Investigating the effect of the micelle structures of block and random copolymers on dye solubilization

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Key words

Amphiphiles, block copolymer, random copolymer, core-shell structure, solubilization amount of dye,

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Experimental section

1. Materials

Five amphiphilic block co-polymers were synthesized (Table 1). Monomers and solvents were prepared as follows. Styrene (St, >99.0%), diphenyl ethyrene (DPE, >98.0%) and methyl-ethyl-ketone (MEK, >99.0%) were purchased from Fujifilm WAKO (Tokyo, Japan). 2.6M n-Butyllithium (n-BuLi), in n-hexane solution was purchased from KANTO Chemical (Tokyo, Japan). n-butyl acrylate (n-BMA, 98%), t-butyl methacrylate (t-BMA, 99%) and α-methyl styrene (α-MeSt, 99%) were purchased from Merck (Darmstadt, Germany). Methacrylic acid (MA), Diethylene Glycol (DEG, >99.5%), KOH and 2,2'-Azobis(isobutyronitrile) (AIBN) were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). 4-Cyano-4-(((dodecylthio)carbonothioyl)thio)pentanoic acid as RAFT agent was purchased from Merck. AIBN was purified by recrystallization using methanol. Oil orange SS (1-(o-Tolylazo)-2-naphthol) was used as an organic dye. It was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Pyrene was used as a fluorescence dye which purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). All water used was purified by treating in reverse osmosis unit followed by a Millipore unit (18 mΩ resistivity).

2. Synthetic procedures

Block copolymer

Five amphiphilic block copolymers were synthesized using a microreactor. The block copolymers were constructed using St, n-BMA and MA monomers. The synthetic procedure was described in previous report.[1] Structure of amphiphilic block copolymers and preparation composition was shown in Figure S1.

Random copolymer

St (0.773g, 0.0076mol), MA (0.227g, 0.0026mol), RAFT agent (0.08g, 0.0002mol) and AIBN (0.0066g, 0.00004mol) were dissolved in 10 mL of isopropanol. The solution was degassed through freeze-pump-thaw cycling, followed by stirring at 60°C for 24 h under N_2 atmosphere. Then, 50 mL of benzene was added, and the mixture was freeze-dried to recover the product as yellow flakes. The yield was 12%.

The obtained product was dissolved in Diethyl ether-d10 (99.5+%, Fujifilm Wako, Tokyo, Japan) and characterized by ^{1}H NMR (JNM-ECA500, JEOL, Tokyo, Japan). The product was eluted by THF at 1.0 mL/min for size exclusion chromatography using HLC-8220 (TOSOH, Tokyo, Japan) system equipped with TSKgel G5000, G4000, G3000 and G2000 columns (TOSOH, Tokyo, Japan). The number and weight average molecular weights were determined using polystyrene standards. The NMR chart is shown in Figure S2. The composition was determined to be 10.3 MA group to 10 St group by NMR. The molecular weights of M_n, M_w and M_n/M_w were determined by GPC; M_n was 1,307, M_w was 1,571 and M_w/M_n was 1.201, respectively. Using a M_w of 1,571 and a ratio of St to MA of 10:10.3, the acid value was calculated to be 301.

3. Preparation of polymer solution

A 0.5 wt.% aqueous solution of BL02 and BL04 was prepared by neutralizing 1.2 times the molar amount of the acidic group of the polymer with an aqueous solution of KOH. BL04 showed a slight residual melt. Therefore, it was removed with a 0.45μm membrane filter during the measurement. A 0.1 wt.% aqueous

solution was dissolved. On the other hand, BL01, BL03, and BL05 could not obtain 0.1 wt.% aqueous solution by this method. Therefore, after dissolving in diethylene glycol, the solution was alkali-neutralized to obtain 0.5 wt.% aqueous solution. The polymer concentration of the diethylene glycol solution was set to 28 wt.%, and heating at about 100°C for 30 minutes was required during dissolution.

RD01 and RD04 were prepared as aqueous solutions in the same manner as BL02. RD02 and RD03 were dissolved directly in water because they originally were neutralized with amines. The aqueous solution prepared by this method was used for the subsequent evaluation.

No precipitation was observed for the aqueous solutions in which the polymer was dissolved for over a week, indicating that the micelles were stable. A zeta potential of the micelles showed in Table 2.

4. Measurement of small angle X-ray scattering (SAXS)

The structures of polymer micelles were evaluated by SAXS at a beam line BL03XU in a synchrotron facility SPring-8, Japan. PILATUS 1M detector was placed 2 m away from the sample. The wavelength of the incident beam (λ) was 0.10 nm. The setup provided q ranges of 0.07-2.0 nm^{-1} where q is the magnitude of the scattering vector (q = ($4\pi/\lambda$) sin θ, where 2θ is the scattering angle), and the range corresponds to a length scale of 3.1−89.8 nm in real space. The camera length and the wavelength of the incident X-ray were set to 2.0 m and 0.1 nm, respectively.

A micelle solution was packed glass capillary (1 mm Φ) and set in the sample chamber. SAXS from a sample solution was measured at an exposure time of 10 or 100 sec. The resulting 2D SAXS images were converted to 1D I(q) versus q profiles by circular averaging. Here, $I(q)$ was the scattering intensity at q, and to obtain the excess scattering intensity $I(q)$ by micelles, the scattering from the background due to the buffer, the cell was subtracted. The scattering experiments were carried out at C = 5 mg/mL for most of the samples. Scattering intensity curves were fitted with an open-source Igor software. [2] The scattering intensity of the polymer solution can be expressed by the following formula:

$$I(\text{q})=P(\text{q})S(\text{q})+bkg. \tag{1}$$

P(q) is a form factor. S(q) is a structure factor, and it can be neglected in dilution system. The bkg is a background intensity. Curve fitting was performed using the following equation.

Monodisperse sphere: Calculates the form factor for polydisperse spherical particles with Gaussian size distribution. Calculates the form factor, P(q), for a monodisperse spherical particle with uniform scattering length density. The form factor is normalized by the particle volume as described below.

$$P(q) = \frac{scale}{V}\left[\frac{3V(\Delta\rho)(\sin(qr)-qr\cos(qr))}{(qr)^3}\right]^2+bkg \tag{2}$$

Scattering contrast ($\Delta\rho$) is a difference of scattering length density between spheres and solvent. The strength of the vertical axis can be adjusted by the scale value.

1) Gauss sphere model: Calculate the scattering of a sphere with uniform scattering length density (2). It is assumed that the spheres have a Gaussian distribution.

$$f(R) = \frac{1}{\sigma\sqrt{2\pi}}exp\left[-\frac{1}{2\sigma^2}(R - R_{avg})^2\right] \tag{3}$$

f(R) is a normalized Gaussian distribution. R_{avg} is an average radius specified. P(q) was multiplied by f(R) to give it a Gaussian distribution. The scattering amplitude for a sphere is as follows.

$$F(x) = \frac{3[\sin(x)-x(\cos(x)]}{x^3} \tag{4}$$

I(q) can be written as follows.

$$I(q) = \left(\frac{4\pi}{3}\right)^2 N_0 \Delta\rho^2 \int_0^\infty f(R)R^6 F(qR)^2 dR \tag{5}$$

N_0 is the total number of particles per unit volume.

2) Polydisperse Gaussian coil: The calculation was done considering the extent of the polymer chain in a good solvent.

$$I(q) = scale \frac{2\left[(1+Ux)^{-1/U}+x-1\right]}{(1+U)x^2} + bkg \tag{6}$$

$$U = \frac{M_w}{M_n} - 1 \tag{7}$$

$$x = \frac{R_g^2 q^2}{1+2U} \tag{8}$$

3) Core shell sphere model: Calculates the form factor, P(q), for a monodisperse spherical particle with a core-shell structure. The form factor is normalized by the total particle volume. The function calculated is:

$$P(q) = \frac{scale}{V_s}\left[\frac{3V_c(\rho_c-\rho_s)j_1(qr_c)}{qr_c} + \frac{3V_s(\rho_s-\rho_{solv})j_1(qr_s)}{qr_s}\right]^2 + bkg \tag{9}$$

$$j_1(x) = \frac{(\sin(x)-x\cos(x))}{x^2} \tag{10}$$

$$r_s = r_c + t \tag{11}$$
$$V_i = \frac{4\pi}{3}r_i^3 \tag{12}$$

Scale is a scale factor and *bkg* is a form factor of background. V_c and V_s are volume of core and shell, ρ_c and ρ_s are contrast of core and shell, r_c and r_s are core radii and whole sphere radii, and *t* is the shell thickness.

4) Poly Core model: Calculate the form factor of polydisperse spherical particles with a core-shell structure which have a polydisperse core with a constant shell thickness. The form factor is normalized by the average particle volume.

$$P(q) = \frac{scale}{V}[3\langle V\rangle\langle F(qr)\rangle^2]. \tag{13}$$

where $\langle F$(qr)$\rangle$ is the particle scattering amplitude, appropriately averaged over the Schulz distribution of radii. The returned form factor is normalized by the average particle volume *<V>*:

$$\langle V\rangle = \frac{4\pi}{3}\langle r^3\rangle \tag{14}$$

$$\langle r^3\rangle = \frac{(z+3)(z+2)}{(z+1)^2}\langle r\rangle \tag{15}$$

$$z = \frac{1}{p^2} - 1 \tag{16}$$

and z is the width parameter of the Schulz distribution, p is a polydispersity calculated as σ/R_c.

5. Measurement of dye's solubilization amount in polymer solution

The organic dye as a 1 wt.% acetone solution (2 mL) was coated into the sample tube by air-drying for 3 hours. Thereafter, 20 ml of an aqueous polymer solutions were added, and the mixtures were shaken at 25°C. Samples were extracted at regular time-intervals for UV measurement. An extracted aqueous polymer solution at each time was mixed with 2 ml of ethanol, thereafter coarse particles had been removed with a 0.20 μm membrane filter, and the intensity at 494 nm in UV spectrum was measured using V-670 (Jasco, Tokyo, Japan). The amount of solubilization was calculated by a calibration curve prepared with a water/ethanol equivalent mixture solution in advance.

The solubilization test measured the amount of dye solubilized from the dry film into the aqueous solution, and the solubilization rate was calculated with reference to the theoretical formula for measuring the solubility of solids. Noyes and Whitney proposed the following equation as the rate of solution of solid substances (A.A. Noyes, W.R. Whitney, The rate of solution of solid substances in their own solutions, J. Am. Chem. Soc., 1897, 19, 930-934.).

$$\frac{dC}{dt} = k(C_s - C) \tag{17}$$

C is a concentration at time t and C_s is a saturated solubility. K is a constant. Eq. 17 was later revised by Bruner and Tolloczko.

$$\frac{dC}{dt} = k_1 S(C_s - C) \tag{18}$$

S is a surface area. In our case, the area of dye coating is constant, so the solubilization rate constant k was evaluated from Eq. 17 (Figure S5, Table S1). Since BL01 and BL05 were not yet saturated even after 68 hours, the maximum value was used as the saturated solubilization amount. Therefore, these k values are overestimated. RD03 and RD04 showed the minimum calculable values because solubilization was nearly saturated early in the measurement.

6. Fluorescence measurements

The critical micelle concentration (cmc) was determined using pyrene as a fluorescence probe.

A stock solution of pyrene (5×10^{-4} mol/l) was prepared in ethanol. The stock solution (3 μl) was added in the sample tube and dried under reduced pressure for 1 hour. After 3 ml polymer solution was added in the sample tubes followed by were shaken at 25°C for 1 day. Thus, micelles with varying concentration were equilibrated with a saturated aqueous solution of pyrene for 1 day under shielded light to load pyrene molecules into the micelle core. Fluorescence measurements were carried out using a FP-8600 JASCO fluorometer (Japan) at an excitation wavelength of 330 nm, and the detection of fluorescence was performed at 373 and 383 nm, which correspond to the wavelength of the (0,0) band (band I) and the (0,2) band (band III) of pyrene fluorescence, respectively. Experiments were done at 25 and 35 °C. These ratios are known to indicate the hydrophobicity of the environment. The hydrophobicity inside the micelles was evaluated.

7. Size distribution measurement

Hydrodynamic diameters and polydispersity of polymeric micelles were determined by dynamic light scattering (DLS) measurements using a 632 nm laser from a scattering angle of 173º (Zetasizer Nano ZSP, Malvern Panalyrtical, Worcestershire, UK). The DLS measurements were performed in water for the micelle at the micelle concentration above 1 mg/ml. The rate of decay in the photon correlation function was analyzed using the cumulant method and by applying the Stokes-Einstein equation to obtain a cumulant diameter.

8. Static light scattering

When a photon collides with a molecule, some of the energy from the photon is used to initiate what is called an oscillating dipole within the molecule. This energy is subsequently re-emitted by the molecule in all directions as light. The principles behind light scattering can be used to measure a number of properties related to the molecule. Static light scattering (SLS) is a non-invasive technique used for characterizing macromolecules in solution. A beam of monochromatic light is directed through a sample and the intensity of the light scattered by the molecules is measured using Zetasizer Nano ZSP. SLS makes use of the time-averaged intensity of scattered light, from which the weight-averaged molecular weight and second virial coefficient can be determined.

The intensity of scattered light that a macromolecule produces is proportional to the product of the weight-average molecular weight and the concentration of the macromolecule. In a batch (cuvette-based) SLS measurement, the sample is prepared at a series of different concentrations. The light scattering intensity from each sample is measured. The scattering intensities are referenced against a light scattering standard, typically toluene. The results are used to build a Debye plot using the Zimm equation (shown below). Refractive index was required for measurement, and it was measured by Otsuka DRM-3000 (Otsuka Electronics, Osaka, Japan).

The properties of the light scattered by molecules and particles vary depending on the object doing the scattering as defined in the Rayleigh equation.

$$\frac{KC}{R_\theta} = \left(\frac{1}{M} + 2A_2C\right)\frac{1}{P_\theta}$$

where C is the sample concentration, θ is the measurement angle, R_θ is the Rayleigh ratio (the ratio of scattered light intensity to incident light intensity), A_2 is the second virial coefficient, M_w is the weight average molecular weight. K is more complex term and P_θ is the angular scattering dependence defined as:

$$K = \frac{2\pi^2}{\lambda_0^4 N_A}\left(n_0\frac{dn}{dc}\right)^2$$

where λ_0 is the laser wavelength in a vacuum, N_A is Avogadro's number, n_0 is the refractive index of the solvent, dn/dc is the refractive index increment of the sample.

$$P_\theta = 1 + \frac{16\pi^2 n_0^2 R_g^2}{3\lambda_0^3}sin^2\left(\frac{\theta}{2}\right)$$

where R_g is the molecule's radius of gyration. The Rayleigh equation tells us that the intensity of scattered

light at a given angle is dependent on a number of factors including both molecular weight and molecular size of the sample under study. As can be seen in the Rayleigh equation, molecules with higher molecular weight and larger sizes will scatter more light. While the increase in the intensity of light scattered is linear with molecular weight, it is non-linear with respect to size. So, if we know all of the other factors in the Rayleigh equation, we can measure the intensity of the scattered light (related to R_θ) and calculate the sample's molecular weight. For isotropic scatterers such as proteins, P_θ is approximately equal to 1 and can be ignored. In a Debye plot, KC/R_θ is plotted as a function of sample concentration and the sample molecular weight and A_2 are then calculated from the y-intercept and slope, respectively. A_2 has previously been shown to have a strong correlation with sample solubility.

For polymer micelles, the number of micelle associations can be determined by dividing the molecular weight of the micelle by the molecular weight of one molecule determined. Because static light scattering is extremely contamination-averse, the samples were prepared with distilled water, and the instruments used were washed with distilled water well in advance and dried. Before the measurement, the intensity distribution of dynamic light scattering confirmed that there were no coarse particles in the sample, and if there were, the sample was filtered. Block copolymer aqueous solution prepared was diluted to 0.1 wt.%, 0.2 wt.%, 0.3 wt.%, 0.4 wt.%, and 0.5 wt.%.

a
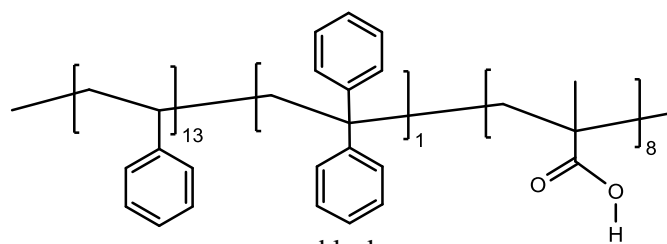
block

BL01
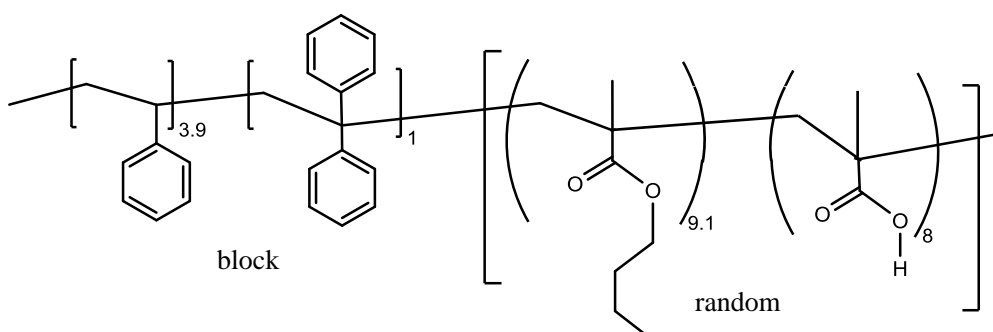
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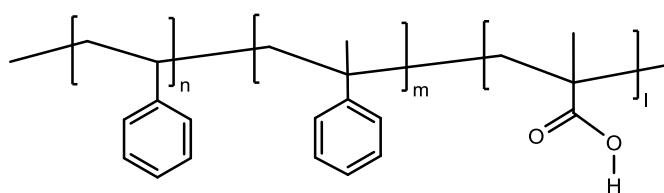
random

BL02

BL03: n=6.8, m=2, l=5

BL04: n=8, m=2, l=13

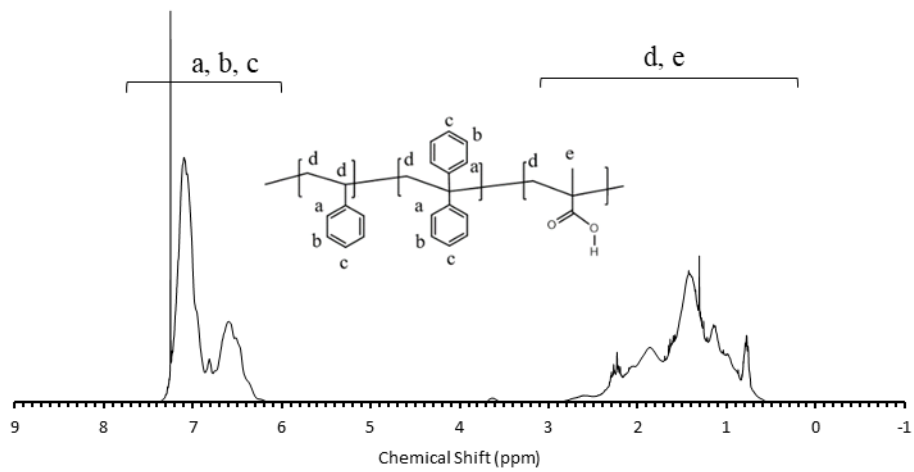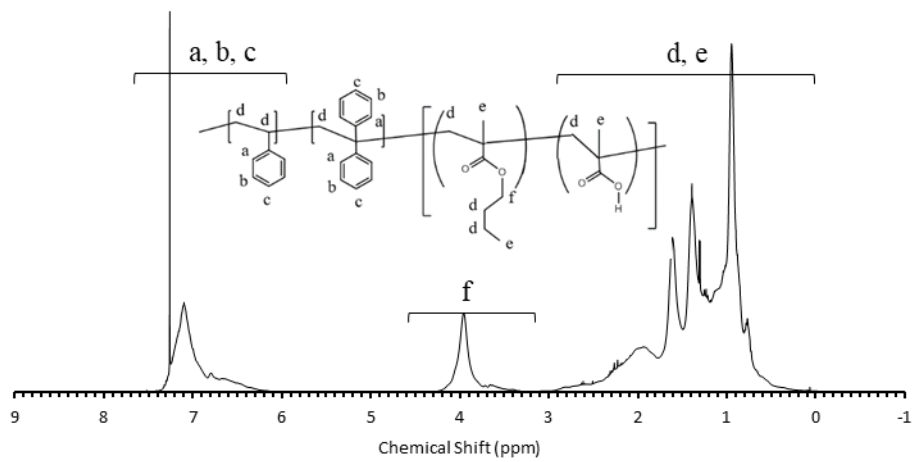**BL05**: n=24, m=2, l=14

b
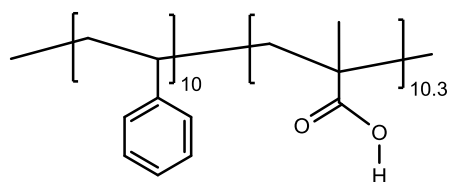
random

RD04

c
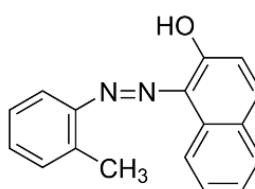
Oil Orange SS

Figure S1. Structures and compositions of a) the amphiphilic block copolymers b) RD04, and c) dye.
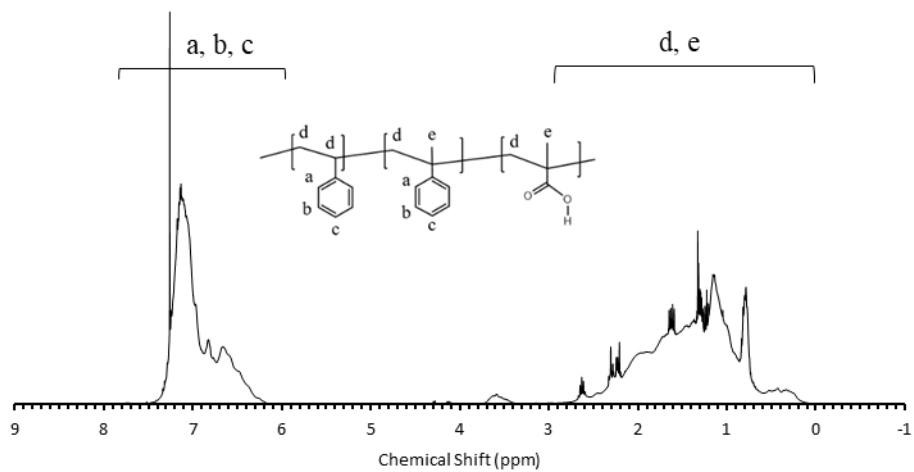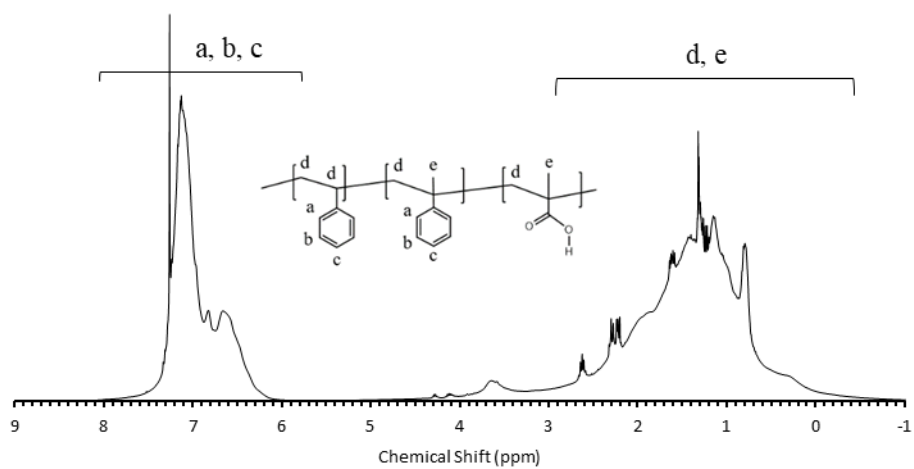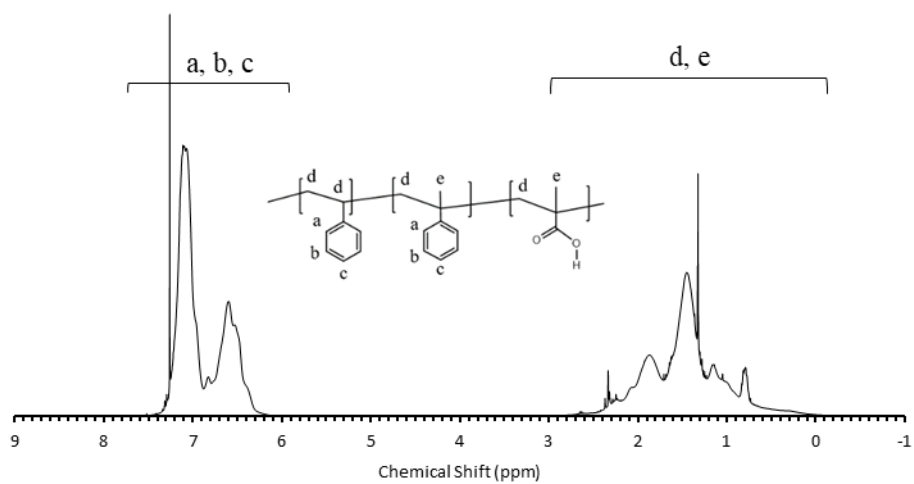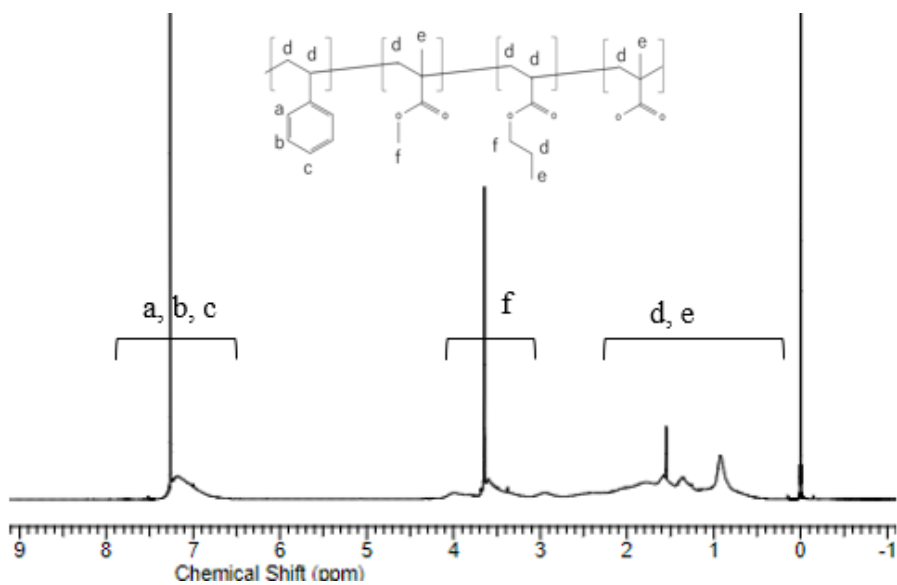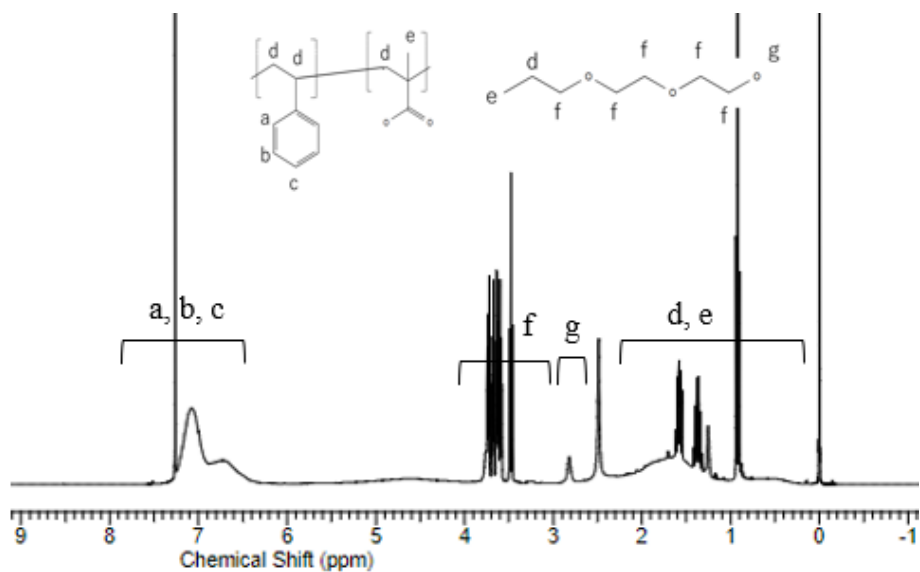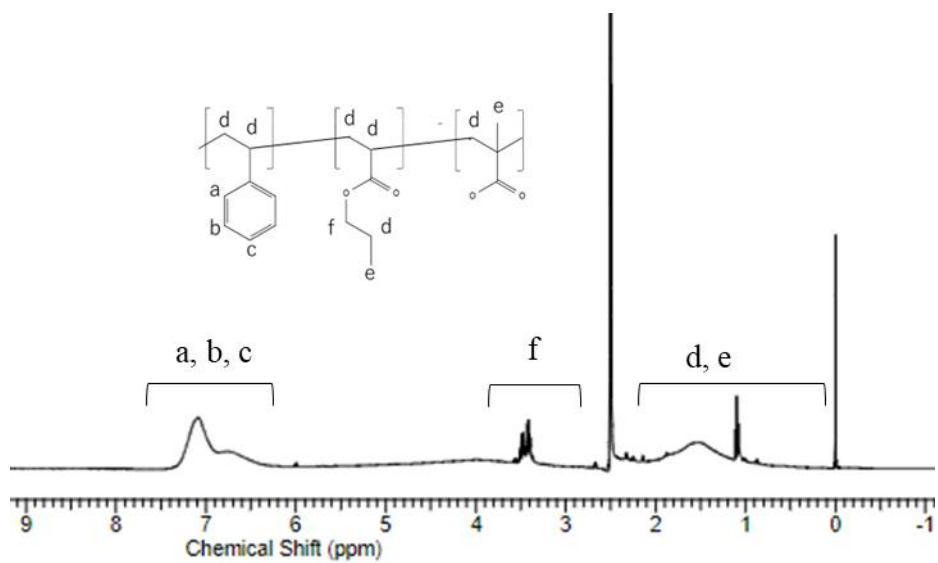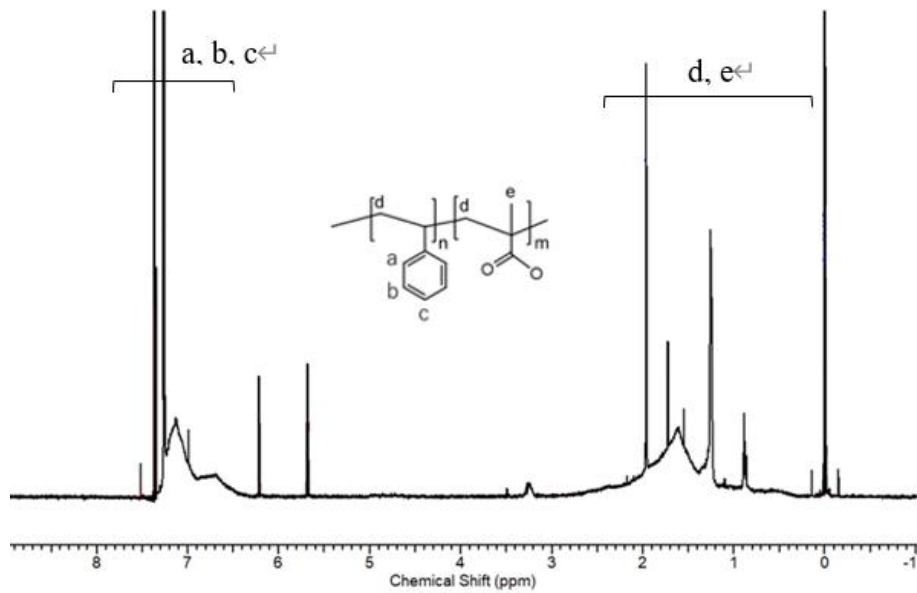
a

b

c

d

e

f

g

h

i

Figure S2. 1HNMR spectra of a) BL01, b) BL02, c) BL03, d) BL04, e) BL05, f) RD01, g) RD02, h) RD03, and i) RD04.
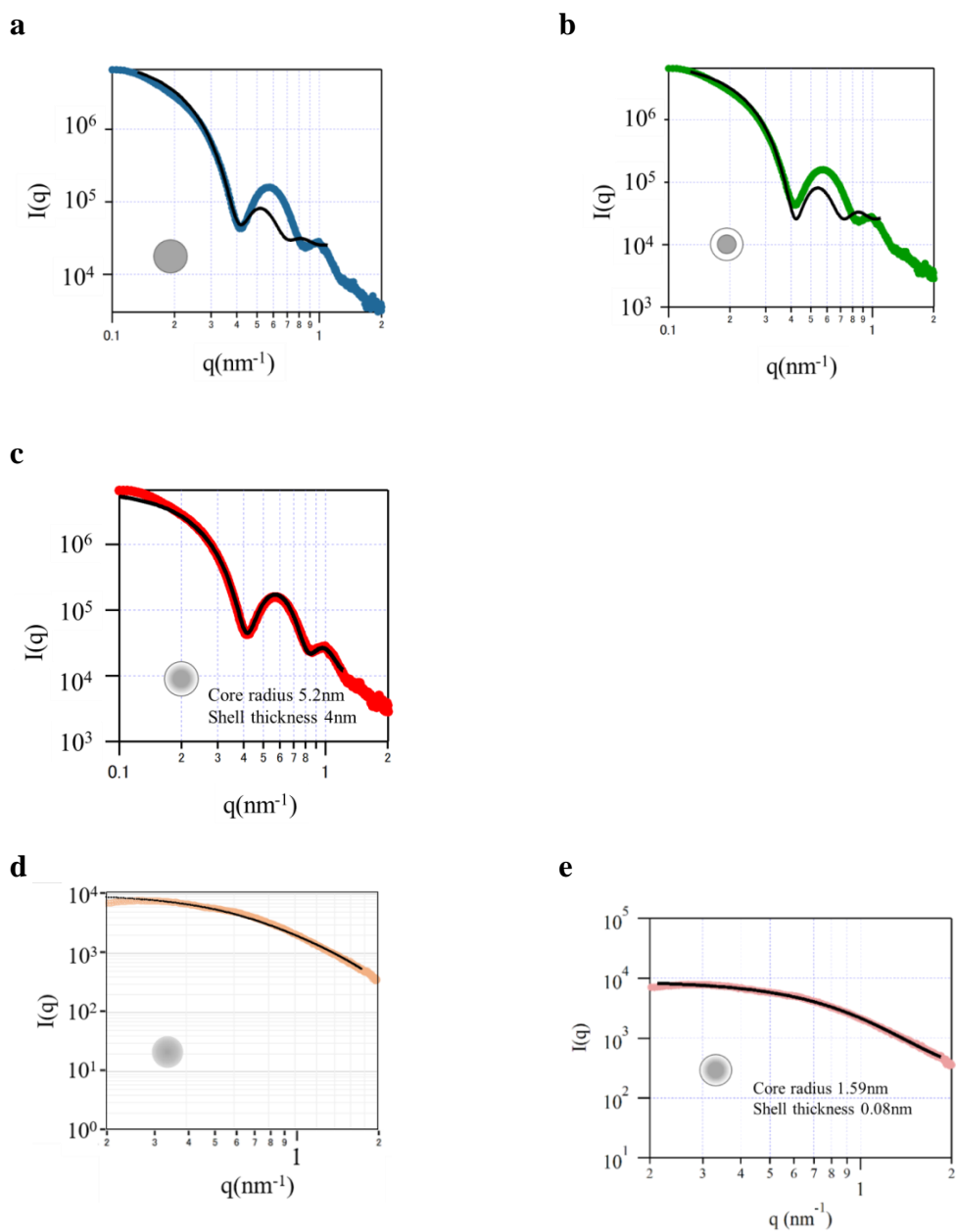
Figure S3. Curve fitting of BL01 by the: a) Gaussian sphere, b) core shell sphere, and c) poly-core models. Curve fitting of RD01 by the d) polydisperse Gaussian coil and e) poly-core models.

a) Gauss sphere model (spheres with a Gaussian size Distribution): Scattering model of the distributed single sphere.

b) Core shell sphere model:[3] Scattering model of the core-shell structure.

c, e) Poly-core model (Polydisperse core with the constant shell thickness model):[4] Scattering model of the distributed core-shell structure.

d) Polydisperse Gaussian coil model:[5] Scattering model of the distributed random coil.
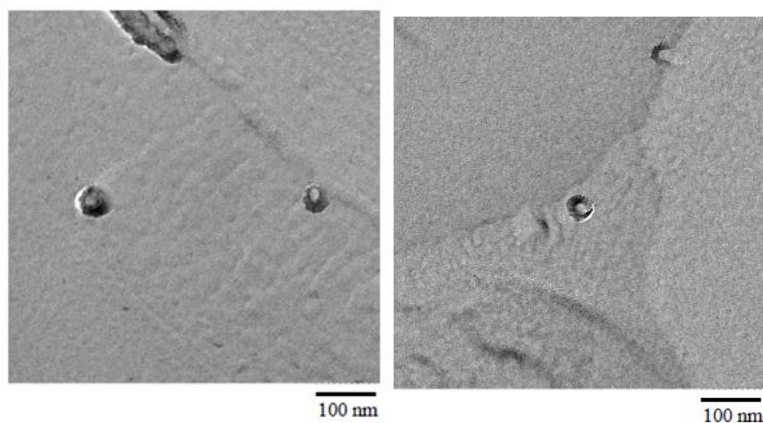
Figure S4. Images of BL05 micelle by Freeze Fracture TEM method. A 5.0wt.% BL05 aqueous solution was frozen in liquid propane, and the cross section was exposed using a glass knife. After platinum was deposited at a 45-degree angle, carbon was deposited at a 90-degree angle. After dissolving the sample with bleach and washing with ultrapure water, the replica image was observed by TEM.
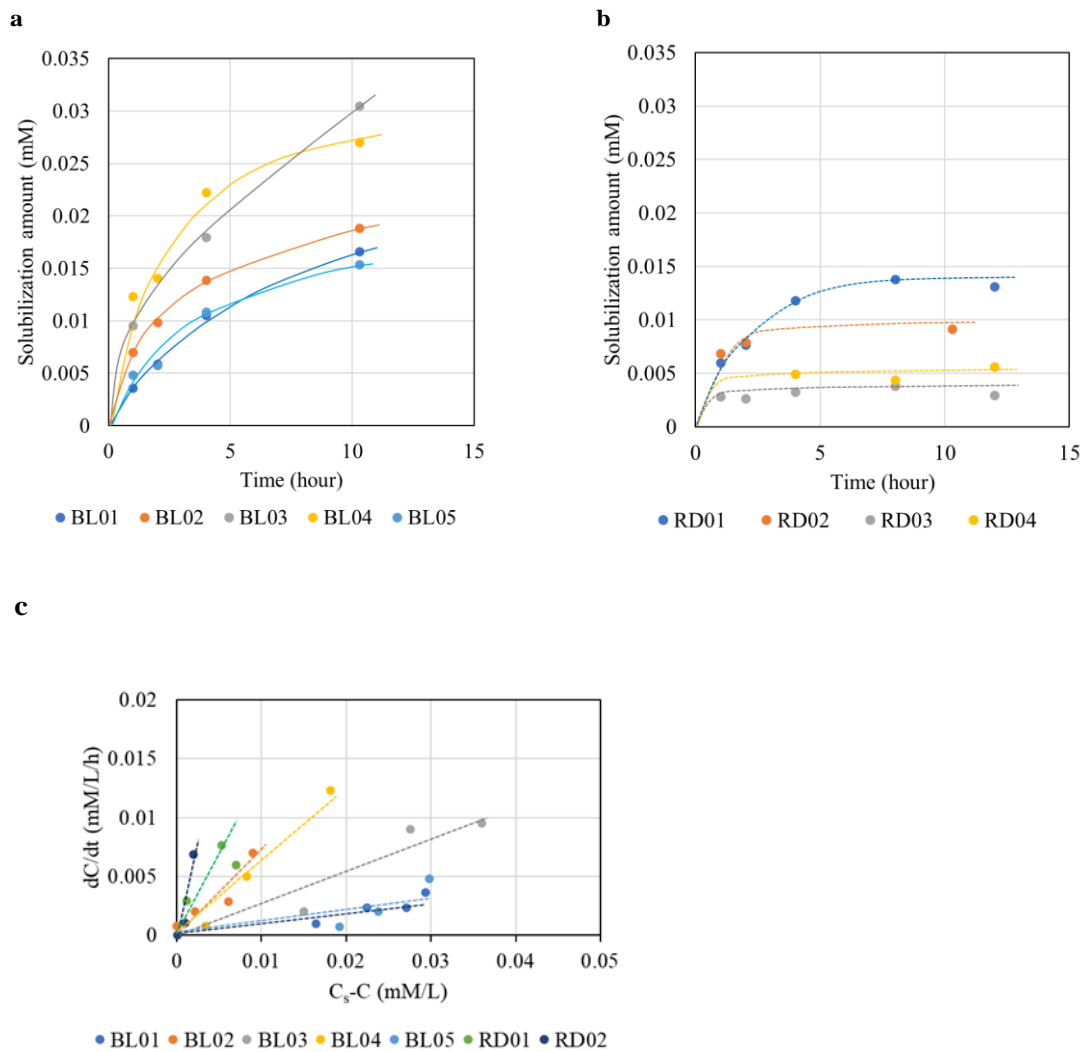
a

b

c

Figure S5 Enlarged view of initial stage for solubilization curve of Oil Orange SS. The polymer micelles of a) Block copolymers and b) Random copolymers, and c) calculation of solubilization rate constant from Eq. 17.
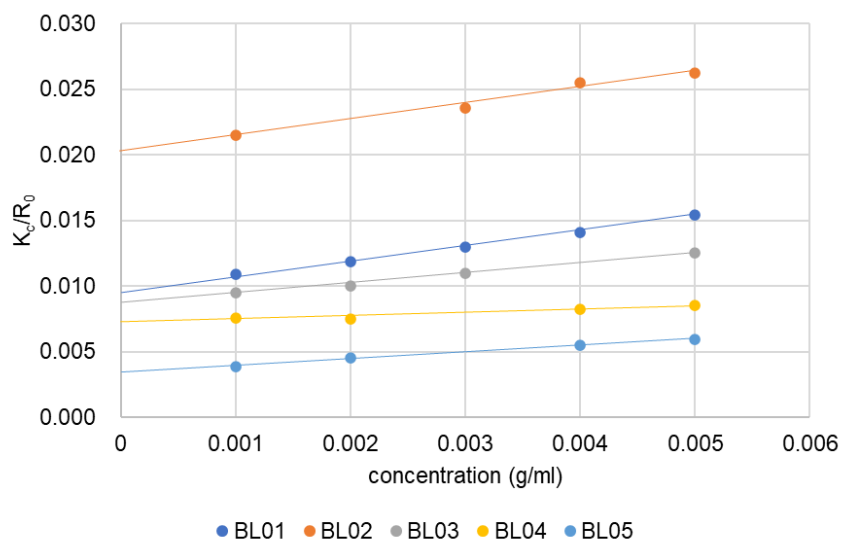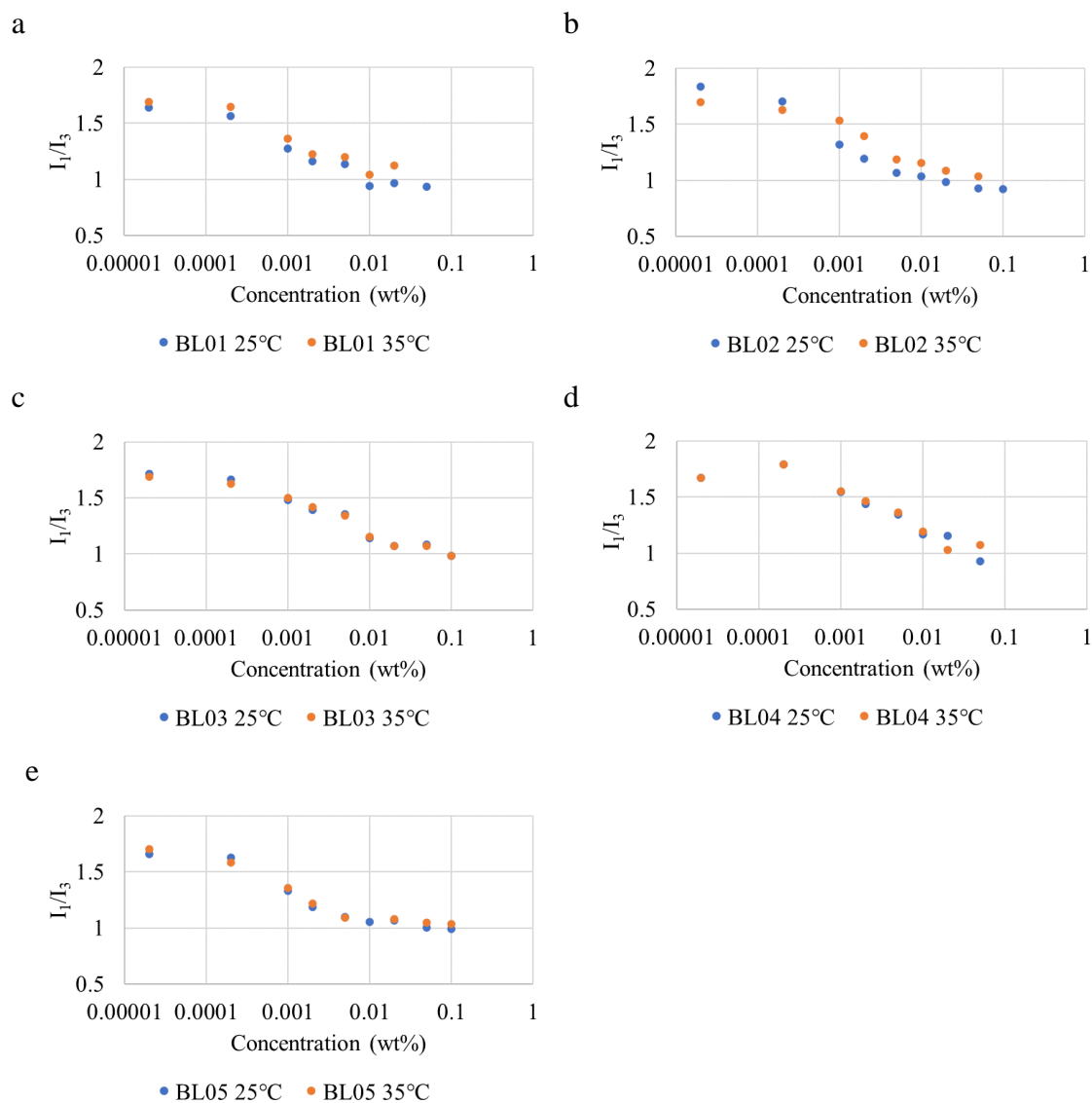
Figure S6. Debye plots of the block polymers.

Figure S7. Variation of the I_1/I_3 ratio in the polymer solution as a function of temperature (25 and 35 °C) a) BL01, b) BL02, c) BL03, d) BL04, and e) BL05.

Table S1. Solubilization rate constant of organic dye into polymers

polymers	k (h^{-1})
BL01	0.10
BL02	0.69
BL03	0.27
BL04	0.65
BL05	0.11
RD01	1.09
RD02	2.92
RD03	>0.80
RD04	>0.56

*: k was calculated from Noyes and Whitney equation.

$$\frac{dC}{dt} = k(C_s - C)$$

Table S2. Amount of dye solubilized per micelle

polymer	number of micelles in 0.5wt.% solution	amount of dye solubilized	dye per micelle
	mol	mol	
BL01	4.84×10^{-5}	3.89×10^{-5}	0.80
BL02	1.01×10^{-4}	2.45×10^{-5}	0.24
BL03	4.33×10^{-5}	5.25×10^{-5}	1.21
BL04	3.57×10^{-5}	3.58×10^{-5}	1.00
BL05	1.72×10^{-5}	4.03×10^{-5}	2.35
RD01	2.13×10^{-4}	1.31×10^{-5}	0.06
RD02	2.1×10^{-5}	8.9×10^{-6}	0.42
RD03	4.88×10^{-4}	2.26×10^{-6}	5×10^{-3}

Table S3. Z average particle size with PDI before and after dye solubilization

polymers	Particle size (Z average/nm)			
	Before solubilization		After solubilization	
	Size	PDI	Size	PDI
BL01	15.7	0.19	30.2	0.38
BL02	10.1	0.38	30.1	0.57
BL03	26.0	0.40	26.9	0.40
BL04	20.0	0.36	30.2	0.70
BL05	21.6	0.21	26.1	0.31

Reference

1. M. Asada, H. Tanaka, Y. Suwa, S. Irifune, S. Osawa and H. Otsuka. Appl. Sci., 2023, 13(3), 1834.

2. S. R. Kline, J. Appl. Cryst., 2006, 39 (6), 895-900.

3. A. Guinier, and G. Fournet, "Small-Angle Scattering of X-Rays", John Wiley and Sons, New York, 1955.

4. Bartlett, P.; Ottewill, R. H. J. Chem. Phys., 1992, 96, 3306.

5. J.S. Higgins, and H.C. Benoit, polymers and Neutron Scattering, Oxford Science Publications, 1996.