

Amphiphilic Copolymer with Pendant Pyrenebutyryl Hydrazide Group: Synthesis, Characterization, and Recognition for Carbonate Anion

Zhaoqiang Wu, Lingzhi Meng, Cheng Li, Xiaoju Lu, Lifan Zhang, Yongbing He

Department of Chemistry, Wuhan University, Wuhan 430072, People's Republic of China

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ABSTRACT: Novel hydrophobic monomer *N*-(1-pyrenebutyryl)-*N'*-methacryloyl hydrazide (PyMAHy) was synthesized and copolymerized with *N*-vinylpyrrolidone (NVP) to get a series of amphiphilic copolymers. FTIR, ¹H NMR, TEM, gel permeation chromatography–multi-angle laser light scattering, UV–vis spectroscopy, and fluorescence spectroscopy were used to characterize these polymers. Results of fluorescence measurements show that these copolymers have good recognition ability to CO₃²⁻ anion and the fluorescence emission intensity was quenched linearly upon

the addition of CO₃²⁻ anion. The recognition for CO₃²⁻ anion was also proved by the results of TEM for the significantly difference in the morphology of copolymer before and after adding CO₃²⁻ anion into copolymer Tris–HCl buffer solution. Results suggest the potential of these amphiphilic copolymers as fluorogenic chemosensor materials. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 101: 2371–2376, 2006

Key words: amphiphilic copolymer; poly(*N*-vinylpyrrolidone); fluorescence; CO₃²⁻ anion

INTRODUCTION

During the past decade, research on polymers combined with fluorophore has attracted increasing interests because of the following two main reasons. First, photochemical processes can be greatly modulated owing to microenvironmental effects. Second, the chromophores can act as “reporters” to provide useful information on the conformation and dynamic properties of the polymers.¹ Fluorophore-labeled copolymers had been used to study the interactions with liposomes,² latex film formation,³ aggregation,^{4,5} electron transfer phenomena,^{6,7} and curing kinetics.⁸

Anions play a fundamental role in a wide range of chemical and biological processes.⁹ Among these anions, carbonate is a physiologically essential polyanion.¹⁰ Furthermore, carbonate is the major constituent of several natural fluids, and the CO₃²⁻/CO₂/HCO₃⁻ equilibrium is very important because it regulates the pH of seawater and controls the circulation of CO₂ around the biosphere, the lithosphere, the atmosphere, and the oceans.¹¹ Hutzler reported that cytochrome enzymes *O*-dealkylase activity appeared to be attenuated in the presence of carbonate.¹² There were some reports about recognition for CO₃²⁻ anions based on, such as, macromonocyclic polyamines and others.^{10,13} To our best

knowledge, the concerning amphiphilic copolymer recognition for CO₃²⁻ anions has rarely been reported.

Here, we report the synthesis of amphiphilic fluorescent copolymers by free-radical copolymerizations of hydrophilic monomer NVP and hydrophobic monomer *N*-(1-pyrenebutyryl)-*N'*-methacryloyl hydrazide (PyMAHy). In these copolymers, poly(*N*-vinylpyrrolidone) (PVP) was selected as the hydrophilic segment because PVP has been proven to be biocompatible.^{14,15} And PyMAHy was chosen as hydrophobic segments in amphiphilic fluorescent copolymers for the following advantages: carbonylhydrazide groups in PyMAHy act as recognition units¹⁶ and pyrene group in PyMAHy has a long singlet lifetime, readily forms excimers, acts as an energy acceptor via nonradiative energy transfer from several dyes, and the vibronic band structure of its emission is sensitive to the environment.¹⁷

In this study, the recognition ability of amphiphilic copolymers to CO₃²⁻ anion was experimentally quantified via fluorescence titration in Tris–HCl buffers. TEM was also used to study the recognition through the morphological change of copolymer before and after adding CO₃²⁻ anions in copolymer Tris–HCl buffer solution. Results show that these copolymers have good recognition ability to CO₃²⁻ anion.

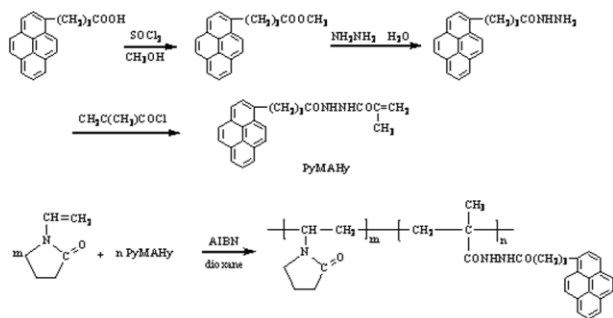
Correspondence to: L. Z. Meng (lzhmeng@whu.edu.cn).

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EXPERIMENTAL

Materials

NVP was obtained from Acros, 1-pyrenebutyl acid was purchased from Aldrich, Tris(hydroxymethyl)



Scheme 1 Synthesis of PyMAHy and copolymer.

aminomethane, 85% hydrazine hydrate, *N,N*-dimethylformamide (DMF), dioxane, and thionyl chloride were purchased from Shanghai Chemical Reagents.

NVP was purified by distillation under reduced pressure to remove the inhibitors before use. Azobis(isobutyronitrile) (AIBN) was recrystallized from methanol. Thionyl chloride was purified by distillation before use. Methacryloyl chloride was synthesized in our laboratory. Methanol was dried and distilled before use, according standard practice. Tris-HCl buffers (pH = 7.4) were prepared by mixing 0.05 mol/L of aqueous tris(hydroxymethyl)aminomethane solution with 1.0 mol/L HCl to the required pH value.

Synthesis of hydrophobic monomer *N*-(1-pyrenebutyryl)-*N'*-methacryloyl hydrazide (PyMAHy)

The synthesis of 1-pyrenebutyric acid hydrazide was prompted by earlier studies in our laboratory.¹⁶ The synthesis outline of hydrophobic monomer PyMAHy is as shown in Scheme 1. In brief, to a solution of 1-pyrenebutyric acid hydrazide (0.7 g, 2.32 mmol), triethylamine (0.5 mL, 3.75 mmol) in a mixture of dry toluene (30 mL) and DMF (0.5 mL) kept at 0 °C under nitrogen atmosphere, methacryloyl chloride (0.3 mL, 3 mmol) was added slowly with a syringe to the stirred reaction mixture. After being stirred overnight at room temperature, the resulting mixture was filtered to remove $\text{NEt}_3\cdot\text{HCl}$. The filtrate was washed in turn with 0.1N HCl, brine, saturated $\text{H}_2\text{O}/\text{NaHCO}_3$, H_2O , and dried with MgSO_4 . The solvent was removed under reduced pressure. The crude products were purified by chromatography (silica gel, eluted with CHCl_3) to give PyMAHy as yellow crystals, yield: 58%.

^1H NMR (CDCl_3 , δ ppm): 1.89 (s, 3H, CH_3), 2.19 (t, 2H, $\text{CH}_2\text{CH}_2\text{CO}$), 2.34 (m, 2H, $\text{CH}_2\text{CH}_2\text{CO}$), 3.31 (t, 2H, PyCH_2), 5.35 (d, 1H, $\text{CH}_2=$), 5.76 (d, 1H, $\text{CH}_2=$), 7.8–8.2 (m, 9H, PyH), 8.42 and 8.51 (each b, 1H, NHNH). IR (KBr pellet, cm^{-1}): 3334.0 (w), 3219.4 (s), 3035.5 (m), 2956.8 (w), 2928.7 (w), 1695.7 (s), 1648.8 (s), 1429.3 (w) and 839.1 (s). FAB-MS m/z (RI): 371 (48, M

+ 1), 271 (63, $M - 99$, $\text{Py}(\text{CH}_2)_3\text{CO}^+$), 215 (52, $M - 155$, PyCH_2^+). Elemental Anal Calcd for $\text{C}_{24}\text{H}_{22}\text{N}_2\text{O}_2$ (%): C 77.83, H 5.94, N 7.56. Found (%): C 77.78, H 5.97, N 7.90.

Synthesis of amphiphilic copolymers

The amphiphilic fluorescent copolymers were prepared by radical polymerization using AIBN as initiator. The general synthetic outline for polymerization is shown in Scheme 1. The polymerization procedure is as follows: NVP (3.6 g, 32 mmol), AIBN (28 mg, 0.17 mmol), and different amounts of hydrophobic monomer PyMAHy were dissolved in 8 mL dry dioxane. The solution was degassed by bubbling with nitrogen for 20 min. The reaction mixture was stirred and heated to 60 °C for 24 h and then cooled to room temperature. The products were recovered by precipitation in fivefold diethyl ether. Purified by repeated reprecipitation from dioxane into diethyl ether three times, and dried in vacuum until a constant weight; yield: 53%.

^1H NMR (CDCl_3 , δ ppm): 1.18 (b, CCH_3); 1.61, 2.20, and 3.18 (b, CH_2 of PVP and $\text{Py}(\text{CH}_2)_3\text{CO}$); 1.96 and 3.64 (b, CH_2 and CH of main chain); 7.8–8.4 (b, PyH). IR (KBr pellet, cm^{-1}): 3434.1 (w), 2958.9 (m), 1653.2 (s), 1462.8 (m), 1423.3 (m), 1289.5 (s), 843.9 (m).

Characterization

Steady-state fluorescence spectra were obtained on a Shimadzu RF-5301PC spectrometer (Japan). The infrared spectra were performed on a Nicolet 670 FTIR spectro-photometer (USA). The mass spectra were recorded on a ZAB-HF-3F spectrometer (England). Elemental analysis was determined by a Flash 1112 series elemental autoanalyzer (Italy). The size and morphology of copolymer aggregation were recorded by a JEM-100CXII transmission electron microscope (Japan) operated at 80 kV. TEM specimens were prepared by aspirating a drop of sample aqueous solution onto a formvar-membrane-coated Cu grid and self-dried in air at room temperature. ^1H NMR spectra were recorded on a Varian Mercury VX-300 MHz spectrometer (USA). UV-vis spectra were taken on a TU-1901 spectrometer (China), using DMF as solvent.

Gel permeation chromatography–multi-angle laser light scattering (GPC–MALLS) analysis was carried out on DAWN[®]DSP multi-angle laser photometer with a pump P100 (Thermo Separation Products, San Jose, USA) equipped with TSK-GEL G6000 PWXL with a G4000 PWXL column (7.8 mm \times 300 mm) for aqueous solutions, and differential refractive index detector (RI-150) at 25 °C.

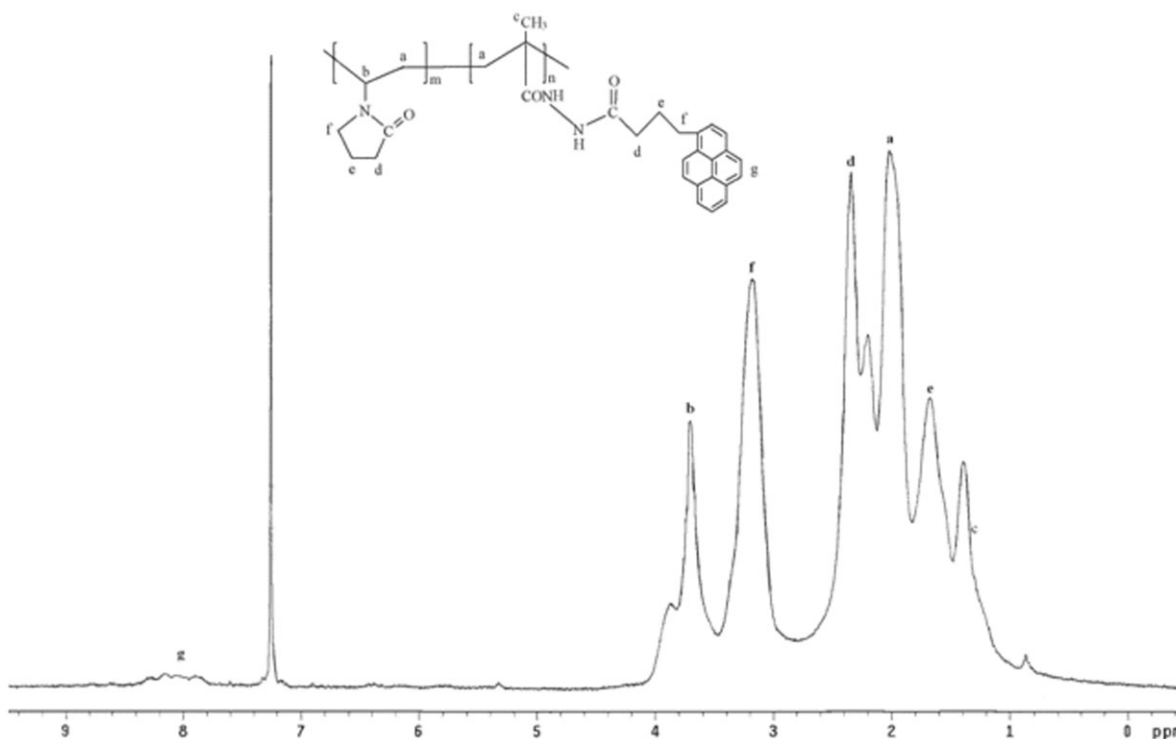


Figure 1 ^1H NMR spectrum of **P2** in CDCl_3 .

RESULTS AND DISCUSSION

Polymer synthesis and characterization

A series of copolymers **P1**, **P2**, and **P3** were prepared by the free-radical copolymerizations with various comonomer mole ratios of PyMAHy to NVP in dioxane. These copolymers are amphiphilic and all easily dissolved in water and organic agents such as CHCl_3 and DMF. FTIR and ^1H NMR were used to characterize these polymers. The IR spectra verified the existence of the out-of-plane vibration at 843 cm^{-1} of pyrene groups in **P1**, **P2**, and **P3**. The peak at 1653 cm^{-1} is ascribed to the stretching vibration of $\text{C}=\text{O}$ on PVP ring and PyMAHy. The ^1H NMR spectra of **P1**, **P2**, and **P3** in CDCl_3 are quite similar. The ^1H NMR spectrum of **P2** in CDCl_3 is shown in Figure 1. Signals between 1.1 and 3.8 ppm are assigned to all the protons of the CH_3 , CH_2 , and CH groups. The proton signals of pyrene group in polymers appear at 7.8–8.4 ppm. The results of FTIR and ^1H NMR showed that PyMAHy had successfully copolymerized with NVP.

The exact PyMAHy content χ of the copolymer was determined by UV–vis spectroscopy at 344 nm, using a standard calibration curve experimentally obtained with 1-pyrenebutyl acid/DMF solutions ($\epsilon = 44,680\text{ L mol}^{-1}\cdot\text{cm}^{-1}$). The PyMAHy content depended on the comonomer molar ratios and decreased with decreasing the comonomer molar ratios added to the system. The molecular weights of the polymers were determined by GPC–MALLS. The compositions and molec-

ular weights of these polymers are summarized in Table I.

Figure 2 shows the fluorescent emission spectra of **P1**, **P2**, and **P3** aqueous solution at the same concentration of 0.25 mg/mL. Each polymer displays characteristic hydrophobic monomer emission peak at 377 and 396 nm and excimer peak centered at 475 nm via direct excitation of ground-state pyrene aggregates of polymers in aqueous solution.^{18,19} The fluorescent emission intensity of **P1**, **P2**, and **P3** decreased with the decreasing of PyMAHy content, which resulted from the low concentration of pyrene groups in copolymer at the same concentration. These properties meet the request of fluorescence detecting and can be used to study copolymer self-aggregation behavior, which will be discussed later.

TABLE I
Results of Copolymerization and Characterization of Copolymers

Polymer	PyMAHy/ NVP ^a	χ^b ($\mu\text{mol/g}$)	M_w^c (10^4 g/mol)	M_w/M_n^c
P1	0.006	31.4	6.75	1.93
P2	0.003	18.3	6.72	1.71
P3	0.0015	8.6	6.99	2.23

^aMolar ratio of PyMAHy to NVP in the feed.

^bFrom UV–vis absorption.

^cFrom GPC–MALLS analysis.

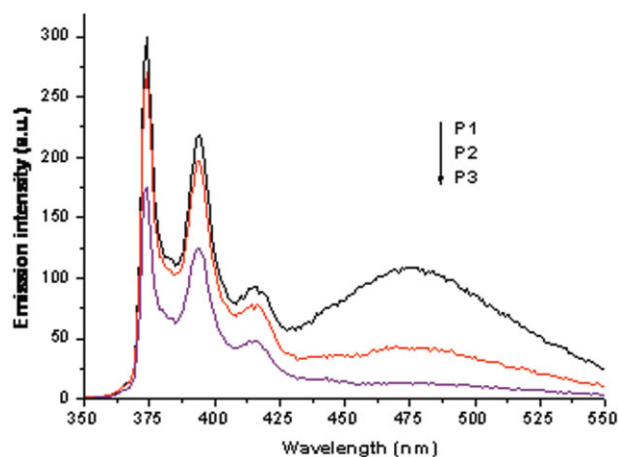


Figure 2 Fluorescent emission spectra of **P1**, **P2**, and **P3** aqueous solutions with the same concentration of 0.25 mg/mL at 25 °C. The emission and excitation slit widths were 1.5 and 3.0 nm, respectively. $\lambda_{\text{ex}} = 342$ nm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

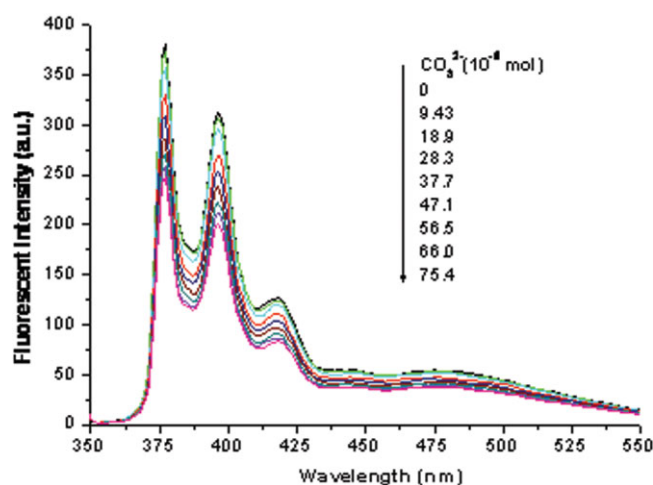


Figure 3 Fluorescence emission spectra of **P1** (0.03 mg/mL) upon addition of CO_3^{2-} anion (3.14 mol/L) in Tris-HCl buffers at 25 °C. The emission and excitation slit widths were 5.0 and 3.0 nm, respectively. $\lambda_{\text{ex}} = 342$ nm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Amphiphilic copolymer recognition for anions

The ability of polymers for recognizing anions was experimentally quantified via fluorescence titration in Tris-HCl buffers (pH = 7.4). Eight kinds of anions such as F^- , Br^- , CH_3COO^- , CO_3^{2-} , H_2PO_4^- , HPO_4^{2-} , PO_4^{3-} , and NO_3^- were added into the Tris-HCl buffer solution of **P1**, **P2**, and **P3**, respectively. All the tests run at 25 °C whether the anions were added or not. Results showed that on the addition of seven kinds of anions, F^- , Br^- , CH_3COO^- , H_2PO_4^- , HPO_4^{2-} , PO_4^{3-} , and NO_3^- , there were no influence on the fluorescence intensity of **P1**, **P2**, and **P3**. To the kind of CO_3^{2-} anion, the fluorescence intensity of **P1**, **P2**, and **P3** decreased upon CO_3^{2-} anion addition. The result showed that **P1**, **P2**, and **P3** have good recognition ability to CO_3^{2-} anion under the experimental condition. The typical fluorescence-quenching curve of **P1** (0.03 mg/mL) upon addition of CO_3^{2-} anion is shown in Figure 3, and the changes of the half-sum of the monomer emission intensity at 377 ($I_{\text{M}377}$) and 396 nm ($I_{\text{M}396}$) dependence on CO_3^{2-} anion concentration is given in Figure 4. It showed that the changes decreased near linearly upon addition of CO_3^{2-} anion. These amphiphilic copolymers with linear emission intensity quenching upon the addition of CO_3^{2-} anion can be used as promising fluorogenic chemosensor materials.

Self-aggregation of amphiphilic copolymer in the presence of CO_3^{2-} anion

The self-aggregation of amphiphilic copolymer in the presence of CO_3^{2-} anions was studied by fluorescence technology and TEM. The emission of pyrene exhibits

a marked medium sensitivity in its vibrational fine structure and the peak height ratio $I_{\text{E}}/I_{\text{M}}$ between excimer (I_{E}) and monomer fluorescence bands (I_{M}) can be used as a measure of the efficiency of the excimer formation.¹⁷ From the typical fluorescence-quenching curve of **P1** (0.03 mg/mL) upon addition of CO_3^{2-} anion (Fig. 3), the $I_{\text{E}}/I_{\text{M}}$ ratios were calculated by taking the ratio of the emission intensity at 475 nm to the half-sum of the emission intensities at 377 and 396 nm. The change of $I_{\text{E}}/I_{\text{M}}$ ratios was observed as a function of CO_3^{2-} anion amount (Fig. 5). Figure 5 showed that the $I_{\text{E}}/I_{\text{M}}$ ratios increased near linearly upon addition of CO_3^{2-} anion, which implied that

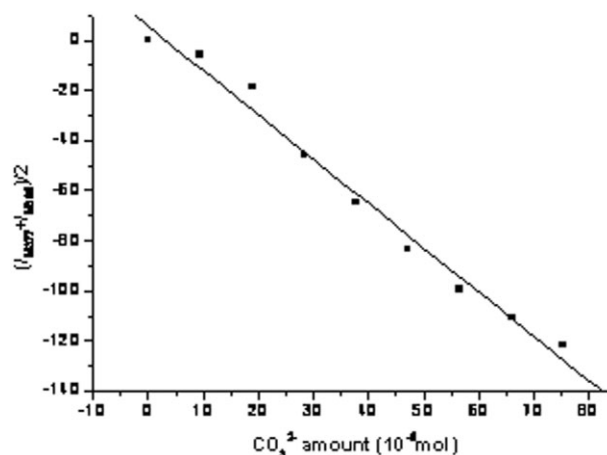


Figure 4 The $(I_{\text{M}377} + I_{\text{M}396})/2$ changes of **P1** (0.03 mg/mL) upon addition of CO_3^{2-} anion (3.14 mol/L) in Tris-HCl buffers at 25 °C. The emission and excitation slit widths were 5.0 and 3.0 nm, respectively. $\lambda_{\text{ex}} = 342$ nm.

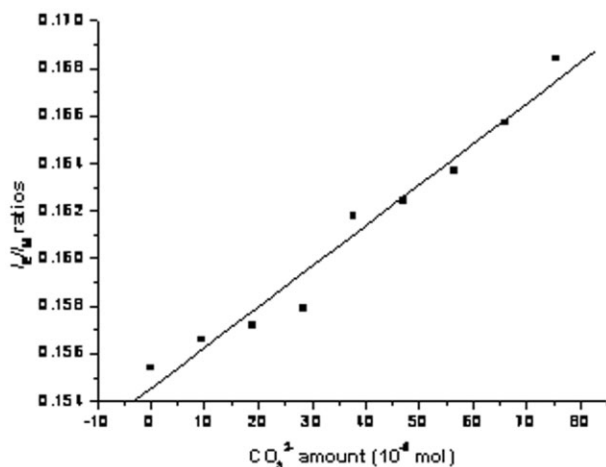


Figure 5 The I_E/I_M ratios of **P1** (0.03 mg/mL) upon addition of CO_3^{2-} anion (3.14 mol/L) in Tris-HCl buffers at 25 °C. The emission and excitation slit widths were 5.0 and 3.0 nm, respectively. $\lambda_{\text{ex}} = 342$ nm.

there were a little more amount of pyrene groups of **P1** in close spatial proximity.² In other words, bigger aggregates are formed in the presence of CO_3^{2-} anions.

The aggregate morphology of **P1** before and after adding CO_3^{2-} anion in Tris-HCl buffer solution was also studied using TEM (shown in Fig. 6). The **P1** stock solution was prepared by dissolving 5 mg of **P1** in 2.5 mL Tris-HCl buffers to give a concentration of 2 mg/mL. A drop of stock solution was aspirated onto a formvar-membrane-coated Cu grid and self-dried in air at room temperature to take a TEM photograph [Fig. 6(A)]. Then, 4 mg Na_2CO_3 were added into the **P1** solution, stirred, and filtered. Then a drop of filtrate was taken out to take another TEM photograph [Fig. 6(B)].

Nearly spherical particles were observed in the absence of CO_3^{2-} anion [as shown in Fig. 6(A)] because of the aggregations of hydrophobic PyMAHy moieties.¹⁹ In the presence of CO_3^{2-} anion, bigger snowflake-like aggregates were observed [as shown in Fig. 6(B)]. It is consistent with the results of fluorescent measurement where bigger aggregates of copolymers were formed in the presence of CO_3^{2-} anion. These factors contribute to the bigger aggregates may be due to hydrophobic-hydrophobic associations of hydrophobic PyMAHy moieties and the hydrogen-bonding power between carbonyl hydrazide groups and CO_3^{2-} anions.

Mechanistic consideration for aggregates formation

The hydrophobic moiety is a bulky group and layer-by-layer packing mechanism²⁰ might be favored for the hydrophobic-hydrophobic interactions of the groups. The layer-by-layer aggregation of the polymer

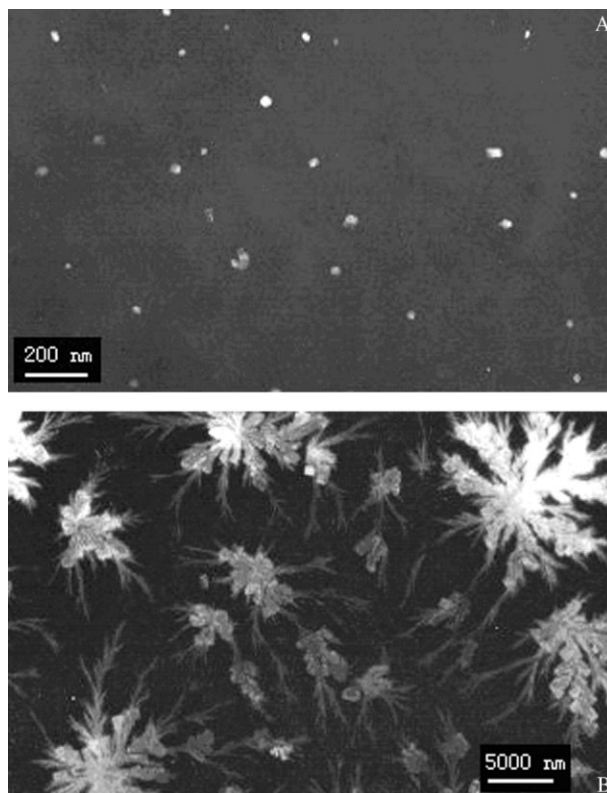


Figure 6 TEM photographs of **P1** (2 mg/mL) before (A) and after (B) adding CO_3^{2-} anion (4 mg) in Tris-HCl buffer solution.

would produce aggregates with “open” hydrophobic faces, which were not well protected by the hydrophilic outer layers. The hydrophobic-hydrophobic associations of these “open hydrophobic faces” served as the driving forces for the further packing to form larger aggregates.²¹ The bulky hydrophobic group PyMAHy in amphiphilic copolymers promoted the formation of the nearly spherical aggregation through layer-by-layer packing mechanism. In the presence of CO_3^{2-} anion, the hydrogen-bonding power between carbonyl hydrazide groups and CO_3^{2-} anions changed the original morphology of copolymer aggregation and through layer-by-layer packing formed snowflake-like aggregates. A schematic presentation of such a process is shown in Figure 7.

CONCLUSIONS

A series of new amphiphilic fluorescent copolymers were synthesized by free-radical copolymerizations.



Figure 7 Proposed mechanism for the formation of snowflake-like aggregates.

Results of fluorescence measurements showed that these polymers have good recognition for CO_3^{2-} anion and the fluorescence emission intensity was quenched linearly upon the addition of CO_3^{2-} anion under the experimental conditions. The recognition for CO_3^{2-} anion was also proved by the results of TEM. These amphiphilic copolymers can be used as promising fluorogenic chemosensor materials.

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