pH-Induced outward Movement of Star Centers within Coumarin-Centered Star-Block Polymer Micelles

Supporting Information

Jinqiang Jiang,*[a, b] Yan Liu,[a] Yunhua Gong,[b] Qiaozhen Shu,[b] Ming Yin,[b] Xiaoya Liu[b], Mingqing Chen[b]

[a] Key Laboratory of Applied Surface & Colloid Chemistry, Ministry of Education. School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an, Shaanxi, 710062, China.

[b] School of Chemical and Material Engineering, Jiangnan University, Wuxi, Jiangsu, 214122, China.

E-mail: jiangjq@snnu.edu.cn

Experimental Details

Materials

7-Hydroxy-4-methylcoumarin, epichlorohydrin, aniline, glycidol, 2-Bromo-2-methylpropionyl bromide (AR, 98%), K_2CO_3, *N, N, N', N', N''*-pentamethyldiethylenetriamine (PMDETA), Cu(I)Br, triphenylphosphine, triethylamine, acetone, chloroform, petroleum ether (30-60 ^oC), ethyl acetate, anhydrous ethanol, dichloromethane, tetrahydrofuran, *N*, *N*-Dimethylformamide, ethyl ether, 1 N volumetric standard solution of hydrochloric acid (HCl), deuterated chloroform ($CDCl_3$), alumina, silica gel 60Å GF254 plates for thin layer chromatography (TLC) were purchased from Sinopharm Chemical Reagent Shanghai Co., Ltd. and used without further purification. Triethylamine was refluxed with potassium hydroxide and distilled just before use. Dioxane was dried by refluxing with the fresh sodium-benzophenone complex under N_2 and distilled just before use. 2-(Dimethylamino) ethyl methacrylate (DMAEMA) and styrene (St) were purchased from Shanghai Aladdin Reagent Co., Ltd. and used after passing through a neutral Al_2O_3 column.

Methods

^{1}H-NMR spectra were obtained with an AVANCE III 300 MHz Digital NMR spectrometer, using $CDCl_3$ as solvent.

GPC was performed sing a Waters system equipped with a refractive index and a photodiode array detector; with THF as eluent (0.5 mL min^{-1}) and polystyrene standards used for calibration.

FTIR spectra were recorded on a Paragon 1000 instrument by KBr sample holder method.

Fluorescence spectra were recorded on a RF5301PC spectrofluorimeter (Shimadzu). All spectra were taken at room temperature with an integration time of 0.1 s and a bandpass of 3 nm. Emission spectra were obtained by exciting the polymer micellar solution at 320 nm. We average the results from three repetitions of each emission and excitation measurement. The spectra are consistent between repetitions, with a standard deviation below 2%.

Absorbance spectra were recorded on a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.).

The Zeta potential measurements were conducted at room temperature and using a Zeta sizer

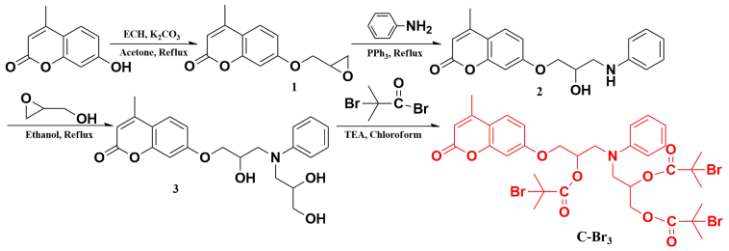
Nano-ZS, ZEN 3600 model of Malvern and a Universal "Dip" cell (ZEN 1002).

The transmittance of the polymer micelles with different pHs was determined by measuring the transmittance at 621 nm using a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.).

Dynamic light scattering (DLS) measurements were performed with an ALV-5000/E dynamic light scattering instrument at 90°. Samples were filtered through 0.2 μm syringe filters (Whatman Anotop) into glass cells (Spectrocell). Prior to sample loading, the cells were soaked overnight in 70/30 v/v sulfuric acid/hydrogen peroxide solution and thoroughly cleaned with filtered Milli-Q water. Measurements were taken at 25 °C, as maintained by a Neslab circulating bath. The errors in the measurements of micellar sizes from the DLS diagrams are within 5% around the mean value over 10 measurements for each sample corresponding to a cumulative time of 1 min. The particle sizes and size distribution were calculated using CONTIN algorithms.

TEM studies were performed with a JEOL JEM-2100 microscope operating at 200KV. The samples were prepared by directly dropping the solution of micelles onto carbon-coated copper grids and dried at room temperature overnight without staining before measurement.

Polymer synthesis



Scheme 1. The synthetic route to the coumarin centered ATRP initiator **C-Br$_3$**.

Synthesis of 4-methyl-7-(oxiran-2-ylmethoxy)-2H-1-benzopyran-2-one (1). A mixture of 7-hydroxy-4-methylcoumarin (35.2 g, 0.2 mol), epichlorohydrin (32 mL, 0.4 mol) and anhydrous potassium carbonate (41.4 g, 0.25 mol) in acetone (200 mL) was heated under reflux for 48 hours. Then the solvent was evaporated to gave a residue, which was partitioned between H_2O (200 mL) and CH_2Cl_2 (500 mL). The organic phase was washed with H_2O (200 mL) and evaporated. Crystallization of the residue from ethanol gave 1 (41.8 g, 90%) as white solid.

^{1}H-NMR ($CDCl_3$) δ: 2.41 (3H, 4-Me), 2.79 (1H, dd, 3′-H), 2.94 (1H, dd, 3′-H) , 3.38 (1H, m, 2′-H), 3.97 (1H, dd, OCH2), 4.34 (1H, dd, OCH2), 6.19 (1H, q, 3-H), 7.65 (1H, d, 5-H), 7.03 (2H, m, 6-, 8-H). Anal. Calcd for C13H12O4: C, 67.24; H, 5.21. Found: C, 67.09; H, 5.23.

Synthesis of 4-methyl-7-(2-hydroxy-3-(phenylamino)propoxy)-2H-1-benzopyran-2-one (2). A mixture of **1** (23.2 g, 0.1 mol), aniline (40 mL, 0.4 mol) and triphenylphosphine (0.1 g) was stirred at 120 °C for 12 h. Then aniline was evaporated with water to give a very sticky half solid, which was washed with petroleum ether to give a light yellow solid (2) (29.6 g, 91%).

^{1}H-NMR ($CDCl_3$) δ: 2.41 (3H, 4-Me), 3.25-3.22 (m, 1H), 3.63-3.56 (m, 3H), 4.78 (d, 1H), 5.06 (t, 1H), 6.14 (1H, CH=), 6.98-6.68 (5H, ArH), 7.29-7.21 (2H, ArH), 7.50 (1H, ArH). Anal. Calcd for C19H19NO4: C, 70.14; H, 5.89. Found: C, 70.02; H, 5.93.

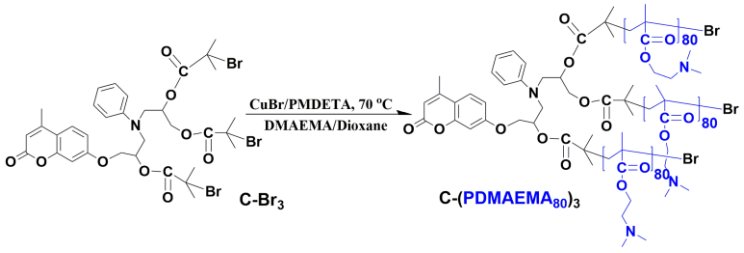
Synthesis of 4-methyl-7-(3-((2, 3-dihydroxypropyl)(phenyl)amino)-2-hydroxypropoxy)-2H-1-benzopyran-2-one (3). A mixture of **2** (13 g, 0.04 mol), glycidol (7 mL, 0.1 mol) and ethanol (50 mL) was refluxed for 12 hours. Then solvent was evaporated and the system was washed with the petroleum ether to give a yellow sticky half solid. The residual solid was resolved in THF and

precipitated from an excess of petroleum ether, filtered, and dried at 40 ^{o}C under vacuum for 24 h (yield: 80%).

^{1}H-NMR ($CDCl_3$) δ: 2.41 (3H, 4-Me), 3.25-3.22 (m, 1H), 3.63-3.56 (m, 6H), 4.78-4.85 (d, 2H), 5.06-5.12 (t, 2H), 6.14 (1H, CH=), 6.98-6.68 (5H, ArH), 7.29-7.21 (2H, ArH), 7.50 (1H, ArH). Anal. Calcd for C22H25NO6: C, 66.15; H, 6.31. Found: C, 66.08; H, 6.28.

Synthesis of Coumarin-containing star ATRP initiator (C-Br$_3$). A mixture of **3** (16 g, 0.04 mol), triethylamine (10 mL, 0.08 mol) and dry chloroform (100 mL) was stirred at 0 ^{o}C for 2 h, and 2-bromo-2-methylpropionyl bromide (40.1 g, 0.12 mol) in chloroform (50 mL) was added dropwise over a period of 2 h. Then, the system was kept stirring for 12 h at room temperature, the resulting insoluble amine hydrobromide salt was removed by filtration, and the spare 2-bromo-2-methylpropionyl bromide was extracted with a K_2CO_3 water solution and water respectively. The chloroform solvent was evaporated and the residue was washed with petroleum ether to give a yellow solid. The crude product was recrystallized from ethanol to afford yellow powdery crystals in 80% yield. ^{1}H-NMR ($CDCl_3$), δ: 7.50 (1H, ArH), 7.29-7.21 (2H, ArH), 6.98-6.68 (5H, ArH), 6.14 (1H, CH=), 5.30-5.47 (2H, -CHOCO), 4,46-4.59、4.13-4.25 (4H, -OCH_2-, -CH_2OCO), 3.64-3.92 (4H, -CH_2-N-CH_2-), 2.39 (3H, CH_3-Ar), 1.80-1.95 (18H, -$C(CH_3)_2$). Anal. Calcd for C34H40B33NO9: C, 48.25; H, 4.76. Found: C, 48.17; H, 4.80.

Synthesis of Coumarin-centered hydrophilic star polymer of C-(PDMAEMA$_{80}$)$_3$. In a typical polymerization, **C-Br$_3$** (84.3 mg, 0.1 mmol), Cu(I)Br (85.2 g, 0.6 mmol), PMDETA (52.0 mg, 0.3 mmol), DMAEMA (4.266 g, 0.027 mol) and dioxane (5 mL) were quickly added into a 25 mL ampoule. Then, the mixture was degassed three times using the freeze-pump-thaw procedure and sealed under vacuum. After 30 min stirring at room temperature, the ampoule was placed in a preheated oil bath (70 ^{o}C) for 24 h. The solution was passed through a neutral Al_2O_3 column with chloroform as eluent to remove the catalyst. The coumarin star centered polymer of **C-(PDMAEMA$_{80}$)$_3$** was collected by precipitation twice into ethyl ether. Yield: 89%. The composition of the polymer was analyzed according to the calibration curve of the coumarin center in dioxane. Mn(UV) =3.84×10^{4}g mol^{-1}, Mn(GPC)=3.95×10^4 g mol^{-1}, Mw/Mn(GPC)=1.23.



Scheme 2. The synthetic route to the hydrophilic star polymer of **C-(PDMAEMA$_{80}$)$_3$**.

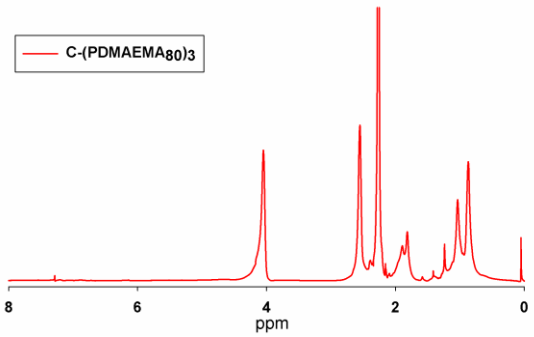
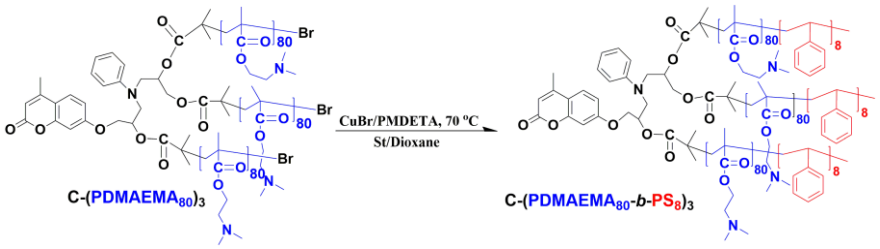


Figure 1. ^{1}H-NMR spectrum of **C-(PDMAEMA$_{80}$)$_3$** in $CDCl_3$.

Synthesis of Coumarin-centered amphiphilic star polymer of C-(PDMAEMA$_{80}$-b-PS$_8$)$_3$.



Scheme 3. The synthetic route to the coumarin-centered amphiphilic star polymer of **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$**.

The amphiphilic star polymer was prepared using **C-(PDMAEMA$_{80}$)$_3$** as macroinitiator, **C-(PDMAEMA$_{80}$)$_3$** (3.85 g, 0.1 mmol), styrene (0.50 g, 4.8 mmol), Cu(I)Br (85.2 g, 0.6 mmol), PMDETA (52.0 mg, 0.3 mmol) dioxane (5 mL) were quickly added into a 25 mL ampoule. Then, the mixture was degassed three times using the freeze-pump-thaw procedure and sealed under vacuum. After 30 min stirring at room temperature, the ampoule was placed in a preheated oil bath (70 ^{o}C) for 24 h. The solution was passed through a neutral Al_2O_3 column with chloroform as eluent to remove the catalyst. The coumarin star centered polymer of **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$** was collected by precipitation twice into ethyl ether. Yield: 92%. The composition of the polymer was analyzed according to the calibration curve of the coumarin center in dioxane. Mn(UV)=4.10×10^4 g mol^{-1}, Mn(GPC)=4.32×10^4 g mol^{-1}, Mw/Mn(GPC)=1.20.

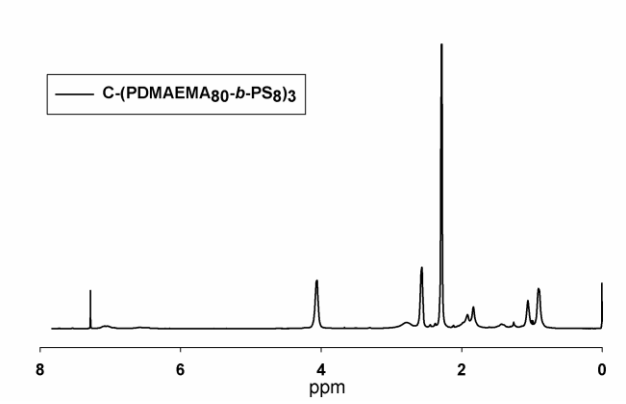


Figure 2. ^{1}H NMR spectrum of **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$** in $CDCl_3$.

Calibration curve of the coumarin center

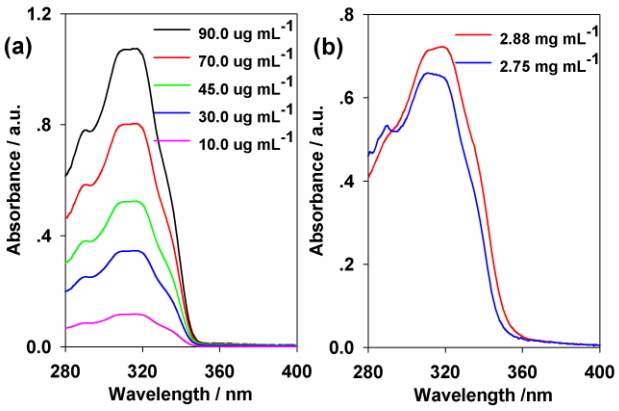


Figure 3. (a) The UV spectra of **C-Br$_3$** in dioxane; (b) the UV spectra of polymers in dioxane, red for **C-(PDMAEMA$_{80}$)$_3$** and blue for **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$**, respectively.

A standard solution (S1) of coumarin center was prepared by adding 9.0 mg **C-Br$_3$** into 50 mL dioxane, and diluted to 100 mL with dioxane after vigorously stirring for 24 h. Then, various amounts of S1 were diluted with dioxane to 70.0 (S2), 45.0 (S3), 30.0 (S4) and 10.0 (S5) μg mL^{-1}, i.e. 8.27×10^{-2}, 5.32×10^{-2}, 3.54×10^{-2} and 1.18×10^{-2} mmol L^{-1}, respectively. And polymers were also dissolved in dioxane at different concentrations, i.e. 2.88 mg mL^{-1} for **C-(PDMAEMA$_{80}$)$_3$** and 2.75 mg mL^{-1} for **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$**, respectively.

As shown in Figure 3a, the characteristic absorbance of coumarin center at 320 nm displayed a good linearity ranging from 1.18×10^{-2} to 1.06×10^{-1} mmol L^{-1} in the dioxane, and a standard curve of $A=9.733\times10^{-2}B$ could be fit (see Figure 4). Then, the average Mns for **C-(PDMAEMA$_{80}$)$_3$** and **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$** were evaluated to be 3.84×10^4 and 4.10×10^4 g mol^{-1} according to the estimated coumarin content by the standard curve, respectively; leading to the estimation of 80 units of DMAEMA in every star polymer arm of **C-(PDMAEMA$_{80}$)$_3$**, and 80 units of DMAEMA and 8 units of St in that of **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$**.

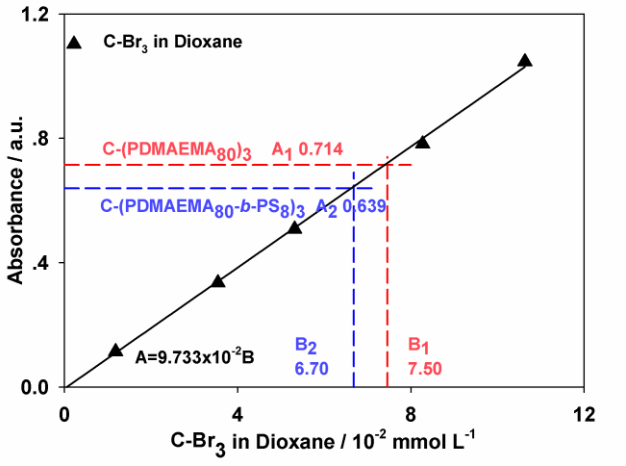


Figure 4. The standard curve of coumarin center fit according to the characteristic absorbance at 320 nm and the estimated coumarin content of polymers in dioxane.

The micellization behavior of C-(PDMAEMA$_{80}$)$_3$ in aqueous solutions.

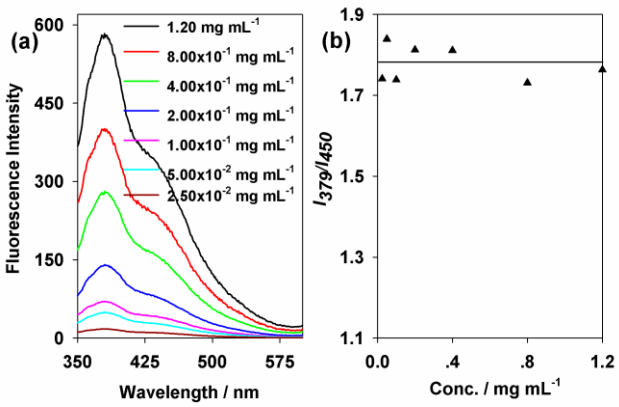


Figure 5. (a) Fluorescence emission spectra of **C-(PDMAEMA$_{80}$)$_3$** in aqueous solutions. (b) The ratio of I_{379}/I_{450} as a function of polymer concentration.

In this experiment, calculated concentrations of **C-(PDMAEMA$_{80}$)$_3$** in deionized waters were

excited at 320 nm to determine its micellization behavior. As shown in Figure 5, the emission spectra of **C-(PDMAEMA$_{80}$)$_3$** in aqueous solutions are characterized by easily detected fluorescence emission centered at 379 nm with a small shoulder around 450 nm. Furthermore, the intensity ratio of I_{379}/I_{450} showed a nearly constant value above 1.70, which indicated that the polymer of **C-(PDMAEMA$_{80}$)$_3$** always remained unimolecular micelles in the aqueous solution because of its much longer PDMAEMA chains, resulting in the dispersion of coumarin centers in the more hydrophilic PDMAEMA environment.

Preparation of amphiphilic polymer micelles of C-(PDMAEMA$_{80}$-b-PS$_8$)$_3$.

The star-block polymer micelle in aqueous solution was expected to be composed of PS inner blocks and hydrophobic coumarin star centers as the hydrophobic core and PDMAEMA as the surrounding hydrophilic corona. The star-block polymer micelle was prepared by the dissolution of **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$** (100 mg) in dioxane (20 mL), a good solvent for both constituents, followed by the progressive addition (1 drop/10 s) of 20 mL of Millipore water at 25 °C under vigorous stirring. The mixed solution was stirred for 12 h and then quenched with 40 mL of deionized water. Stirring was continued at room temperature for another 12 h. Then, the micellar solution was then dialyzed (Spectrum, MW cutoff 3,500) against deionized water while stirring for three days; water was frequently refreshed (every 2 h during day time). Finally, the amphiphilic polymer micelle was diluted to 1.0 mg mL^{-1}.

Protonation of C-(PDMAEMA$_{80}$-b-PS$_8$)$_3$ in aqueous solutions.

The pH-dependent micellar behavior was investigated using an automatic titrator (Titrando 809) from Metrohm. For the titration experiments, 60 mL×1.0 mg mL^{-1} of **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$** aqueous solution was degassed by applying vacuum (50-100 mbar) for 15 min at room temperature in order to minimize bubble formation during the experiments. The measurements were carried out with 1N hydrochloric acid solution as titer, using a homemade thermostatable vessel equipped with a turbidity sensor (Spectrosense electrode, λ=523 nm, Metrohm), a pH sensor (Aquatrode, Metrohm), and a titration unit (Dosino 800, Metrohm). The setup was kept at a constant temperature of 25 ^{o}C. Titration curve were generated by titrating the solution to pH 2 with 1N HCl while measuring the pH after each decrement. The protonation process was also in situ monitored by the zeta potential, optical transmittance measurements, fluorescence spectra instrument.

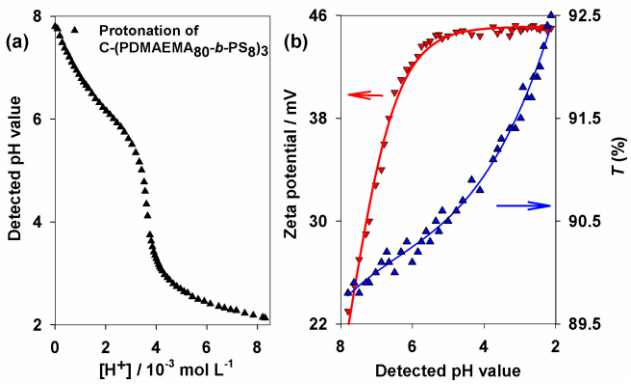


Figure 6. (a) The protonation curve of **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$** in aqueous solution (1 mg mL^{-1}) with HCl (1 N). (b) The zeta potential (red triangles) and optical transmittance (blue triangles) changes of micelle solution as a function of detected pH values.

As shown in Figure 6a, the obtained protonation curve primly agrees with those reported for PDMAEMA polymers in the literatures. And along with the protonation, the potential values (red triangles in Figure 6b) quickly and continuously increased to more than 40 mV upon the decreasing to around pH 6.0; then, the potentials remained nearly constant, despite the fact that the degree of protonation increased substantially. And in the whole protonation procedure, the optical transmittance (blue triangles in Figure 6b) increased gradually.

And as shown in Figure 7a, the emission spectra of **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$** in aqueous solution show easily detected dual fluorescence emissions centered at 384 nm and 440 nm, resembling the isolate and the packed state of coumarin centers, respectively; and both intensities declined by different levels along with the protonation. Furthermore, as shown in Figure 7b, the dependence of I_{384}/I_{440} upon the protonation began with the lowest value around 1.10 from pH 7.80 (A) to pH 5.75 (C), indicating the slightly detachment of the coumarin moieties in the hydrophobic micelle core; and increased sharply to 1.30 (D) at the break point of pH 5.75 (C), suggesting the solubilization of the coumarin centers into the hydrophilic micelle shell from the hydrophobic micelle core; then increased quickly as a result of the increasing acidity, meaning the further outward moving and isolate of coumarin moieties in the micelle shell.

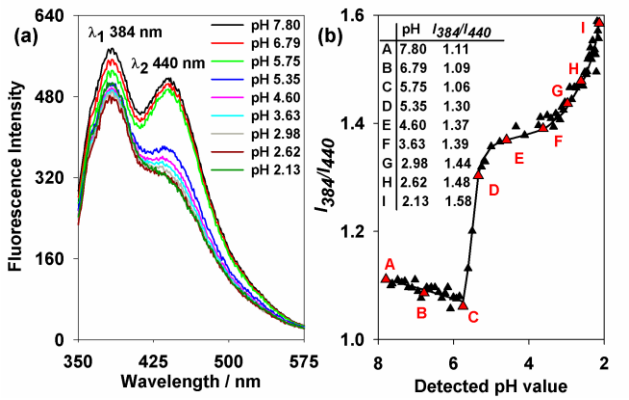


Figure 7. (a) The emission spectra of polymer micelles excited at 320 nm in different pH aqueous solutions (1 mg mL^{-1}). (b) The intensity ratio changes of I_{384}/I_{440} as a function of detected pH, the red triangles from A to I referring to the samples for DLS and TEM observations.

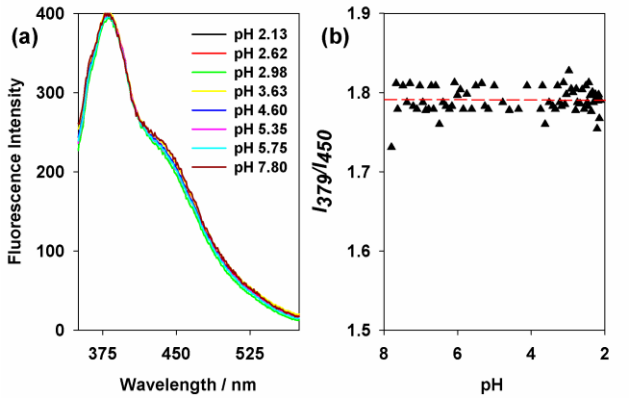


Figure 8. (a) The emission spectra of **C-(PDMAEMA$_{80}$)$_3$** excited at 320 nm in different pH aqueous solutions (0.8 mg mL^{-1}). (b) The intensity ratio changes of I_{379}/I_{450} as a function of detected pH.

In order to further confirm the pH dependence of coumarin centers in polymer micelles, the parent star polymer of **C-(PDMAEMA$_{80}$)$_3$** was also protonated and monitored with fluorescence

spectra instrument. As shown in Figure 8a, compared with **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$**, one of the dual emissions at 450 nm weakened to a small shoulder, and another emission showed a blue shift to 379 nm. Furthermore, the intensity ratio of I_{379}/I_{450} showed a nearly constant value above 1.70, which indicated that the polymer of **C-(PDMAEMA$_{80}$)$_3$** always remained unimolecular micelles upon the protonation because of its much longer PDMAEMA chains, resulting in the dispersion of coumarin centers in the more hydrophilic PDMAEMA environment.

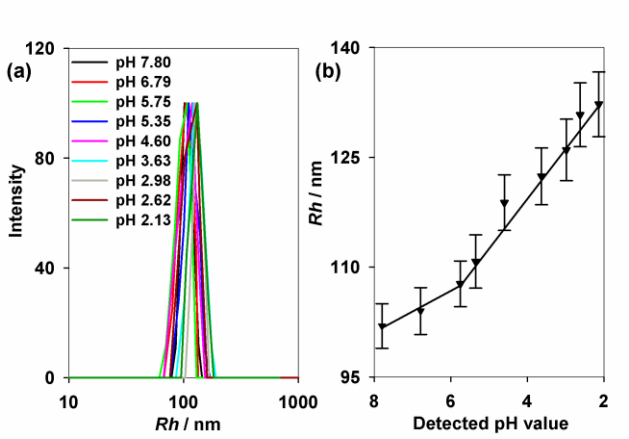


Figure 9. (a) Size distributions of polymer micelles at different pH solutions. (b) The average size dependence as a function of detected pH values.

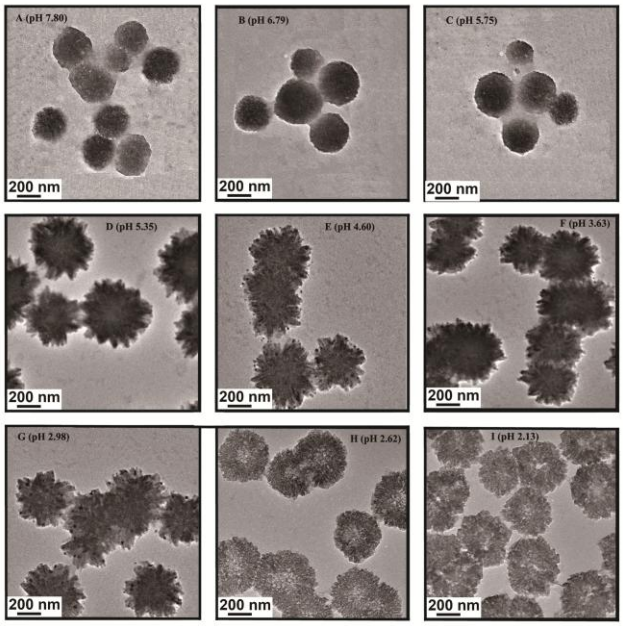


Figure 10. TEM images for polymer micelles at different pH solutions.

And nine samples of **C-(PDMAEMA$_{80}$-*b*-PS$_8$)$_3$** in aqueous solution (5 mL × 1 mg mL^{-1}) at different pHs were chosen to be further investigated by DLS and TEM instrument according to the I_{384}/I_{440} ratios estimated from the fluorescence spectra. As shown in Figure 9, the average hydrodynamic radius (Rh) linearly and slightly increased from 101.9 to 107.7 nm as the pH values decreased from 7.80 to 5.75; then increased a little quickly to 132.4 nm as a result of the increasing acidity to pH 2.13. The morphology changes were also directly observed by TEM. As shown in Figure 10, the spherical and rather compact micellar structures (A, B and C) were

observed in neutral or weak acidic conditions, and the diameters of these micelles are in the range of 180-250 nm. Then, along with further acidity to pH 5.35, 4.60, 3.63 and 2.98, the micelle morphologies (D, E, F and G, respectively) were observed as a novel nanostructure of spiky-shelled micelles. The needles corresponded to the restricted arrangement of the protonated PDMAEMA segments. Finally, at the strong acid condition of pH 2.62 and 2.13, the completely protonated PDMAEMA segments diverged along the helix-like direction resulting in the hollowed loose micelles (H and I, respectively). These micelle morphology transitions primly agree with the dependence of I_{384}/I_{440} upon the protonation.