Optimal Synthesis of Polyelectrolyte Nanogels by Electrostatic Assembly

Directed Polymerization for Dye loading and Release

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Materials

Diblock copolymer PAA_{83}-b-PEO_{250} [poly(acrylic acid-b-ethylene oxide)] with polydispersity index 1.09, $PAMPSNa_{50}$-b-PEO_{238} [poly (2-acrylamido-2-methyl-1-propane sulfonic acid sodium-b-ethylene oxide)] with polydispersity index 1.35 were purchased from Polymer Source and used as the template. Photoinitiator 2-hydroxy-2-methyl-1-propanone (HMP) was purchased from TCI and used as received. Cationic monomer 2-(Dimethylamino) ethyl methacrylate (DMAEMA), N, N'-Methylenebisacrylamide (MBA) were purchased from Sigma Aldrich. Rhodamine B, Cresol red, and New Coccine were purchased from Sigma Aldrich. Deuterium oxide (D_2O) was purchased from Adamas. 3-(Trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (TSP) was purchased from Sigma Aldrich and used as internal standard for ^1H NMR. All other chemicals were of analytical grade and were used without further purification. Ultrapure water was produced with a Milli-Q water purification system.

Characterizations

^1H-NMR spectra were recorded on a BRUKER AVANCE 400 spectrometer operating at 400 MHz. Reaction solution was dissolved in D_2O after adding NaCl to a final concentration of 3M and the spectrum was scanned 32 times at 25 ^oC. UV spectra for all the samples were recorded on a SHIMADZU 1800 spectrophotometer.

Light scattering

The Dynamic light scattering (DLS) measurement was performed with an ALV light scattering apparatus, equipped with a 21 mW He-Ne laser operating at a wavelength of 632.8 nm. Measurements were done at a detection angle of 90°, unless stated otherwise.

All measurements were performed at room temperature. The CUMULANT method was used to analyse the mean apparent hydrodynamic radius (R_h),[1] which is

$$R_h = kTq^2/6\pi\eta\Gamma$$

Where k is the Boltzmann constant, T is the absolute temperature, q is the scattering vector, η is the viscosity of the solvent, Γ is the measured average decay rate of the correlation function. The CONTIN method is used to analyze the size distribution of micelles hydrodynamic radius.[2-3]

For angular-dependent DLS, ten correlation functions $g_2(t)$ were recorded from 45° to 120° at intervals of 15°, to evaluate the angular dependence of the diffusion coefficient.

Preparation of nanogel

Typically, PAA-*b*-PEO (8.2 mg), cationic DMAEMA monomer (6.3 mg, 0.04 mmol), MBA (0.62 mg, 0.004 mmol), and HMP (0.131 mg, 8×10^{-4} mmol) were dissolved in water (2 mL) in a 10 mL Schlenk tube. For all synthesis, reaction was done at a 1:1 stoichiometric ratio of chargeable groups. The solution was adjusted to pH 6.5 using 3.0 M HCl. The tube was sealed and de-oxygenated by bubbling with nitrogen for 30 min. Then the tube was exposed to UV light for 3h. The reaction was stopped by exposure to air. The synthesis at different salt concentrations was carried out according to the above-mentioned recipe and reaction conditions, but using different salt concentrations. The nanogel was separated from the template by centrifugation using 2 M NaCl as eluent for six times with an Amicon Ultra centrifugal filter (Millipore, Molecular weight cut off = 100 kDa). The solution inside the tube was collected and dialyzed against water to remove the salt, and then used for further characterizations.

Determination of monomer conversion and nanogel yield

1. Determination of monomer conversion

Solution after polymerization was collected and lyophilized, then the powder was dissolved in D_2O for ^1H NMR measurement. The un-polymerized monomer display typical chemical shift of carbon-carbon double bond in ^1H NMR spectra, and the amount is determined by calculating the peak integrals with TSP as an internal standard. The monomer conversion is obtained by subtracting the total monomer amount by the un-polymerized monomer amount.

2. Determination of nanogel yield

The polymerized monomers converted into either free polymer, or cross-linked networks. The free polymers are moved out during the centrifugation process with an Amicon Ultra centrifugal filter. The filtrate outside was collected and lyophilized, followed by dissolving in D_2O and adding NaCl to a final concentration of 3M for ^1H NMR measurement. The free polymer amount is calculated based on the integrals of PDMAEMA characteristic peaks at 2.7-3.2 ppm, form which nanogel yield (amount of monomers resided in the nanogels) is determined.

Dye Loading and Release Study

Anionic dye (NC) was titrated into the PDMAEMA nanogel solution (0.1 mM positive charge, 5 mM citrate-buffer at pH 3.0), followed by 1-minute vortex, and then tested by light scattering. The release behavior of NC from nanogel was carried out by dialysis method. Briefly, the prepared NC-loaded nanogel was divided into different equivalent aliquots. Each 1 mL of the aliquot sample was placed inside a dialysis tube (molecular weight cut off 100 kDa) and then immersed into 20 mL of an aqueous medium with different pH or salt concentration. At desired intervals, 1 mL of the solution outside the dialysis tube was taken out followed by the addition of an equal amount of fresh

solution to keep the constant volume of the medium. The amount of NC released from nanogel was measured by UV-vis spectroscopy at 507 nm at 25 °C. The cumulative release of the dye was calculated as a percentage of the total dye loaded in nanogels and plotted with time. A calibration curve was drawn by measuring a series of NC solutions with known concentrations.

PAA$_{83}$-*b*-PEO$_{250}$ PAMPS$_{50}$-*b*-PEO$_{238}$ DMAEMA   MBA
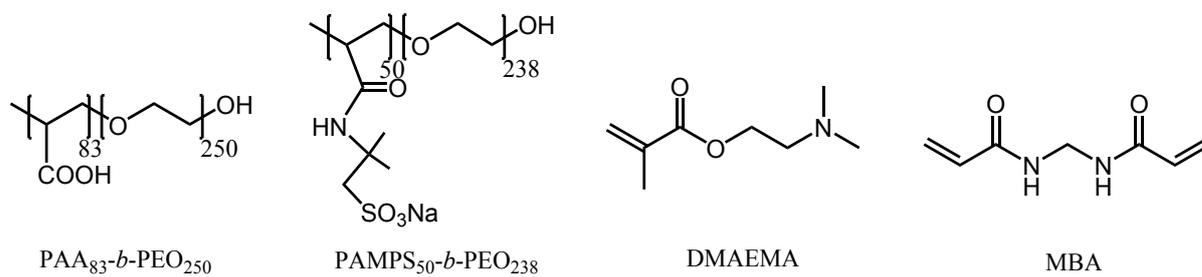
Figure S1. Chemical structures of template, monomer, and crosslinker.
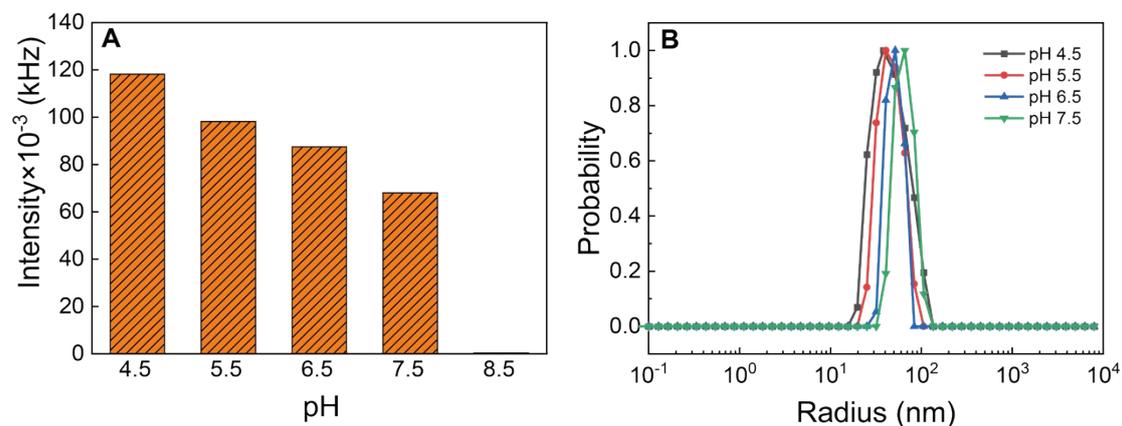
Figure S2. (A) Scattering intensity of particles prepared at different pH in the presence of PAMPS-*b*-PEO as template. (B) Size and size distribution of nanogels prepared at different pH with PAMPS-*b*-PEO template.

Table S1. Monomer conversion and nanogel yield of PDMAEMA nanogel prepared at different pH

pH	Conversion	Yield
4.5	82.4%	37.7%
5.5	79.5%	41.5%
6.5	90.5%	70.6%
7.5	90.0%	48.7%
8.5	86.7%	8.3%
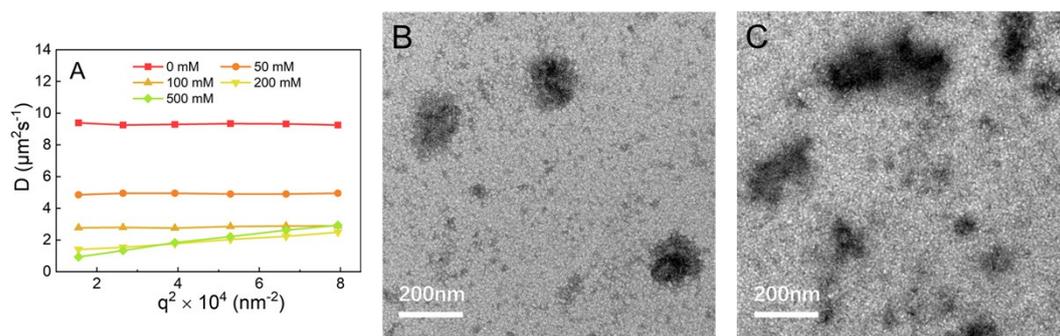
Figure S3. (A) Angular dependence of the diffusion coefficient of PDMAEMA nanogels prepared at different salt concentrations. TEM images of PDMAEMA nanogels prepared at (B) 100 mM and (C) 500 mM salt concentrations. Phosphotungstic acid was applied as stain agent. Nanogel synthesis was carried out at pH 6.5, charge mixing ratio 1/1, and cross-linker 10%.

Table S2. Monomer conversion and nanogel yield of PDMAEMA nanogel prepared at different salt concentration

Salt concentration	Conversion	Yield
0	90.6%	69.0%
50	90.3%	75.5%
100	91.7%	79.8%
200	84.3%	57.3%
500	74.6%	36.3%

Table S3. Monomer conversion and nanogel yield of PDMAEMA nanogel prepared

at different cross-linker fractions

Cross-linker fractions	Conversion	Yield
10%	90.5%	70.6%
15%	91.2%	74.6%
20%	92.7%	78.9%
30%	94.4%	82.4%
50%	95.2%	87.5%

Rhodamine B   New Coccine   Cresol red
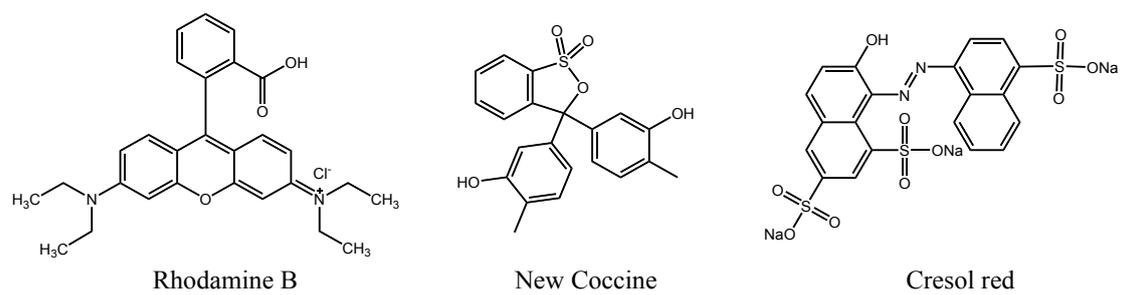
Figure S4. Chemical structures of different dyes used in this study.

Figure S5
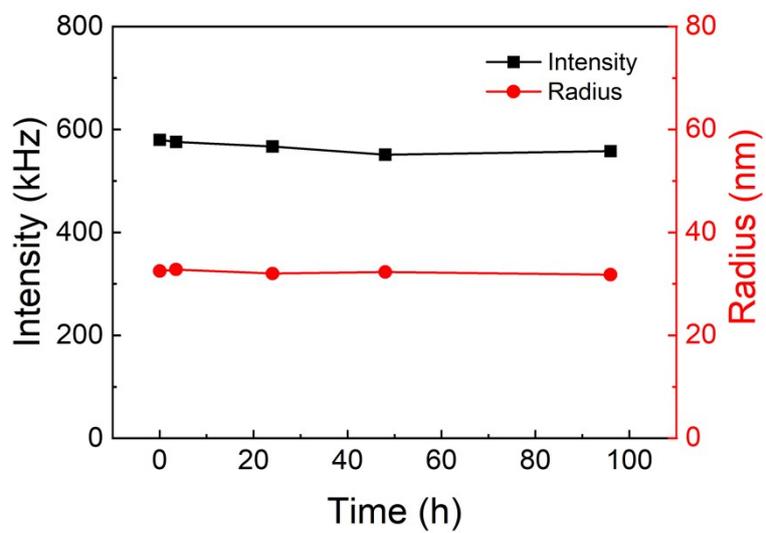
Figure S5. Light scattering intensity and hydrodynamic radius over time of NC-loaded

PDMAEMA nanogel prepared at a charge ratio of 0.91/1.

Figure S6
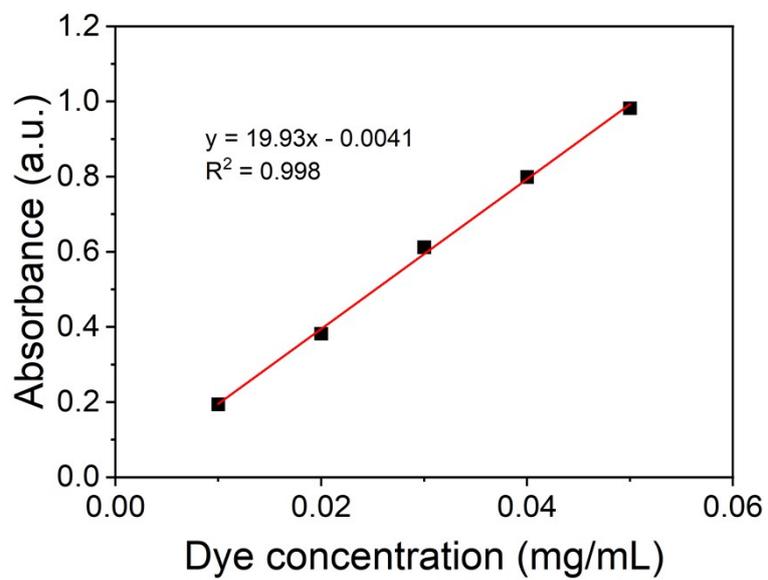
Figure S6. The standard curve of NC dye.

Reference:

1. Scotti, A.; Liu, W.; Hyatt, J. S.; Herman, E. S.; Choi, H. S.; Kim, J. W.; Lyon, L. A.; Gasser, U. and Fernandez-Nieves, A., *J. Chem. Phys.* 2015, **57**, 4814-4820.
2. PROVENCHER, S. W., *Comput. Phys. Commun.* 1982, **27**, 213-227.
3. PROVENCHER, S. W., *Comput. Phys. Commun.* 1982, **27**, 229-242.