diameters of the vesicles increased. It was observed that the diameters at pH = 6.0 were comparable to that at pH = 7.4. This alteration may have happened because of the protonation of PDEA segment.This particular property has great potential and applications since most drug loading and controlled release are conducted within this pH range.

KEYWORDS:Block copolymer, self-assembly, pH responsive, PGMA, PDEA

I. INTRODUCTION

Block copolymers can self-assemble into a variety of morphologies both in solvent and in bulk.[1^{-1} 0] The vesicles and micelles prepared from self-assembly of amphiphilic block copolymers are widely used as drug loading and delivery vehicles and drew great attentions in the past decade.[11-20]

As most popular candidate for drug loading and delivery agent with excellent biocompatibility, poly(2-(diethylamino)ethylmethacrylate) (PDEA) with pH-stimuli responsiveness had drawn great attentions.[21-23]However, the PDEA becomes protonated and soluble in aqueous medium when pH remains less than 6.5 which makes it unfit for application as hydrophobic block and as adrug loading agent alone.To consider the vesicles as potentialdrug carriers and nano-reactors, the tunable permeability of membranes is greatly preferred.Using stimuli-responsive block copolymers as the buildingblocks of vesicles is one elegant way to modify the permeability. Later on it becomes easy to tune the releasing properties of the vesicles. In the past decade, development ofintelligent vesicles responding to pH, temperature, redox, andlight became one of the important topics in the vesicleresearch.However, the stimulus often leads to dissolving and disruption of the particles14. To avoid disruption, keeping the morphologies of the self-assemblies under stimulus, cross-linking of vesicle membrane or micelle cores are needed. Although, it was observed that the Polystyrene (PS) can also fix the morphological issues related to the self-assembly under proper stimulation to prepare breathing vesicles[24]. Previous research indicated that the dynamically stable morphology was attained at new condition when appropriately heated[25]. Based on the gelation reaction of poly(3-(trimethoxysilyl)propylmethacrylate) (PTMPM), Du et al. producedpH-responsive stable vesicles by

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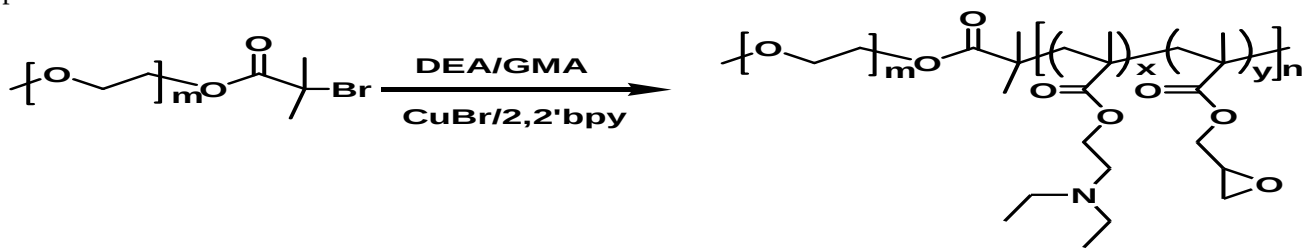
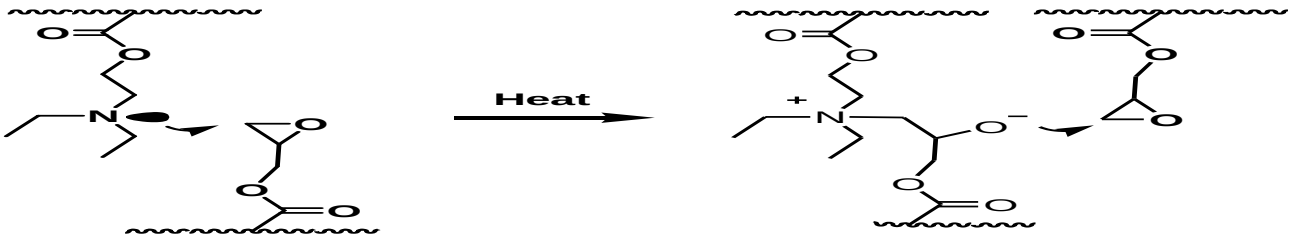
incorporating pH sensitive PDEA to the PTMPM[26]. Peng et al. prepared thermo-responsive organic-inorganic hybrid vesicles from PEO-b-poly(N-Isopropyl acrylamide-r-TMPM) (NIPAM-r-TMPM)PEO-b-P (NIPAM-r-TMPM)11. Du et al. reported core cross-linked vesicles via UV exposure from 7-Hydroxy-4-methylcoumarin based monomers[27]. As a versatile cross-linkable polymer, PGMA has been used in both cross-linking the morphologies of the self-assemblies[28,29] and preparation of polymer brushes[10,30].In this paper, membrane cross-linked vesicles were prepared from amphiphilic block copolymer of PEO-b-P(DEA-co-GMA), as shown in Scheme 1. With both epoxy groups and tertiary amine on the hydrophobic block, self-catalyzed cross-linking was triggered by mild heating following the Scheme 2. The vesicles showed pH responsiveness while keeping the morphologies of the vesicles intact. Moreover, the permeability of the vesicle membrane was tuned by tuning the molar ratio of cross-linkable GMA unit and pH.



Scheme 1. Schematic illustration for preparation of POE-b-P(DEA-co-GMA).



Scheme 2. Self-triggered cross-linking of P(DEA-co-GMA) at the vesicle membrane.

II. EXPERIMENTAL SECTION

Materials:

Poly (ethylene glycol) methyl ether(PEO; Mn ca. 2000 Da; Mw/Mn = 1.06) and 2-(diethylamino)ethyl methacrylate (DEA)were purchased from Maya Chemical Co. Glycidyl methacrylate (GMA) was supplied by TCI. PEO was dried under vacuum overnight before using. 2-Bromoisobutyryl Bromide and 2,2'-Bipyridine (bpy; 99%)were obtained from Aladdin industrial Co. DEAand GMA were passed through a column filled with basic alumina to remove the inhibitors. CuBr was washed by acetic acid and ethanol before use. Other chemicals and reagent were purchased from Kermel Chemicals and used as received.

Preparation of PEO-b-P(DEA-co-GMA) by ATRP:

Initially, PEO-Br macroinitiator was prepared via the reaction of PEO with excess 2-bromoisobutyryl bromide according to the literature report[31] and confirmed by 1H NMR. Later, the PEO-Br was used as macroinitiator to prepare block copolymer as illustrated below and the reaction mechanism is shown in

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Scheme 1. PEO-Br macroinitiator (0.20 g, 0.1mmol), PMDETA (21 uL, 0.1 mmol), DEA (2.22g, 12.0mmol), GMA (0.43 g, 3.0 mmol) were dissolved in 3 mL dioxane in a 25 mL reaction tube. Then the tube was deoxidized by three freeze-evacuate-thaw cycles. In the next stage of the experiment, 14.4 mg (0.1 mmol) CuBr was added into the flask in solid state and the flask was deoxidized again. The flask was immersed in an oil bathat 50oC under continuous stirring. The reactionwaspolymerizedfor 3h and terminated by cooling to roomtemperature and exposing to air. The polymer solution wasdiluted with DCM and passed through a column filled with neutral alumina to remove CuBr. The block copolymer was precipitated in petroleum ether three times and lyophilized in dioxane. The composition of the block copolymer was calculated by comparing according peak areas on 1H NMR and the conversion of monomers were almost 100%.

Preparationof vesicles and their self-catalyzed cross-linking:

General procedure for self-assembly of PEO45-b-P(DEA-co-GMA) and cross-linking: First, 10 mg of copolymer was dissolvedin 2 mLTHF to obtain a 5 mg/mL solution in a vial, then deionized water was added dropwise under continuous stirring until the water volume ratio was up to 80%. After stirring for 12h, 10 mg of ethylene diamine was added into the vesicle solution and stirred for another 12h to cross-link the membrane of the vesicles.

The hydrodynamic diameter and the size distribution of themembrane cross-linked vesicles prepared from PEO45-b-P(DEA-co-GMA) at various pH were evaluated by dynamic light scattering (DLS).The pH responsiveness of the cross-linked vesicles was characterized by comparing the hydrodynamic diameter of the vesicles in different pH along with aqueous dispersions by DLS. At the beginning, the THF was removed by dialyzing the vesicles against water, and then the vesicles were diluted into 0.1 mg/mL. Dynamic diameter of the vesicles at various pH was characterized by DLS.

Characterization:

Gelpermission chromatography (GPC) was performed on anAgilentSECequipment with a set of Styragel columns and a refractive index detector. Theexperiments were carried out at 30□ C with THF as eluent at 1.0 mL/min flow rate and were calibrated by polystyrene standard.1H NMR spectra were recorded on a Bruker 600MHZ spectrometer (AvanceIII,Bruker Co. Switzerland) in CDCl3 at room temperature. Fourier-transform infrared (FT-IR) spectroscopy scan was obtained on a Varian 600-IR spectrometer. The samples were dissolved in dichloromethane and dropped on KBr plates with 2 mm thickness for characterization. Transmission electron microscope (TEM) characterization was carried out on a JEM^{-1} 00SX microscope and the images were recorded by a digital camera.The dispersed samples were dropped onto carbon-coated grids for TEM observation. Size measurement of the vesicles (DLS) was carried outon a DelsaNanoC instrument (Beckman Coulter Inc).

III. RESULTS AND DISCUSSION

Synthesis of self-catalyzed cross-linkable block copolymerPEO45-b-P(DEA-co-GMA):

During this study, the PEO45-Br was synthesized through reaction of hydroxyl terminated PEO45 with excess of 2-bromoisobutyryl bromideand confirmed by 1H NMR, as shown in Figure 1. By comparing the peak area of (a) (3.38 ppm) derived from PEO45 and (d) (1.94 ppm) derived from 2-bromoisobutyryl bromide, it was confirmed that all hydroxyl groups were consumed. The structure of PEO45-Br was also confirmed by FT-IR as shown in Figure 2. A peak appeared at 1730 cm^{-1} represented the formation of ester group, simultaneously no absorbance between 3200^{-1} and 3700 cm^{-1} was detected, which confirmed that all hydroxyl groups were consumed.

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In the next stage, PEO45-Br was used as macroinitiator to synthesize PEO45-b-P(DEA-co-GMA) block copolymer through ATRP as it can well mediate the controlled radical polymerization of DEA and GMA. After purified by precipitation and dried under vacuum, the structure of the block copolymer was characterized by 1H NMR and FT-IR. The 1H NMR of PEO45-b-P(DEA118-co-GMA33) as an example is shown in Figure 2 (top). The block ratio of PDEA and PDEA can be determined by comparing peak areas of (b) (3.76 ppm) with (e) (4.01)and (j) (3.20 ppm).A series of PEO45-b-P (DEA-co-GMA) block copolymers with different PDEA to PGMA ratios were synthesized in this work and their structural parameters are listed in Table 1. The structure of the block copolymer was also characterized by FT-IR, as shown in Figure 2 (bottom). The absorbance of carbonyl group at 1730 cm^{-1} increased, which proved the polymerization of methyl acrylate. The wide absorbance at 3110 cm^{-1} - 2770 cm^{-1} was derived from C-H stretching on both PDEA and PGMA. At the same time, a small absorbance appeared at 907 cm^{-1}, which is derived from epoxy groups of the block copolymer.

The GPC characterizations were performed to determine the PDI of the block copolymer and characterized the stability of the block copolymer during ATRP. The GPC evolution of PEO45-Br and PEO45-b-P(DEA118-co-GMA33)were illustrated in Figure 3 as an example. After ATRP the peak moved to lower elution time, indicated the increase of molecular weight. More importantly, the GPC curve of PEO45-b-P(DEA118-co-GMA33) was also symmetrical confirming the fact that the epoxy ring was stable during polymerization although self-triggered cross-linking was noticed in bulk state at 40oC. This ensured the good control of block copolymer preparation and drug loading, and provided possible cross-linking without addition of cross-linker of amine.

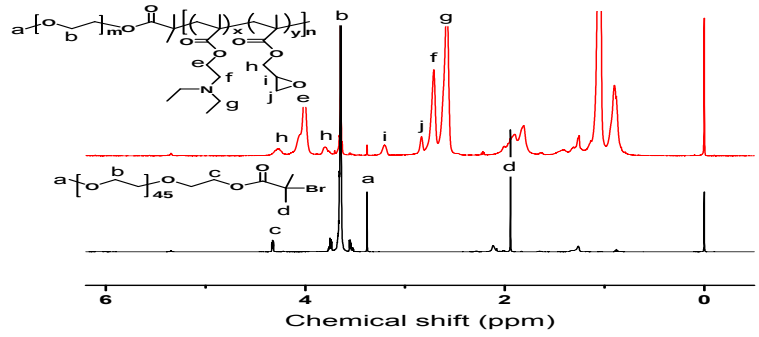

Figure 1. 1H NMR spectra of PEO-Br and PEO-b-P(DEA118-co-GMA33).

Polymers[a]	M_n	M_w/M_n	Morphology	Diameter[b]
PEO_{45}-*b*-P(DEA_{133}-*co*-GMA_{16})	28.9×10^3	1.12	Vesicles	352 nm
PEO_{45}-*b*-P(DEA_{118}-*co*-GMA_{33})	28.5×10^3	1.15	Vesicles	314 nm
PEO_{45}-*b*-P(DEA_{105}-*co*-GMA_{48})	28.2×10^3	1.14	Vesicles	166 nm

[a] determined by ^{1}H NMR ; [b] characterized by DLS at pH=7.4

Table 1. PEO45-b-P(DEA-co-GMA) block copolymers synthesized in this research and their characteristics.

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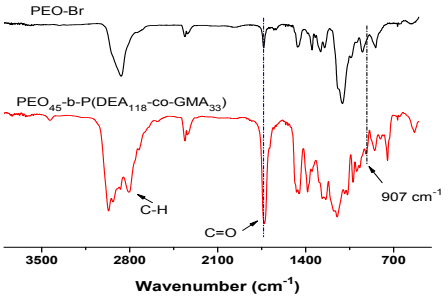

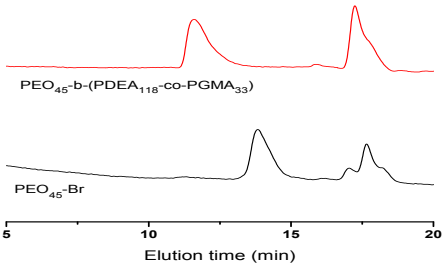

Figure 2.FT-IR spectra of PEO45-Br and**Figure 3.** GPC evolution of PEO45-Br and PEO45-b-P(DEA118-co-GMA33).PEO45-b-P(DEA118-co-GMA33).

Self-assembly of PEO-b-P(DEA-co-GMA) copolymers inTHF/water mixture solvent:

The self-assembly processes were conducted at room temperature and deionized water was added into the solution until the volume ratio of water was up to 80%. After stirring for 24 hours, the ethylene diamine cross-linked withthe PGMA domains to form the self-assemblies. The morphologies of the self-assemblies were observed by TEM after cross-linking. The TEM images of the vesicles prepared from PEO45-b-P(DEA133-co-GMA16) and PEO45-b-P(DEA105-co-GMA48) are shown in Figure 4. As shown in Figure 4, the average diameter of the vesicles prepared from PEO45-b-P(DEA133-co-GMA16) is 110 nm, and the average diameter of vesicles prepared from PEO45-b-P(DEA105-co-GMA48) is 119 nm which are comparable to that of PEO45-b-P(DEA133-co-GMA16), possibly due to similar hydrophilic/hydrophobic ratios.

To characterize the pH responsiveness of the vesicles, the hydrodynamic diameters dependent on PGMA ratio were determined by DLS using various pH values in aqueous dispersion. As shown in Figure 5, the diameter of the vesicles prepared from PEO45-b-P(DEA105-co-GMA48) is little larger but comparable to TEM observations (166 nm at pH = 7.4). On the contrary, the diameters of the other two samples differed a lot, the diameter of the vesicles from PEO45-b-P(DEA118-co-GMA33) was found to be 314 nm at pH = 7.4, and the diameter of PEO45-b-P(DEA133-co-GMA16) vesicles was 352 nm. The reason for larger hydrodynamic was due to the hydrophilic properties of PDEA in the vesicle membrane. Moreover, the diameters of the vesicles showed typical pH responsiveness in the pH range of 4.0-9.8. Allthree samples showed similar trend with variation of pH, as shown in Figure 6. At pH 9.8, the PDEA was insoluble in water and resulted in a smaller diameter. However, the vesicles with higher PDEA ratios showed much larger diameters indicating that PDEA is more hydrophilic than PGMA segment. With decreased pH of 7.4, the diameters of the vesicles increased due to increased hydrophilicity of PDEA segment. The PEO45-b-P(DEA105-co-GMA48) vesicles increased around 15% when pH was changed from 9.8 to 7.4 whereas the PEO45-b-P(DEA118-co-GMA33) and PEO45-b-P(DEA133-co-GMA16) increased 38% and 44% respectively because of higher PDEA ratios and lower cross-linking density. When the pH further changed to 6.0, the diameters of all three vesicles were found to be almost intact.

The comparable vesicle diameters between pH 7.4 and 6.0 has important practical applications since most of the drug deliveries are conducted within this pH range.

Interestingly, further decrement in pH to 4.0, obvious reduction in vesicle diameter was found rather than increment which is evident in Figure 6.The possible reason may be due to the alteration in solubility of PDEA in water by protonation at low pH. It was observed that reduction of pH value to 4.0 allowed part of

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the PDEA segment belonging to the vesicle membranes to dissolve in water. In parallel, it was observed that the intensity of the dissolved PDEA segments was very low compared to cross-linked vesicle membranes. As a result, the diameters of the vesicles decreased because the hydrodynamic diameter of the vesicles did not take the dissolved polymer segment into account.

At the same time, the vesicles did not disaggregate which proved that the PGMA have cross-linked the vesicle membrane successfully. The DLS curves of PEO45-b-P (DEA118-co-GMA33) vesicles at various pH are shown in Figure 6 as an example. These kind of responsive vesicles with pH-responsiveness have great advantages in anti-cancer drug loading and delivery since the pH in tumour is lower than other parts of human body. But the DOX turned during the drug loading and release, the most plausible reason may be due to the reaction of the epoxy ring of the PGMA segment and the amino group on DOX. Intensive study on the drug loading and release is already ongoing.

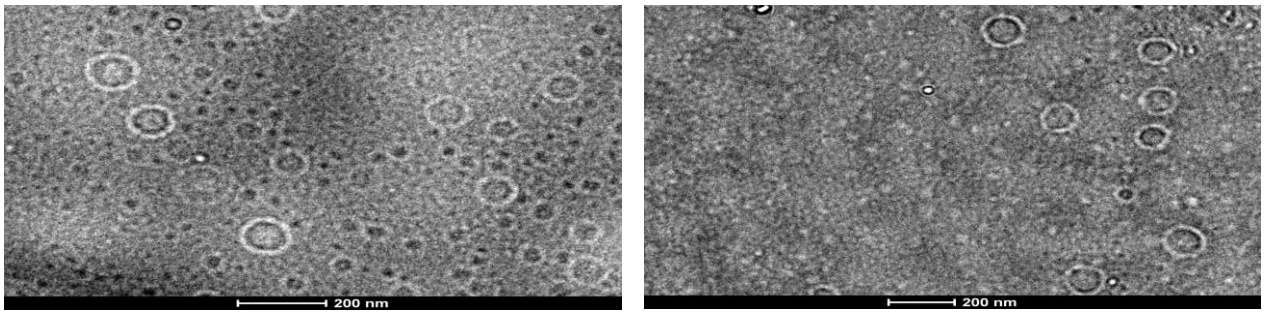

Figure 4. TEM images of vesicles self-assembled from (left) PEO45-b-P(DEA133-co-GMA16)and (right) PEO45-b-P(DEA105-co-GMA48).

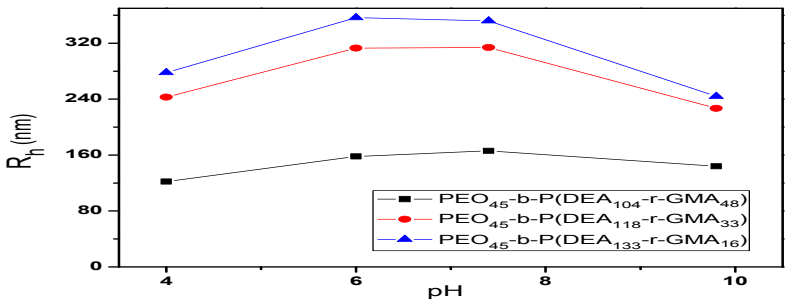

Figure 5.pH dependence vesicles self-assembled from PEO45-b-P(DEA-r-GMA) with different DEA/GMA ratios.

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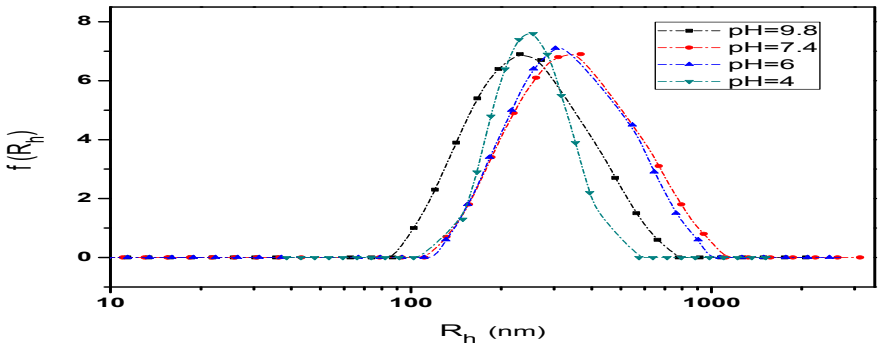

Figure 6.DLS curves vesicles from self-assembly of PEO45-b-P(DEA118-r-GMA33) at various pH.

IV. CONCLUSION

A series of amphiphilicblock copolymers with pH responsiveness were synthesized via ATRP and self-assembly of these block copolymers was conducted in THF/H2O. The hydrophobic block of the copolymer was composed from cross-linkable PGMA and pH responsive PDEA. The obtained results suggest that the block copolymers self-assembled into vesicles with the diameters regulated easily by tuning the pH of the solvent maintaining the stable morphologies of the vesicles. This kind of polymer self-assemblies have great potential application in anti-cancer drug loading and delivery and further studies were under progress.

V. ACKNOLEDGEMENT

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