Synthesis of Fluorescent Methoxy Poly(ethylene glycol)-*b*-Poly(ethyl cyanoacrylate)–2-(*N*-carbazolyl) Ethyl Methacrylate Copolymer via Living Oxyanion-Initiated Polymerization

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ABSTRACT: The fluorescent amphiphilic block copolymer methoxy poly(ethylene glycol) (mPEG)-*b*-poly(ethyl cyanoacrylate) (PECA)–2-(*N*-carbazolyl) ethyl methacrylate (CzEMA) was synthesized via living oxyanion-initiated polymerization. mPEG-*b*-PECA–CzEMA was characterized by gel permeation chromatography, ^{1}H-NMR, and Fourier transform infrared spectroscopy. The results indicate that the polymerization was well controlled with a narrow molecular weight distribution. The mPEG-*b*-PECA–CzEMA nanoparticles prepared by nanoprecipitation techniques showed a narrow size distribution with an average diameter of less than 100 nm. The mPEG-*b*-PECA–CzEMA exhibited a strong carbazole fluorescence. Furthermore, it was found that the fluorescence intensity of mPEG-*b*-PECA–CzEMA was sensitive to a change in solvent. The results indicate that a subtle change in the state of the polymer micellar association may have altered the state of carbazole groups, which was responsible for the fluorescence emission.
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Key words: block copolymers; fluorescence; living polymerization; nanoparticle

INTRODUCTION

Live-cell optical imaging has provided a wealth of information regarding biological mechanisms and has dramatically transformed the biological sciences in the past few decades.[1–6] Fluorescent labeling dyes have been considered as a simple and useful tool for tracking drugs or polymers by their high sensitivity. There have been a number of studies based on dye-labeled drugs or proteins as tracers for the targeting and diagnosis of tumors in the biomedical fields.[7–10] Carbazol is a photosensitizer with promising applications in the bioimaging for its high quantum yield, and carbazol is also the most inexpensive of the fluorescent dyes. Thus, the ability to integrate the fluorescence and photosensitivity of carbazol for developing carbazolyl-substituted polymers is very encouraging. Poly[2-(*N*-carbazolyl) ethyl methacrylate] (PCzEMA) is well known as a precursor for photographical materials with valuable optical, photoconductive, and other useful applications, such as in organic light-emitting diodes.[11–13] PCzEMA has been synthesized from the corresponding methacrylate monomer by free-radical polymerization with 2,2′-azobisisobutyronitrile as the initiator.[14] However, it is difficult to control its molecular weight (MW) and architecture. The intrinsic wide polydispersity and the difficulty in purifying polymers are the critical problems that affect the performance of these polymers. It is desirable to establish precise synthetic methodologies to control the MW, polydispersity, and composition to manifest unique optical properties. Well-defined PCzEMA copolymers synthesized via reversible addition–fragmentation chain transfer[13] polymerization and atom transfer radical polymerization[15] have been reported. It should be noted that such living polymerizations as atom transfer radical polymerization involving metal catalysts and the presence of catalyst residues might impair the copolymers' performance.

Oxyanion-initiated polymerization, as an effective living polymerization, has unique advantages, such as its ability to produce well-defined functional polymers under less stringent conditions than conventional anionic polymerization and its easier purification method from raw products.[16–23] Therefore, this living process provides a new approach for the synthesis of block copolymers or macromonomers with controlled MWs and MW distributions. We synthesized a series of poly(ethyl cyanoacrylate) (PECA)-*b*-poly(ethylene glycol) (PEG)-*b*-PECA amphiphilic triblock copolymers via an oxyanion-

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initiated polymerization with sodium alcoholate terminated PEG as a macroinitiator, which could provide a delivery system for dexamethasone and other hydrophobic drugs.[24,25] The integrity of the block copolymer micelles is important for their effectiveness and successful delivery of the incorporated drugs.

In this study, we synthesized fluorescent methoxy poly(ethylene glycol) (mPEG)-*b*-PECA–2-(*N*-carbazolyl) ethyl methacrylate (CzEMA) block copolymer via an oxyanion-initiated polymerization. The polymer nanoparticles (NPs) were prepared by a nanoprecipitation technique. The fluorescence properties of mPEG-*b*-PECA–CzEMA NPs were also investigated and are discussed.

EXPERIMENTAL

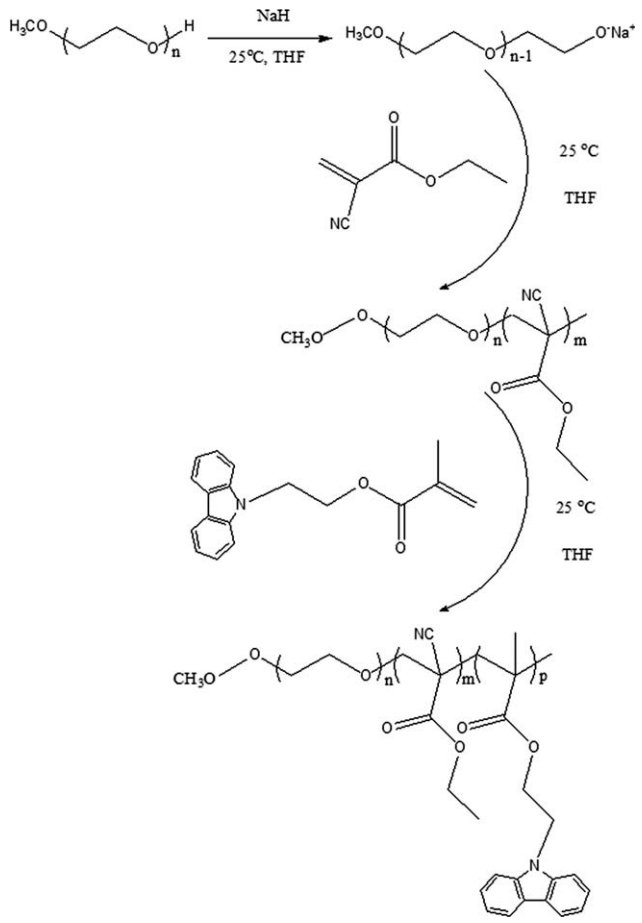
Materials

Ethyl cyanoacrylate (ECA) was purchased from Beijing East Chemical Reagent Co. (Beijing, China). mPEG [number-average molecular weight (M_n) = 5 kDa] and NaH were supplied by Sigma-Aldrich (St Louis, MO, USA) Anhydrous ethyl ether and tetrahydrofuran (THF) were purchased from Tianjin Kewei Chemical Reagent Co. (Tianjin, China). CzEMA was synthesized as reported elsewhere.[13] THF was dried with sodium wire for 3 days and subsequently refluxed in the presence of potassium. It was distilled under nitrogen just before use. All of the other chemicals were analytical grade.

Synthesis and characterization of mPEG-*b*-PECA–CzEMA

mPEG-*b*-PECA–CzEMA was synthesized according to Lin et al.[24] (Scheme 1). Briefly, anhydrous THF and dry NaH powder were stirred at room temperature, and then, mPEG in an amount equivalent to the molar amount of NaH was subsequently added to the flask. The reaction mixture was stirred at 25°C for 1 h to yield sodium alcoholate initiators. The required amount of ECA monomer was added to the reactor. After the reaction proceeded at 25°C for 2 h, CzEMA was added and stirred at 25°C for 2 h, and then, the reaction was quenched with methanol. The obtained copolymer solution was precipitated in 150 mL of anhydrous ethyl ether (precooled to 0°C) and filtered. After it was washed three times, the cake was finally dried in a vacuum oven at 40°C for approximately 48 h.

Fourier transform infrared (FTIR) spectroscopy (Bio-Rad FT3000, Hercules, CA, USA) was used to confirm the structure of mPEG-*b*-PECA–CzEMA. The samples were pressed into KBr pellets (1 : 100 w/w), and the results were analyzed by Infrared Data Management Software (Hercules, CA, USA).



Scheme 1 Reaction scheme for the synthesis of mPEG-*b*-PECA–CzEMA.

^{1}H-NMR spectra were recorded on a Varian INOVA 500-MHZ nuclear magnetic resonance instrument (Palo Alto, CA, USA) with $CDCl_3$ as a solvent and tetramethylsilane as an internal standard.

Gel permeation chromatography (GPC) analyses were conducted with an Agilent (Palo Alto, CA, USA) with standard polystyrene samples as the MW references and THF as the eluent at a flow rate of 1.0 mL/min.

Preparation and characterization of the mPEG-*b*-PECA–CzEMA NPs

The mPEG-*b*-PECA–CzEMA NPs were prepared by nanoprecipitation techniques.[24] Briefly, 100 mg of mPEG-*b*-PECA–CzEMA was dissolved in 3 mL of acetone. This solution was added dropwise to 10 mL of deionized water under magnetic stirring at 500 rpm. The NPs were formed immediately, and acetone was removed through 3 h of evaporation at room temperature. The resulting dispersion was centrifuged for 30 min at 4500 rpm to eliminate the aggregated particles. The supernatant fluid was freeze-dried for further use.

The size and size distribution of the mPEG-*b*-PECA–CzEMA NPs were measured by a laser

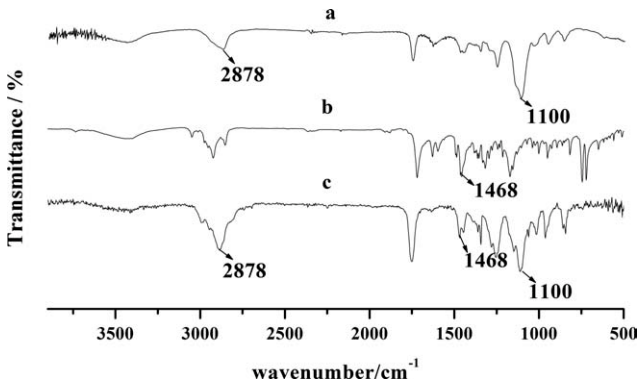


Figure 1 FTIR spectra of (a) mPEG-*b*-PECA, (b) CzEMA, and (c) mPEG-*b*-PECA–CzEMA.

particle size analyzer (Brookhaven Instruments Corp. 90 Plus particle sizer, Holtsville, NY, USA) at 25°C and a scattering angle of 90°.

The measurements of fluorescence were performed on a Varian Cary Eclipse fluorometer with an excitation wavelength of 335 nm. Both excitation and emission bands were set at 3.5 nm in these experimental conditions. All of the experiments were carried out at 25°C.

RESULTS AND DISCUSSION

Synthesis and characterization of mPEG-*b*-PECA–CzEMA

A fluorescent copolymer, mPEG-*b*-PECA–CzEMA, was prepared by sequential monomer addition with a sodium alcoholate terminated mPEG as aninitiator (see Scheme 1). mPEG was first reacted with NaH in THF; this generated a sodium alcoholate. ECA was added to the reactor with stirring at 25°C for 2 h, and then, the second monomer was injected into the flask. The reactant was stirred continuously for 2 h before the reaction was quenched.

Figure 1 presents the FTIR spectra of mPEG-*b*-PECA, CzEMA, and mPEG-*b*-PECA–CzEMA. As shown in Figure 1(c), a new and strong carbazol band at 1468 cm^{-1} was attributed to the CzEMA residues. A strong C—O—C stretching band at 1100 cm^{-1} was attributed to the mPEG segments. The overlapping of the aliphatic CH stretching band of mPEG and that of ECA at 2878 cm^{-1} was also observed. As a result, we believe that mPEG-*b*-PECA–CzEMA containing mPEG-*b*-PECA and CzEMA segments was successfully obtained.

In this study, ^{1}H-NMR spectroscopy was used to determine the relative composition of the mPEG–PECA and CzEMA segments. The peaks assigned to protons of the mPEG–PECA and CzEMA units can be clearly seen in Figure 2. The H_{f1} and H_{f2} protons of mPEG-*b*-PECA–CzEMA at 2.0 ppm were shifted compared to those of CzEMA at 5.51 and 6.13 ppm, respectively. The characteristic peaks of CzEMA could be found in the mPEG-*b*-PECA–CzEMA spectrum. A comparison of the peak integrals obtained for the H_a signals of mPEG block with the H_d associated with the PECA block and H_4 and H_5 associated with CzEMA allowed M_n to be calculated. ^{1}H-NMR measurement confirmed that for mPEG-*b*-PECA–CzEMA, the MW of the copolymer was very close to the calculated values, and the results are shown in Table I; these suggest a high initiator efficiency for the CzEMA monomer.

As shown in Figure 3, a single and sharp peak appeared; this suggested that all of the mPEG chains reacted with the monomer, and the copolymer had a

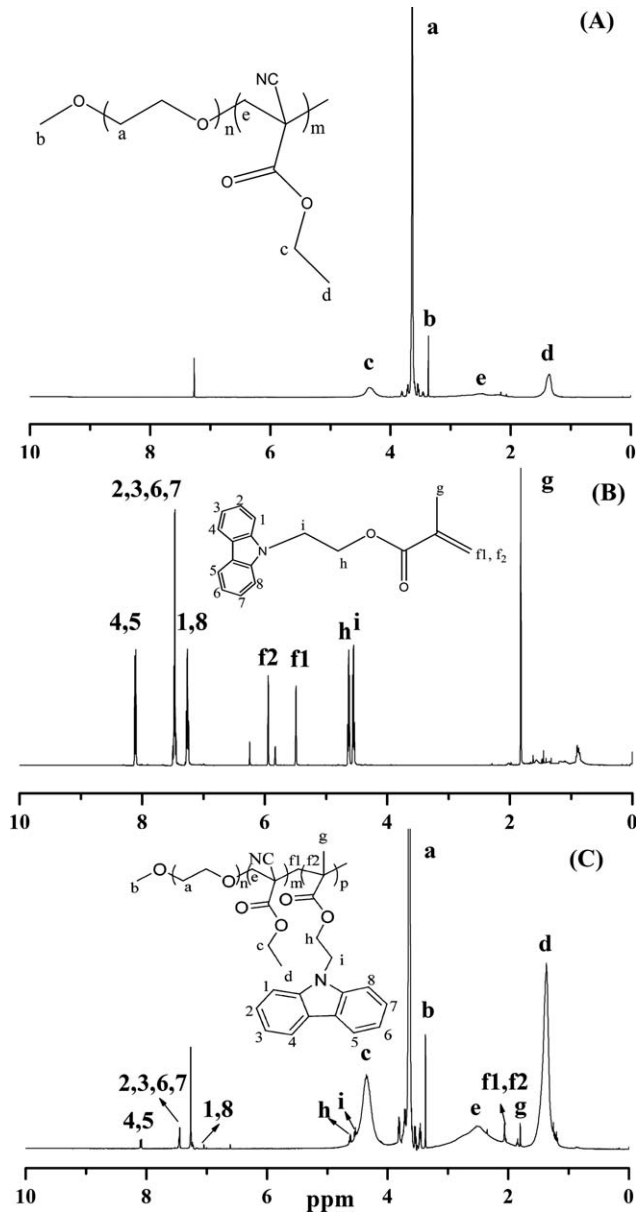


Figure 2 ^{1}H-NMR spectra of (A) mPEG-*b*-PECA, (B) CzEMA, and (C) mPEG-*b*-PECA–CzEMA.

TABLE I
mPEG-*b*-PECA–CzEMA Synthesized via Oxyanion-Initiated Polymerization

Sample	M_n (g/mol)			M_w/M_n[c]
	M_n[a]	M_n[b]	M_n[c]	
mPEG-*b*-PECA–CzEMA	10,100	9800	9700	1.33

[a] Calculated theoretically.
[b] Calculated according to the results of ^{1}H-NMR.
[c] Obtained by GPC.

single MW distribution. The weight-average molecular weight (M_w)/M_n value of the polymers was 1.33; this suggested that the mPEG-*b*-PECA–CzEMA was an almost narrow-distribution polymer.

Evaluation of the mPEG-*b*-PECA–CzEMA NPs

The NPs were prepared via a nanoprecipitation technique. As shown in Figure 4, the NPs displayed two peaks with sizes of less than 100 nm. The small size of the micelles not only would allow them to escape from the renal exclusion and reticuloendothelial systems but also would enable them to have a higher vascular permeability at the tumor target site by passive diffusion.

Fluorescence spectra of mPEG-*b*-PECA–CzEMA

Figure 5 shows the fluorescence spectra of carbazol and mPEG-*b*-PECA–CzEMA NPs in water. Compared with that of carbazol, the fluorescence spectra of the mPEG-*b*-PECA–CzEMA NPs exhibited a red-shift of the emission maximum from 359 to 365 nm. The intensity of emission at 600 nm of carbazol was quite weak. However, it increased in the mPEG-*b*-PECA–CzEMA NPs. According to the theory of Zhao et al.,[13] the amphiphilic mPEG-*b*-PECA–CzEMA NPs were micellized in water, which confined the carbazole groups into the core region of micellar aggregates; the neighboring carbazole groups in the side chain of mPEG-*b*-PECA–CzEMA had a strong tendency to induce electronic interaction, such as in *p*-stacked structures, when they were close enough.

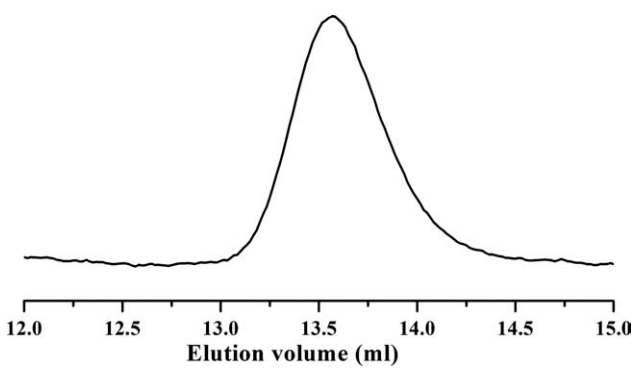


Figure 3 GPC chart of mPEG-*b*-PECA–CzEMA.

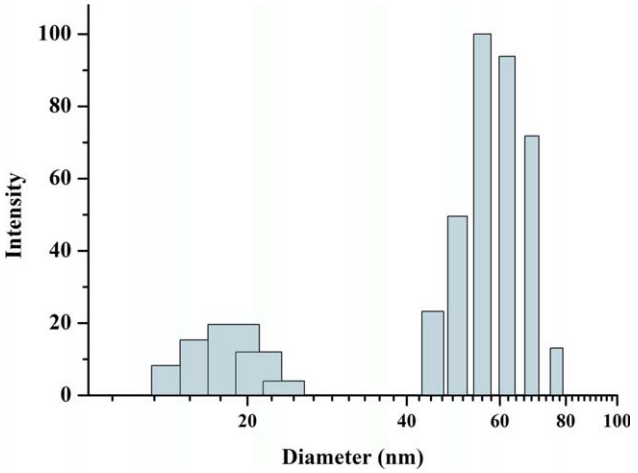


Figure 4 Size distribution of mPEG-*b*-PECA–CzEMA NPs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 6 compares the fluorescence spectra of mPEG-*b*-PECA–CzEMA, carbazol, and CzEMA in acetone. The conjugated effect of double bonds and carbazol led to a fluorescence structural self-quenching effect. As a result, the intensity of CzEMA must have decreased. In the fluorescence spectra of mPEG-*b*-PECA–CzEMA, a strong carbazole fluorescence was obtained; this implied that the desired composition was achieved because mPEG-*b*-PECA–CzEMA had no self-quenching sites in the polymer chain after CzEMA coupled to mPEG-*b*-PECA. In addition, the emission maximum of mPEG-*b*-PECA–CzEMA was almost 1.5 times stronger than that of carbazol.

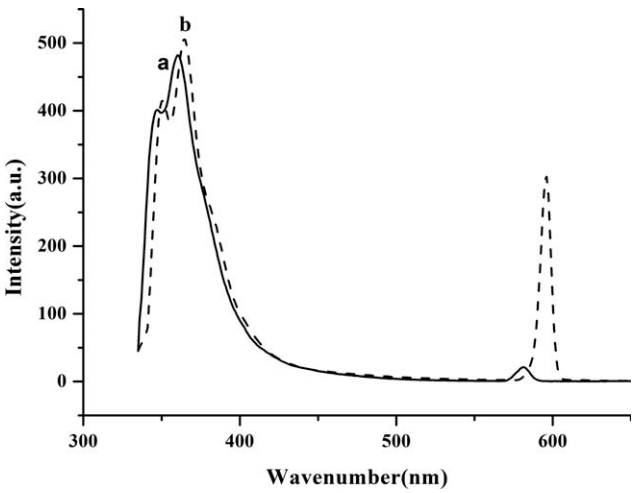


Figure 5 Fluorescence emission spectra of (a) carbazol and (b) mPEG-*b*-PECA–CzEMA NPs in water. The excitation wavelength was 335 nm, and [CZ] residue was 6×10^{-6} mol/L.

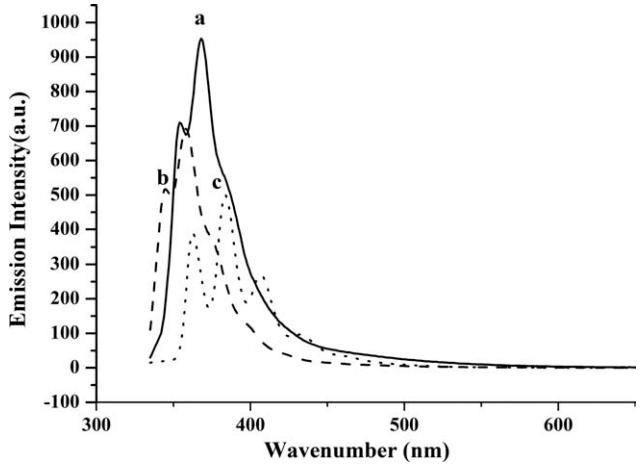


Figure 6 Excitation and emission spectra of (a) mPEG-*b*-PECA–CzEMA, (b) carbazol, and (c) CzEMA in acetone. The excitation wavelength was 335 nm, and Carbazolyl residue was 6×10^{-6} mol/L.

In a comparison of Figure 6 with Figure 5, one can see that mPEG-*b*-PECA–CzEMA also exhibited stronger fluorescence in the acetone solution than in water. The intensity of emission at 600 nm of mPEG-*b*-PECA–CzEMA disappeared in acetone. The amphiphilic copolymer could self-assemble into core/shell NPs in water, composed of an inner core of PECA segments and CzEMA and an outer shell of PEG segments, which was coiled tightly; the chromophores were hidden in the core and showed a fluorescence of lower intensity. Acetone solutions with NPs coils expanded, and the fluorescence-exposed solutions showed intensive fluorescence. This result suggests that a change in the packing of carbazol groups may have occurred and drastically altered the fluorescence association state of the chromophore.

CONCLUSIONS

A well-defined mPEG-*b*-PECA–CzEMA with a narrow MW distribution was synthesized via an oxyanion-initiated polymerization. The mPEG-*b*-PECA–CzEMA NPs were prepared by a nanoprecipitation technique, with an average size of less than 100 nm. The mPEG-*b*-PECA–CzEMA exhibited a strong carbazole fluorescence. The micellization behavior of amphiphilic mPEG-*b*-PECA–CzEMA NPs in the water enhanced the emission intensity at 600 nm compared to carbazol. The mPEG-*b*-PECA–CzEMA NPs exhibited an intensive fluorescence in acetone solution and a decreased intensity in water as a result of a change in the packing of carbazol groups. mPEG-*b*-PECA–CzEMA provided a fluorochromer, which may have a range of biotechnology applications.

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