Preparation of polymeric vesicles with tertiary amine and epoxy functional groups via polymerization-induced self-assembly

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Abstract. Polymeric vesicles with tertiary amine and epoxy functional groups were fabricated via the reversible addition-fragmentation chain transfer (RAFT) dispersion polymerization of 2-(diisopropylamino) ethyl methacrylate (DIPEMA) and glycidyl methacrylate (GlyMA) in an ethanol-water mixture by using poly (ethylene oxide)-4-(4-Cyanopentanoic acid) dithiobenzoate (mPEG45-CPADB) as the macro-chain-transfer agent (macro-CTA). Biocompatible polymer mPEG45 with molecular weight of 2000 and 4-(4-Cyanopentanoic acid) dithiobenzoate (CPADB) were used for the macro-CTA synthesizing via the esterification reaction. Investigation of the degree of polymerization (DP) of the hydrophobic block P(DIPEMA-co-GlyMA) and the solid content indicated that both have significant effect on the particle morphology and particle diameter. 1H NMR and FTIR analysis indicated the existing of tertiary amine and epoxy functional groups on the produced vesicles.

Keywords: Polymeric vesicles; polymerization-induced self-assembly; reversible addition-fragmentation chain transfer; dispersion polymerization.

1. Introduction

Nanoparticles have attracted significant attention due to their broad applications in areas of catalysis, drug delivery, imaging and nanoreactors.[1-3] Polymerization-induced self-assembly (PISA) is a recently developed one-pot polymerization approach that can generate multiple morphologies such as micelles, as well as worm-like/rod-like and spherical vesicles at high yields and with high solid content (typically 10-50 wt%).[4,5]

The morphology control of block copolymers has always been a hot topic for research on PISA, but it is not simple to generalize the parameters that determine the particle morphologies produced via PISA. Generally the morphologies of the nanoparticles are thought to depend on the block copolymer composition and the solid content, and both of them influence the relative volume fractions of the constituent blocks (packing parameter P, P=v/(al), where v is the volume of the hydrophobic polymer chain, a is the optimal interfacial area per molecule and l is the hydrophobic length normal to the interface). Spherical particles were thought to be formed when P was $\leq 1/3$, while worms and vesicles were expected for $1/3 \leq P \leq 1/2$ and $1/2 \leq P \leq 1$, respectively.[7,8]

In this study, we reported the copolymerization of 2-(diisopropylamino)ethyl methacrylate (DIPEMA) and glycidyl methacrylate (GlyMA) with poly(ethylene oxide)-4-(4-Cyanopentanoic acid) dithiobenzoate (mPEG45-CPADB) as the macro-chain-transfer agent (macro-CTA) in an ethanol/water solvent for the preparation of polymeric particles with tertiary amine and epoxy functional groups. The synthesizing of the macro-CTA (mPEG45-CPADB), and the effect of the degree of polymerization (DP) of the hydrophobic block P(DIPEMA-co-GlyMA) and the solid content on the particle morphologies were studied in detail.

2. Experiment

2.1 Materials

The α-methoxy-ω-hydroxypoly(ethylene oxide) (mPEG45) with a number average molecular weight (Mn) of 2000 was purchased from Tokyo Chemical Industry Co. Ltd. and was used as received. The 4-(4-Cyanopentanoic acid) dithiobenzoate (CPADB), 4-(dimethylamino)pyridine (DMAP), and dicyclohexylcarbodiimide (DCC) compounds were purchased from Aladdin and also used as received. The monomers 2-(diisopropylamino)-ethyl methacrylate (DIPEMA, Aladdin, 97%) and glycidyl methacrylate (GlyMA, Aladdin) were purified by passing the compounds through a column of Al_2O_3 to remove the inhibitor prior to use. Initiator N,N'-Azobis(isobutyronitrile) (AIBN, Tianjin Chemical Reagent Co. Ltd.) was purified via recrystallization from ethanol.

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2.2 Preparation of polymeric vesicles

mPEG$_{45}$-CPADB was used as the macro-CTA agent, and synthesized via an esterification reaction of mPEG$_{45}$ and CPADB at room temperature with DMAP as the catalyst and DCC as the dehydrant according to the reported method.[9,10] A typical protocol was performed as below: DIPEMA (0.3686 g, 1.5930 mmol), GlyMA (0.0614 g, 0.4319 mmol), mPEG$_{45}$-CPADB (0.0616 g, 0.0270 mmol), AIBN (0.0014 g, 0.0085 mmol), and a solvent (2.7937 g, with a mass ratio of ethanol/water=6:4) were added into a glass tube with a magnetic bar. This reaction mixture was degassed via N$_2$ purge in an ice bath for 30 min. After degassing by three pump-N$_2$ purge cycles, the glass tube was sealed under a vacuum, and then the sealed tube was placed in an oven at 70°C under magnetic stirring to polymerize for 7 h. The reaction mixture was quickly cooled to room temperature and then opened to air to quench the polymerization.

2.3 Characterization

The molecular weight (M$_n$) and molecular weight distribution (M$_w$/M$_n$) were measured by the gel permeation chromatograph (GPC, Waters 2695), and THF was used as the eluent and monodispersed polystyrene as the standards. The ^{1}H NMR spectra was done on a Bruker DMX500 spectrometer, where CDCl$_3$ was used as the solvent and tetra-methylsilane was used as an internal reference. The morphologies of the nanoparticles were characterized by transmission electron microscopy (TEM, JEM-2100Plus electron microscope), and stained with phosphotungstic acid before observations. All of the dynamic light scattering measurements were carried out on a commercial dynamic light scattering (DLS) spectrometer (PPS Z3000, Particle Sizing Systems, UK). The Fourier transform infrared (FTIR) spectroscopy was obtained using a PerkinElmer Frontier.

3. Results and discussion

3.1 Synthesis of macro-CTA mPEG45-CPADB

The synthesizing of macro-CTA mPEG$_{45}$-CPADB was conducted according to Scheme 1, and GPC and ^{1}H NMR analysis were done to characterize the produced macro-CTA, as shown in Figure 1. M$_n$ of mPEG$_{45}$-CPADB was M$_n$=2631 with M$_w$/M$_n$ =1.19 (Figure 1A, both M$_n$ and M$_w$/M$_n$ increased a little compared with mPEG$_{45}$), which showed relatively narrow M$_w$/M$_n$ after esterification reaction. ^{1}H NMR spectroscopy result indicated that the esterification reaction was successfully done based on the integral values of signals of ester methylene protons at δ=4.3 (f). The average end-functionality of mPEG$_{45}$-CPADB was 98.3% by calculating the integral ratio of methoxy protons of mPEG at δ= 3.4 (h) and aromatic protons at δ=7.3-8.0 (a，b，c), as shown in Figure 1B.
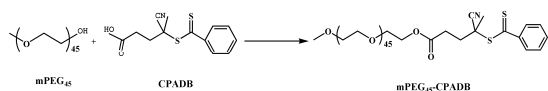
Scheme 1. Synthesis of mPEG45-CPADB macro-CTA.

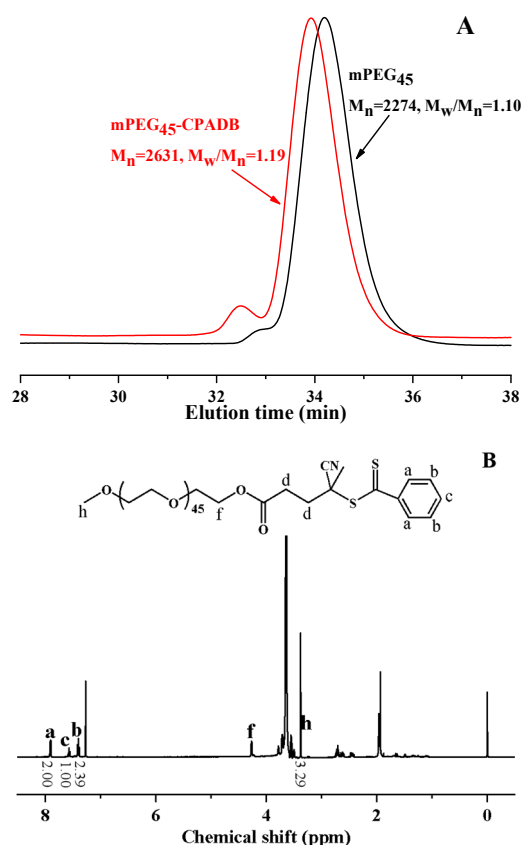**Figure 1.** (A) GPC traces of mPEG$_{45}$ and mPEG$_{45}$-CPADB; (B) ^{1}H NMR spectrum of mPEG$_{45}$-CPADB in CDCl$_3$.

3.2 Effect of DP of hydrophobic block on polymeric vesicles

The mPEG$_{45}$-CPADB produced above was used as the macro-CTA to produce the functional polymeric vesicles, which also acted as the stabilizer to stabilize the produced nonoparticles. During the polymerization, the hydrophilic macro-CTA was chain extended with DIPEMA and GlyMA to form an insoluble block segment. Once the amphiphilic block copolymer chains grew to a certain length, they precipitated out and self-assembled to form nanoparticles, and the nanoparticles aggregated and finally were stabilized by the hydrophilic segment of the macro-CTA. The effect of DP of the hydrophobic block P(DIPEMA-co-GlyMA) on the particle morphology was evaluated.

A series of nanoparticles were prepared via changing DP of the P(DIPEMA-co-GlyMA) block from 40 to 100. Each sample was characterized by its morphology, hydrodynamic diameter and its distribution index (PDI). Figure 2 showed the TEM images of the produced nanoparticles, and Figure 3 showed the particle hydrodynamic diameter and its distribution index (PDI). When DP of P(DIPEMA-co-GlyMA) block was 40, spherical particles of 117 nm were formed, while vesicles could be obtained with DP of P(DIPEMA-co-GlyMA) block equal to or higher than 60. The DLS results of the particle hydrodynamic diameter in Figure 3 showed that the diameter of the particles increased from 117 nm to 593

nm when DP increased from 40 to 100, while the PDI in range of 0.1-0.25. As the increasing of the DP of P(DIPEMA-co-GlyMA) block, more hydrophobic monomer was added to the polymerization system, the degree of swollen of the polymer chains would be increased, which helped the chain mobility and chain reorganization, and also promoted particle fusion, and thus facilitated the vesicle formation.
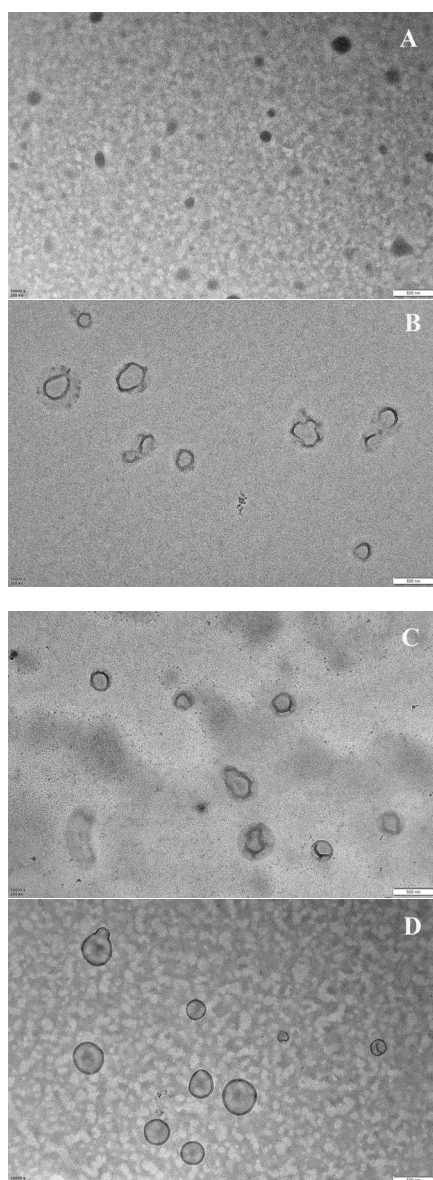

Figure 3. Hydrodynamic diameter and PDI of $mPEG_{45}$-b-P(DIPEMA-co-GlyMA) nanoparticles prepared under different DP of P(DIPEMA-co-GlyMA) block.

3.3 Effect of solid content on polymeric vesicles

The effect of solid content, which was one of the key factors that affected the morphology of the nanoparticles, was evaluated in this polymerization system. Figure 4 shows the TEM images of the nanoparticles produced with solid content changing from 5% to 20%, while Figure 5 illustrates the corresponding hydrodynamic diameter and PDI. It can be seen that pure vesicles could be fabricated when solid content increased to 10%, and the hydrodynamic diameter of the produced nanoparticles increased from 438 nm to 890 nm with solid content increased from 5% to 20%, while the PDI kept in range of 0.5-1.5. With a higher solid content, the number of nanoparticles generated in the nucleation stage would be larger, and the inelastic collision of the nanoparticles became more easily, and thus facilitated the formation of vesicles.

Figure 2. TEM images of $mPEG_{45}$-b-P(DIPEMA-co-GlyMA) nanoparticles prepared under different DP of P(DIPEMA-co-GlyMA) block, (A) DP=40, (B) DP=60, (C) DP=80 and (D) DP=100.
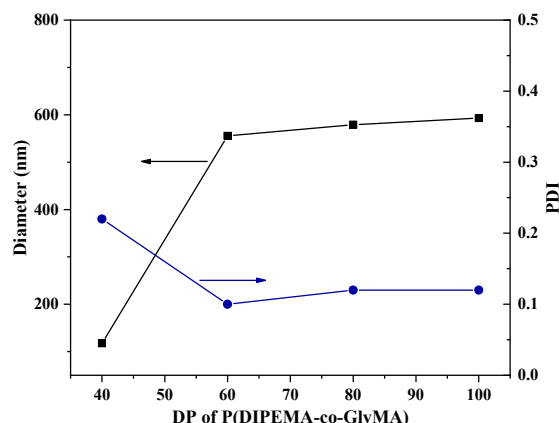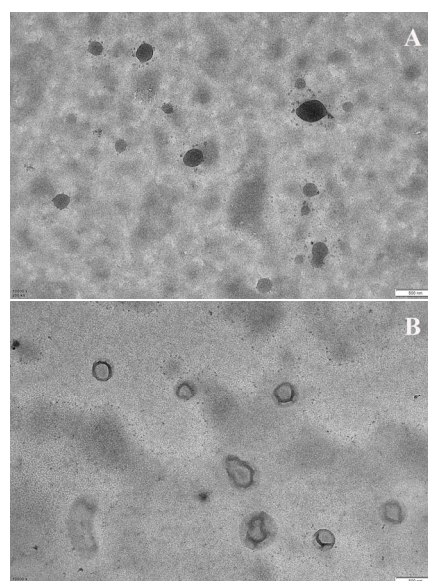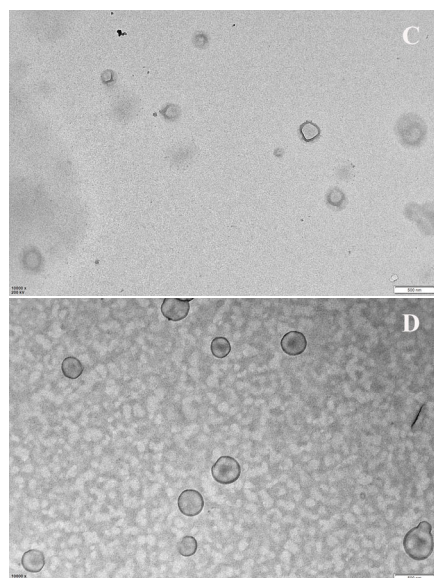
Figure 4. TEM images of mPEG$_{45}$-P(DIPEMA-co-GlyMA) nanoparticles prepared under different solid content, (A) 5%, (B) 10%, (C) 15% and (D) 20%.
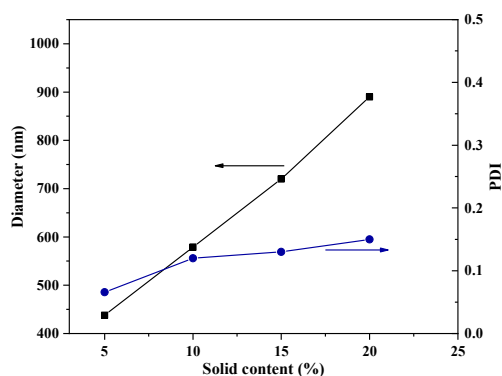

Figure 5. Hydrodynamic diameter and PDI of mPEG$_{45}$-b-P(DIPEMA-co-GlyMA) nanoparticles prepared under different solid content.

3.4 ^{1}H NMR and FTIR analysis of the polymeric vesicles

In current study, DIPEMA and GlyMA were used to copolymerize for the preparation of polymeric vesicles with the tertiary amine and epoxy groups via RAFT dispersion polymerization. The produced mPEG$_{45}$-P(DIPEMA-co-GlyMA) vesicles were isolated by freeze-drying. ^{1}H NMR spectroscopy and FTIR spectroscopy were employed to characterize the chemical structure of the produced block copolymers, as shown in Figure 6. From Figure 6A, the ^{1}H NMR spectra curve, the peak at δ 3.84 (d) attributed to -OCH$_2$- protons of PDIPEMA units, while the peaks at δ 3.2 (i), δ 2.63 and 2.82 (j) can be assigned to the protons of the epoxy group. And the absorption peak at 842 cm^{-1} in Figure 6B also indicated the existing of epoxy groups on the produced vesicles. The tertiary amine group on the PDIPEMA block could enable the vesicles with pH responsibility, and by using this epoxy functional groups on the vesicle membrane, the vesicles could be further functionalized or cross-linking of the vesicle membrane by reacting with other monomers, which would expand its potential application areas.
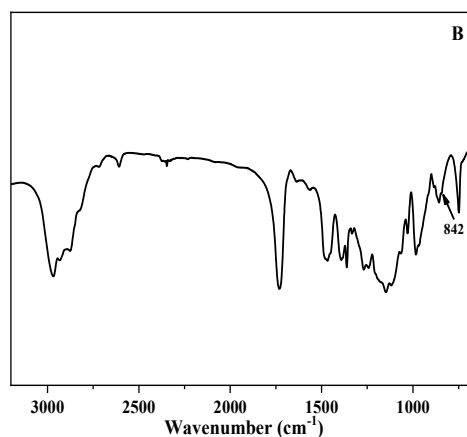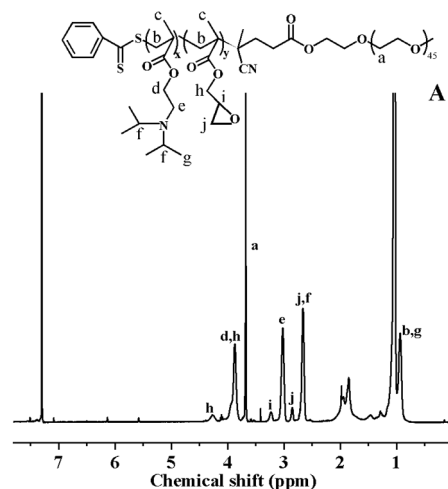

Figure 6. ^{1}H NMR (A) and FTIR (B) spectra of the mPEG$_{45}$-P(DIPEMA-co-GlyMA) vesicles

4. Conclusion

In summary, polymeric vesicles with tertiary amine and epoxy functional groups in range of 500-900 nm were fabricated by using mPEG$_{45}$-CPADB as the macro-CTA agent via the RAFT dispersion polymerization approach. DP of hydrophobic block P(DIPEMA-co-GlyMA) and the solid content both had significant effect on the morphology of the produced particles. Increasing DP of the hydrophobic block P(DIPEMA-co-GlyMA) to 60 or solid content to 10%, good shaped vesicles could be obtained, and the diameter of the vesicles increased accordingly based on the DLS results. ^{1}H NMR and FTIR analysis indicated the existing of tertiary amine and epoxy groups on the produced vesicles.

Acknowledgements

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