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Kondo, Masaaki

2017-04

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Kondo , M , Takahashi , R , Qiu , X-P , Winnik , F M , Terao , K & Sato , T 2017 , ' Small-angle X-ray scattering from the concentrated bulk phase separated from an amphiphilic block-copolymer solution ' , Polymer Journal , vol. 49 , no. 4 , pp. 385-389 . <https://doi.org/10.1038/pj.2016.124>

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<http://hdl.handle.net/10138/308019>

<https://doi.org/10.1038/pj.2016.124>

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## Article

# Small-angle X-ray scattering from the concentrated phase in a phase-separated block-copolymer solution

Masaaki Kondo,<sup>1</sup> Rintaro Takahashi,<sup>1</sup> Xing-Ping Qiu,<sup>2</sup> Françoise M. Winnik,<sup>2,3</sup> Ken Terao,<sup>1</sup> and Takahiro Sato<sup>1</sup>

<sup>1</sup>Department of Macromolecular Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka 560-0043, Japan, <sup>2</sup>Department of Chemistry, University of Montreal, CP 6128 Succursale Centre Ville Montreal, Quebec, Canada H3C 3J7, <sup>3</sup>Department of Chemistry, University of Helsinki, FIN-00014 Helsinki, Finland

Running Head: SAXS from a Block Copolymer Solution

Key Words: Small Angle X-Ray Scattering / Block Copolymer / Phase Separation / Poly(2-oxazoline) /

**ABSTRACT:** Aqueous solutions of the doubly thermosensitive block copolymer poly(2-isopropyl-2-oxazoline)-*b*-poly(2-ethyl-2-oxazoline) heated to 50 °C undergo a macroscopic liquid/liquid phase separation. The small-angle X-ray scattering intensity recorded from the concentrated phase settled on the bottom of a sample indicated that this phase is in the disordered state without any micro-phase separation, although the block copolymer is amphiphilic in water at 50 °C. SAXS measurements performed on the concentrated phase suspended in the form of colloidal droplets in the lean phase indicated that the contribution to the total scattering intensity of individual copolymer chains and their aggregates present in the coexisting concentrated phase of the phase-separated samples was

very small,

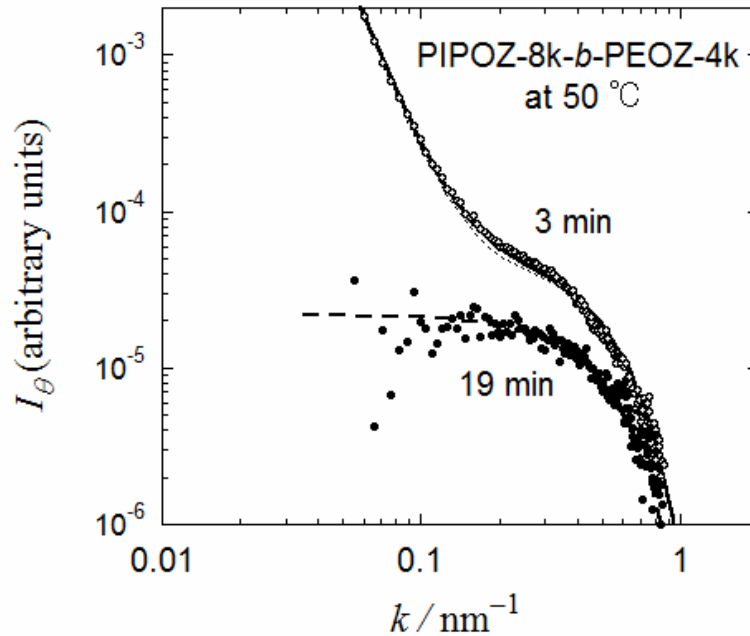
## INTRODUCTION

Block copolymers consisting of a hydrophilic and a thermosensitive block or of **two** thermosensitive blocks form self-assemblies in water upon heating. Extensive studies have been performed recently on the temperature-induced self-assembly of such block copolymers, in view of to their potential applications in drug delivery systems,<sup>1-17</sup> Block copolymers studied so far consist predominantly of a poly(*N*-isopropylacrylamide) (PNIPAM) block and a poly(ethylene glycol).<sup>1-7</sup> With the advent of controlled polymerization techniques, it has become possible to prepare hydrophilic/thermosensitive block copolymers consisting of various kinds of blocks, e.g., PNIPAM-*b*-poly(acrylic acid),<sup>9</sup> PNIPAM-*b*-poly(glycidol),<sup>10</sup> PNIPAM-*b*- poly(*N*-vinyl-2-pyrrolidone),<sup>11</sup>, and doubly thermosensitive block copolymers consisting of two thermosensitive blocks, e.g., block copolymers of vinyl ethers with different pendant groups,<sup>12</sup> block copolymers of polystyrenics and polyacrylates with pendant oxyethylene moieties,<sup>13</sup> and block copolymers of poly(acrylamide)s bearing different alkyl side chains.<sup>15,16</sup>

These hydrophilic/thermosensitive and doubly thermosensitive block copolymers often form self-assembled particles exceeding 100 nm in radius, a value much larger than the contour length of the constituent block copolymer chains. These self-assemblies are not simple spherical micelles.

Recently, we<sup>18</sup> investigated by small-angle X-ray scattering (SAXS) the self-assembled structure formed by a doubly thermosensitive block copolymer poly(2-isopropyl-2-oxazoline)-*b*-poly(2-ethyl-2-oxazoline) (PIPOZ-*b*-PEOZ) at 50 °C, a temperature for which the copolymer is amphiphilic. In aqueous solutions kept at 50 °C, the copolymer forms star-like micelles that further aggregate to form homogeneous concentrated-phase droplets. Eventually, the droplets coalesce into a liquid bulk phase. In Figure 1 we present SAXS results obtained in a previous study of an aqueous solution of PIPOZ-*b*-PEOZ heated at 50 °C

for 3 min (open circles) and 19 min (full circles). Just after heating, the concentrated-phase droplets coexist with star-like micelles in the dilute phase and, after 19-min, the droplet scattering signal disappears, an indication that the droplets have settled to the bottom of the sample outside the volume probed by the X-Ray beam. The dashed and solid curves in the figure are theoretical curves for star-like micelles with an aggregation number of 11 and for a mixture of star-like micelles and polydisperse spherical particles with a copolymer concentration as high as  $0.8 \text{ g/cm}^3$ .<sup>18</sup> As the SAXS profile with open circles has no sharp diffraction peak, it would seem that the concentrated-phase droplets do not undergo microphase separation.



**Figure 1.** SAXS profiles of an aqueous solution of the sample PIPOZ-8k-*b*-PEOZ-4k (the original concentration:  $0.0246 \text{ g/cm}^3$ ) heated at  $50 \text{ }^\circ\text{C}$  for 3 min (open circles) and 19 min (full circles), and fitting results obtained previously (solid and dashed curves).<sup>18</sup> See eq 8 and text following the equation for the thin dotted curve close to the open circles.

In the present study, we carried out SAXS measurements on the coexisting concentrated bulk phase of aqueous PIPOZ-*b*-PEOZ after macroscopic liquid/liquid phase separation. The

aim of this study is twofold. (1) We characterize precisely the coexisting concentrated phase macroscopically separated from the heated aqueous block copolymer solution; (2) We assess whether or not microphase separation occurs in the concentrated phase of aqueous PIPOZ-*b*-PEOZ after prolonged heating at 50 °C. If microphase separation only occurs in the highly concentrated phase after a long time, the observations after a few minutes reported previously were not adequate to conclude that microphase separation does not occur in this system. Very recently, Sato and Takahashi<sup>19</sup> predicted theoretically the coexistence of star-like micelles and a homogeneous concentrated phase in a weakly amphiphilic block copolymer solution. The experimental evaluation of the microphase separation status in the concentrated phase is important to confirm this theoretical prediction.

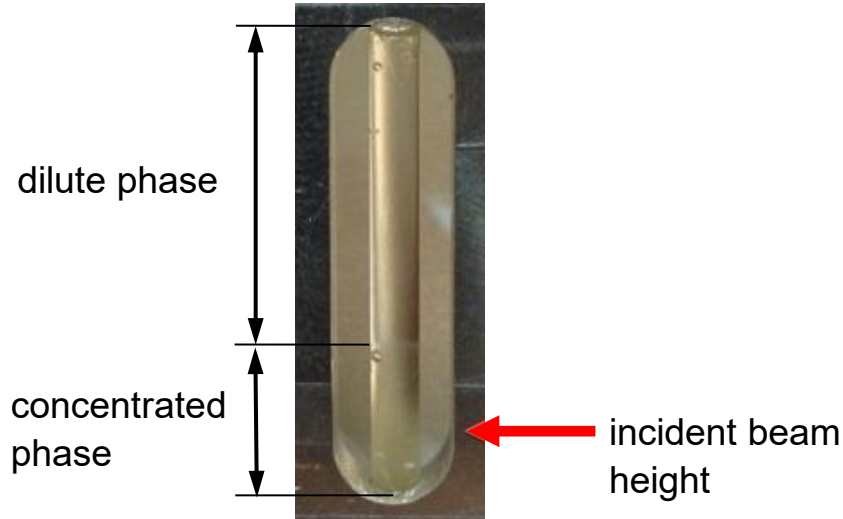
## EXPERIMENTAL PROCEDURE

The PIPOZ-5k-*b*-PEOZ-4k sample used for SAXS measurements was synthesized as described previously.<sup>18</sup> The number average molecular weight  $M_1$  and the molecular weight dispersity of the sample determined by SEC-MALS were 9,100 g/mol and 1.04, respectively. The mole fraction of IPOZ monomer in the copolymer estimated by <sup>1</sup>H NMR was 0.53. SAXS measurements were performed at the BL40B2 beamline in SPring-8 with an incident light of 0.1 nm wavelength and a camera length of 4164 mm. The scattered X-ray intensity was detected by a RIGAKU R-AXIS VII imaging plate detector. The sample was dissolved in water to prepare the test solution of mass concentration  $c = 0.2$  g/cm<sup>3</sup>. The solution was placed in a capillary cell with a 1.9-mm inside diameter. It separated into two macroscopic phases after being kept at 50 °C for 240 min. Then, the incident X-ray beam was put on for 180 sec focused at the separated concentrated phase to obtain the excess scattering intensity  $I_\theta$  of the coexisting concentrated phase over the solvent intensity. As a reference, the SAXS measurement was also performed on the aqueous solution of PIPOZ-5k-*b*-PEOZ-4k before phase separation at 25 °C.

## RESULTS AND DISCUSSION

Figure 2 shows a photograph of a phase-separated sample of PIPOZ-5k-*b*-PEOZ-4k within the capillary cell used for the SAXS measurements. Macroscopic phase separation was attained after the solution was kept at 50 °C for 240 min. The bottom concentrated phase was slightly turbid. The incident X-ray beam was focused at the height indicated by the red arrow in Figure 2. The excess SAXS intensity  $I_\theta$  was measured under these conditions.

Figure 3 presents the angular dependence of  $I_\theta$  (circles) for the concentrated phase at 50 °C, in the configuration shown in Figure 2, as well as for the same solution at 25 °C where it exists as a single phase. The abscissa is the magnitude of the scattering vector  $k$ . Let us first consider the result at 25 °C. At  $k > 0.1 \text{ nm}^{-1}$ , the data points can be fitted to the scattering function for a homogeneous concentrated polymer solution given by<sup>20</sup>



**Figure 2.** Picture of the phase-separated aqueous solution of PIPOZ-5k-*b*-PEOZ-4k ( $c = 0.2 \text{ g/cm}^3$ ) in a capillary cell used for the SAXS measurement.

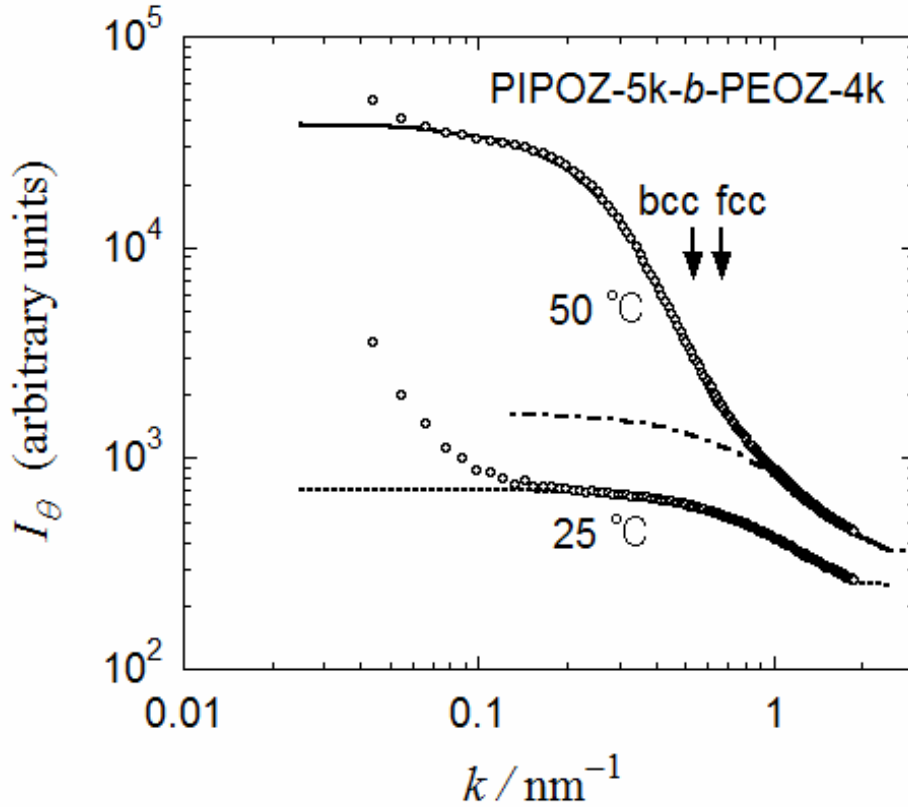
$$I_\theta = \frac{FM_1P_1(k)c}{1 + 2\gamma_{11}M_1P_1(k)c} \quad (1)$$

where  $F$  is an instrumental constant,  $M_1$ ,  $P_1(k)$ , and  $\gamma_{11}$  are, respectively, the molar mass,

particle scattering function, and effective second virial coefficient of the copolymer chain. Assuming a Gaussian chain for the copolymer chain, and ignoring the difference in the excess electron density between the PIPOZ and PEOZ blocks (ca. 10 % difference), we write  $P_1(k)$  as<sup>21, 22</sup>

$$P_1(k) = \frac{2 \left[ \exp(-k^2 \langle S^2 \rangle_1) - 1 + k^2 \langle S^2 \rangle_1 \right]}{(k^2 \langle S^2 \rangle_1)^2 \exp\left(\frac{1}{16} k^2 d_1^2\right)} \quad (2)$$

where  $\langle S^2 \rangle_1$  and  $d_1$  are the mean square radius of gyration and the thickness of the Gaussian chain, respectively. Although water is a good solvent for PIPOZ-*b*-PEOZ at 25 °C, the molar mass  $M_1$  (= 9,100 g/mol) is so low that the excluded volume effect may be neglected.



**Figure 3.** SAXS profiles of an aqueous solution of PIPOZ-5k-*b*-PEOZ-4k in the one-phase state at 25 °C ( $c = 0.2 \text{ g/cm}^3$ ) and of the coexisting concentrated phase in the same test solution phase-separated at 50 °C. The solid curve indicates theoretical values calculated by eqs 3-6 by using  $c = 0.39 \text{ g/cm}^3$ ,  $M_1 = 9,100 \text{ g/mol}$ , and parameters listed in Table 1. The dotted curve indicates theoretical values for the single chain component, calculated by eqs 1



and 2 with  $c = 0.20 \text{ g/cm}^3$ ,  $M_1 = 9,100 \text{ g/mol}$ , See the text for the dot-dash curve.

Sung and Lee<sup>23</sup> reported the unperturbed dimension of PEOZ in THF. We assumed that the unperturbed dimension of PIPOZ-5k-*b*-PEOZ-4k in 25 °C water is approximately the same as that of PEOZ with the same degree of polymerization in THF, i.e.,  $\langle S^2 \rangle_1^{1/2} = 2.3 \text{ nm}$ , and fitted eqs 1 and 2 to the experimental SAXS profile at 25 °C at  $k > 0.15 \text{ nm}^{-1}$ . The dotted curve in Figure 3 shows the fitting result. By this fitting, we determined  $\gamma_{11} = 4.3 \times 10^{-4} \text{ cm}^3\text{g}^{-2}\text{mol}$  and  $d_1^2 = -2.8 \text{ nm}^2$ , as well as the instrumental constant  $F$ , almost uniquely. The value of the effective second virial coefficient indicates that water is a good solvent for PIPOZ-5k-*b*-PEOZ-4k at 25 °C. The negative  $d_1^2$  chosen is physically unreasonable but it has been reported in several previous SAXS studies on polymers in dilute solutions.<sup>24-26</sup> It may be due to the heterogeneous electron density distribution in the thickness direction of the polymer chain.<sup>26, 27</sup>

The scattering function at 25 °C shows a sharp upswing at  $k < 0.15 \text{ nm}^{-1}$ , indicating the existence in the solution of a very small amount of a large scattering component. It is known that polymer solutions sometimes contain minute amounts of “irreversible aggregates” that can only be detected by this scattering technique, even if the solution is thermodynamically in the one phase region in the phase diagram. Because PIPOZ is known as a crystalline polymer, a small amount of PIPOZ-*b*-PEOZ may form semi-crystalline aggregates in the semi-concentrated aqueous solution ( $c = 0.2 \text{ g/cm}^3$ ) used here.

The scattering function for the coexisting concentrated phase at 50 °C in Figure 3 shows a  $k$  dependence similar to that of the single chain before phase separation at 25 °C at  $k \gtrsim 1 \text{ nm}^{-1}$ . It has a strong contribution decaying at lower  $k$ , indicating the existence of a larger aggregated component present in the concentrated phase along with the single-chain component, contributing to  $I_\theta$  at high  $k$ . Thus, we tried to fit  $I_\theta$  at 50 °C by<sup>28</sup>

$$I_{\theta} = \frac{FM_1c \left[ w_1P_1(k) + w_2m_{w,2}P_2(k) + 2w_1w_2m_{w,2}P_1(k)P_2(k)(\gamma_{11} + \gamma_{22} - 2\gamma_{12})M_1c \right]}{\left[ 1 + 2w_1P_1(k)\gamma_{11}M_1c \right] \left[ 1 + 2w_2m_{w,2}P_2(k)\gamma_{22}M_1c \right] - 4w_1w_2m_{w,2}P_1(k)P_2(k)(\gamma_{12}M_1c)^2} \quad (3)$$

where  $w_i$ ,  $M_i$ , and  $P_i(k)$  are, respectively, the weight fraction, the molar mass, and the particle scattering function of the component  $i$  ( $i = 1$  for the single chain and  $i = 2$  for the large aggregating component),  $m_{w,2}$  is the weight-average aggregation number of the component 2, and  $\gamma_{ij}$  is the effective second virial coefficient between the components  $i$  and  $j$  ( $i, j = 1$  and  $2$ ). Component 2 may be responsible for the slight turbidity of the concentrated phase shown in Figure 2. We approximate  $P_2(k)$  of the component 2 by the scattering function for polydisperse spherical particles given by

$$m_{w,2}P_2(k) = \int_0^{\infty} \left[ 3 \times \frac{\sin(kR) - kR \cos(kR)}{(kR)^3} \right]^2 m_2 w(m_2) dm_2 \quad (4)$$

where  $m_2$  is the aggregation number of each spherical particle component,  $R$  is the radius of the sphere with  $m_2$ , and  $w(m_2)$  is the weight fraction of the sphere with  $m_2$  in the total component 2. The former is calculated from  $m_2$  by

$$R = \left( \frac{3m_2M_1}{4\pi N_A c_{in,2}} \right)^{1/3} \quad (5)$$

with the Avogadro constant  $N_A$  and the internal copolymer mass concentration  $c_{in,2}$  of the sphere, and the latter is assumed to be expressed by the log-normal distribution given by

$$w(m_2) = \frac{1}{\sqrt{2\pi \ln(m_{w,2}/m_{n,2})} m_2} \exp \left[ -\frac{\ln^2(m_2/\sqrt{m_{w,2}m_{n,2}})}{2 \ln(m_{w,2}/m_{n,2})} \right] \quad (6)$$

with the number-average aggregation number  $m_{n,2}$  of the component 2.

Among many parameters and functions in eqs 3-6, we chose the same  $M_1$  and  $\langle S^2 \rangle_1^{1/2}$ , as well as  $F$ , as those used at 25 °C. (Because the excluded volume effect can be neglected due to the low  $M_1$  value, the single copolymer chain may keep the unperturbed state even in the poor solvent.) The total copolymer mass concentration  $c$  of the concentrated phase was determined gravimetrically<sup>18</sup> to be 0.39 g/cm<sup>3</sup>. Then, we first fitted  $I_{\theta}$  at high  $k$  by eq 1 to

determine  $\gamma_{11}$  and  $d_1^2$ . Next, we changed  $w_1 (= 1 - w_2)$  and  $m_{w,2}$  to fit the plateau of  $I_\theta$  at low  $k$  by eq 3, and then fitted  $I_\theta$  in the intermediate  $k$  by eq 3 adjusting  $m_{w,2}$ ,  $c_{in,2}$ , and  $m_{w,2}/m_{n,2}$ . According to the change of  $w_1$ , we re-adjusted  $\gamma_{11}$  to fit  $I_\theta$  at high  $k$  again. Since water is a poor solvent for the copolymer at 50 °C, the effective second virial coefficients  $\gamma_{22}$  and  $\gamma_{12}$  were assumed to be zero at the first fitting, and then  $\gamma_{22}$  was slightly adjusted to obtain the best fitting in the low and intermediate  $k$  region;  $\gamma_{12}$  was kept to be zero during the whole fitting because of its insensitiveness to  $I_\theta$ .

The fitting result is indicated by the solid curve in Figure 3, and the parameter values chosen were listed in Table 1. The dash-dot curve in Figure 3 indicates the scattering component for the single chain (the component 1) calculated by eqs 1 and 2 with  $c$  replaced by  $cw_1$  and the same  $w_1$ ,  $M_1$ ,  $\langle S^2 \rangle_1$ ,  $\gamma_{11}$ , and  $d_1^2$  as in the case of the solid curve. (The value of  $d_1^2$  was slightly different from that at 25 °C, which may arise from the dehydration of the PIPOZ block chain at 50 °C.)

**Table 1.** Characteristics of the scattering components in the coexisting concentrated phase at 50 °C.

$i$	component	$w_i$	$m_{w,i}$	$m_{w,i}/m_{n,i}$	$\langle S^2 \rangle_i^{1/2}/\text{nm}$	$d_i^2/\text{nm}^2$	$\gamma_{ii}/\text{cm}^3\text{g}^{-2}\text{mol}^a$
1	single chain	0.9	1	1	2.3 (2.3) <sup>b</sup>	-2.0 (-2.8) <sup>b</sup>	$1.5 \times 10^{-4}$ $(4.3 \times 10^{-4})^b$
2	aggregate	0.1	112	2	6.6 <sup>c</sup>		$1 \times 10^{-6}$

<sup>a</sup>  $\gamma_{12} = 0 \text{ cm}^3\text{g}^{-2}\text{mol}$ . <sup>b</sup> Value at 25 °C. <sup>c</sup> Calculated from  $c_{in,2} = 1.14 \text{ g/cm}^3$ .

As seen from the solid and dash-dot curves, the scattering from the component 2 is predominant in the low  $k$  region, but the weight fraction  $w_2$  is only 10 %, and the single-chain component 1 is predominant in the concentrated phase. The component 2 consists of ca. 100 copolymer chains and has the z-average root-mean-square radius of gyration  $\langle S^2 \rangle_2^{1/2}$  of 6.6 nm.

It has a very high density  $c_{in,2} = 1.14 \text{ g/cm}^3$ , which is identical to the inverse of the partial specific volume of the copolymer. These results imply that the component 2 is the micro-crystallite of the copolymer. Although we do not have evidence for the crystallite formation in our solution, some researchers<sup>29-31</sup> have reported the formation of micro-crystallites in phase-separated aqueous PIPOZ samples.

One may expect micro-phase separation in concentrated solutions of amphiphilic block copolymers.<sup>32</sup> If micro-phase separation would take place in the concentrated phase of aqueous PIPOZ-*b*-PEOZ, it should be possible to observe some diffraction peaks in the SAXS profile. If spherical micelles of aggregation number  $m$  form body-centered cubic (bcc) or face-centered cubic (fcc) lattices in the concentrated phase, a first-order diffraction peak should appear at  $k = k_d$  indicated by the arrows in Figure 3, which was calculated by

$$k_d = 2\pi \left( \frac{N_A c}{m M_1 N} \right)^{1/3} \quad (7)$$

with the number of micelles per unit cell  $N = 2$  for bcc and 4 for fcc, as well as  $M_1 = 9,100 \text{ g/mol}$ ,  $m = 10$ ,<sup>18</sup> and  $c = 0.39 \text{ g/cm}^3$ . There are no peaks in the SAXS profile for the concentrated phase at 50 °C around this  $k$  region. Hence, we conclude that the concentrated phase of the phase-separated solution of PIPOZ-*b*-PEOZ is disordered, and that micro-phase separation does not take place. Although differential scanning calorimetry demonstrated that the dehydration occurs only for the PIPOZ block chain in PIPOZ-*b*-PEOZ in 50 °C water and that star-like micelles form in the dilute phase (cf. Figure 1), the amphiphilicity of PIPOZ-*b*-PEOZ may not be strong enough for micro-phase separation to take place in the concentrated phase. A similar conclusion was reached in a theoretical study of Sato and Takahashi<sup>19</sup> on the coexistence of star-like micelles and a homogeneous concentrated phase in dilute solutions of weakly amphiphilic block copolymers.

In the previous study,<sup>18</sup> we fitted the SAXS profile for the phase-separated dilute solution of a PIPOZ-*b*-PEOZ sample, where the dilute phase contains micelles (the

component S) with a weight-average aggregation number  $m_{w,S}$  and the concentrated phase exists as colloidal droplets (the component L) consisting of  $m_{w,L}$  copolymer chains, by the equation

$$I_{\theta} = FM_1c \left[ w_S m_{w,S} P_S(k) + w_L m_{w,L} P_L(k) \right] \quad (8)$$

In Figure 1, we have reproduced the previous SAXS profile (open circles) for a dilute aqueous solution of PIPOZ-8k-*b*-PEOZ-4k with  $c = 0.0246 \text{ g/cm}^3$  heated at 50 °C for 3 min. At higher and lower  $k$ , the contributions of the first and second terms of eq 8, respectively, are predominant, so we can almost uniquely determine  $w_i$ ,  $m_{w,i}$ , and  $P_i(k)$  ( $i = S, L$ ), by fitting the SAXS profile as shown in Figure 1, which characterizes both the polymer species in the coexisting dilute phase and the colloidal particle of the coexisting concentrated phase in the phase-separated polymer solution.

In the previous study, we calculated  $P_L(k)$  of the concentrated-phase droplets by eqs 4–6. Although eqs 4–6 are the scattering function for uniform density spheres, the concentrated phase is not perfectly uniform, and the individual single chains and aggregating components in the concentrated phase scatter X-ray, as demonstrated in Figure 3. We here examine the contribution of the heterogeneity in the concentrated-phase droplet to  $P_L(k)$ . The scattering function for composite particles is known to consist of the form factor of the sub-particle and the structure factor of the composite particle.<sup>33</sup> When the concentrated-phase droplet is viewed as a sphere consisting of single chains and their aggregates,  $P_L(k)$  may be written as

$$P_L(k) = \frac{w_1 P_1(k) + w_2 m_{w,2} P_2(k)}{w_1 + w_2 m_{w,2}} P_{\text{UDS}}(k) \quad (9)$$

where  $P_1(k)$  and  $P_2(k)$  are *the form factors* of the single chain and aggregates in the concentrated phase, respectively, and  $P_{\text{UDS}}(k)$  is *the structure factor* of the uniform density sphere. The previous study<sup>18</sup> used  $P_L(k)$  calculated by eq 9 with  $P_1(k) = P_2(k) = 1$ , and the previous fitting result is shown by the solid curve in Figure 1.

Assuming that the concentrated-phase droplet in the aqueous solution of the previous sample PIPOZ-8k-*b*-PEOZ-4k contained the aggregating component same as that in the concentrated phase of the aqueous solution of the present sample PIPOZ-5k-*b*-PEOZ-4k, we have calculated  $P_L(k)$  by eq 9 and then  $I_\theta$  by eq 8. As shown by the thin dotted curve in Figure 1, the result is very close to the solid curve, demonstrating that the form factors of the individual single chains and their aggregates in the concentrated-phase droplet little affects the total scattering function. It is difficult to study the heterogeneity inside the concentrated phase in the colloidal droplet state by SAXS.

## CONCLUSION

Small-angle X-ray scattering measurements were performed on the macroscopically phase-separated concentrated phase of an aqueous solution of the thermosensitive block copolymer poly(2-isopropyl-2-oxazoline)-*b*-poly(2-ethyl-2-oxazoline) at 50 °C. The scattering function obtained was fitted by the theoretical model of a mixture of single copolymer chains and very condensed aggregates of chains in the disordered state, demonstrating that micro-phase separation does not take place in the concentrated phase of this amphiphilic block copolymer. This result is consistent with a theoretical prediction<sup>19</sup> that star-like micelles and a homogeneous concentrated phase can coexist in dilute solutions of weakly amphiphilic block copolymers.

Furthermore, it turned out that the contribution to the total scattering intensity of individual copolymer chains and their aggregates in the coexisting concentrated-phase droplet is very small in the case of the phase-separated polymer solutions where the concentrated phase exists in the form of colloidal droplets. This confirms the validity of the method for characterizing the coexisting concentrated phase dispersed as colloidal particles in a phase-separated solution by SAXS used previously.<sup>18</sup>

## ACKNOWLEDGMENT

The synchrotron radiation experiments were performed at the BL40B2 in SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) (Proposal No. 2013B1647).

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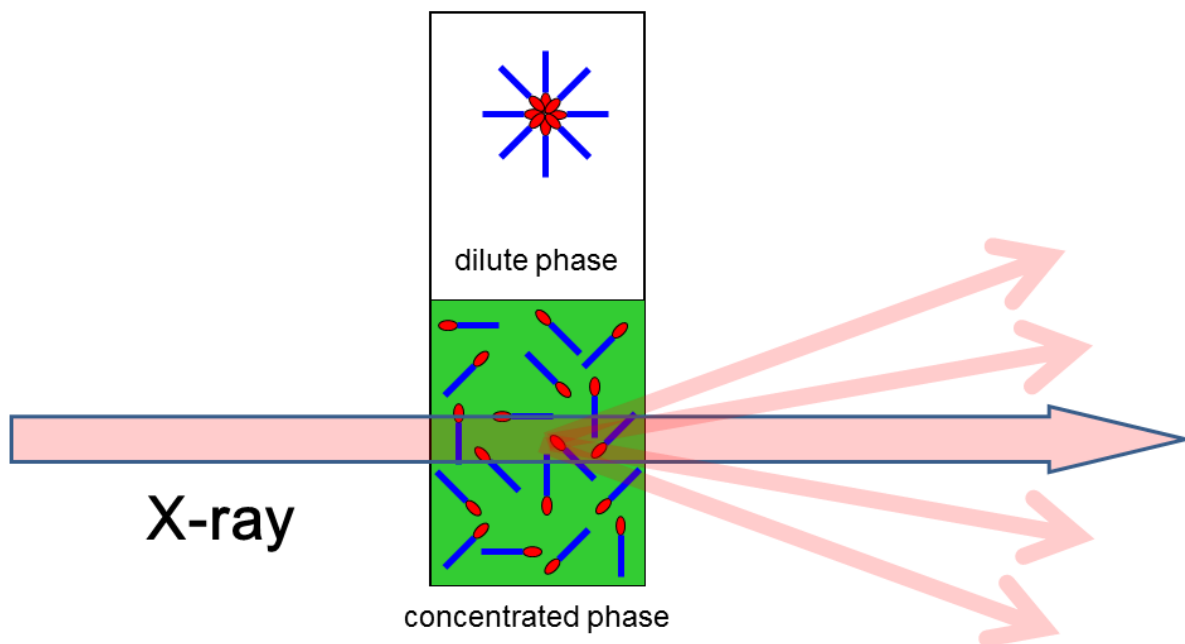


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## Graphical Abstract

“Small-angle X-ray scattering from the concentrated phase in a phase-separated block-copolymer solution” by Masaaki Kondo, Rintaro Takahashi, Xing-Ping Qiu, Françoise M. Winnik, Ken Terao, and Takahiro Sato



The small-angle X-ray scattering intensity from the coexisting concentrated phase was measured in a phase-separated aqueous solution of the thermosensitive block copolymer poly(2-isopropyl-2-oxazoline)-*b*-poly(2-ethyl-2-oxazoline) under the amphiphilic condition (50 °C). It turned out that the concentrated phase is disordered with no sign of phase separation. In addition, the scattering from individual copolymer chains and their aggregates in the concentrated phase hardly contributes to the total scattering intensity of the phase-separated colloidal solution of the block copolymer.