Synthesis of Fine Hydrogel Microspheres and Capsules from Thermoresponsive Coacervate

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ABSTRACT: Poly(4-hydroxybutyl vinyl ether) [poly(HOBVE)] and the related copolymers, which showed thermally induced phase separation in water, are synthesized via living cationic copolymerization. When the aqueous solution of copoly(HOBVE) is heated, coacervate droplets are observed in micrograph. This is the thermoresponsive copolymer exhibiting coacervation accompanied by the liquid−liquid-phase separations though unlike precipitation. To obtain hydrogel microspheres from the droplets, highly random copolymers of HOBVE and 2-(vinyloxy)ethyl methacrylate (VEM), that is poly(HOBVE_{1-x}-*r*-VEM_x), are synthesized by sequential living cationic copolymerization, which possess evenly photofunctional units. The initially formed coacervate droplets slightly above phase separation temperature can be cross-linked by UV (254 nm) irradiating in water, to form fine hydrogel microspheres. The obtained hydrogel microspheres show the thermoresponsiveness and adsorption/desorption effect, whose size and form are confirmed by the measurement of dynamic light scattering and by atomic force microscopy, respectively. On the basis of the results between structure of the copolymer and the polydispersity index of obtained hydrogel microsphere, it is important that the sequence distribution of VEM as cross-linking point in the copolymer is controlled, to prepare the fine hydrogel microspheres or capsules from thermoresponsive coacervate. In addition, including the coacervate droplet in a core allows one to synthesize the hydrogel capsule.

Introduction

Recent studies have witnessed the appearance of a vast number of synthetic functional polymers, including an increasing number of stimuli-responsive polymers.[1−6] Stimuli-responsiveness of polymers refers to relatively large and sharp physical or chemical changes in solution, on the surface, or in cross-linked polymers systems in response to a slight external stimulus toward a desired outcome. For physical stimuli-responsive polymers, thermoresponsive polymers such as poly(*N*-isopropylacrylamide) (PNIPAM) have been well-examined by many chemists.[7] The polymers are soluble in water at low temperatures but become insoluble above a threshold temperature (T_{PS}), known as the lower critical solution temperature (LCST). A coil−globule transition[8] occurs at the temperature, leading to precipitation of the PNIPAM aggregates or shrinkage of the cross-linked hydorogel. In such thermoresponsive polymers, there are the polymers exhibiting coacervation accompanied by the liquid−liquid-phase separations though unlike precipitation. Examples exist of themoresponsive coacervation from elastin as biomacrmolecules,[9] copoly(*N*,*N*-dimethylacrylamide),[10,11] poly(*N*-vinylamide-*co*-vinyl acetate),[12] poly(isobutyl vinyl ether-*r*-2-hydroxyethyl vinyl ether),[13] and poly(*N*-isopropylacrylamide-*co*-2-hydroxyisopropylacrylamide).[14] The key to form the coacervate, i.e., coacervation, is partial dehydration from the copolymer above T_{PS}.[10,13,14] The coacervation can be observed by the formation of droplets comprised of a concentration polymer phase, which is valuable bioscience in the purifications of bioactive molecules and tissue engineering.[15,16]

Among coacervations, two types of classification have used. One classification is simple coacervation or segregation phase separation treated in this study and the other is complex coacervation or associative phase separation.[17,18] Simple coacervation is caused by changing the solvent composition or the solution temperature, whereas complex coacervation is induced by ion complexes between polyelectrolytes and natural polymers such as chitosan/poly(ethylene glycol)−alginate.[19] In the case of simple coacervation such as synthetic thermoresponsive polymers, coacervate droplets are unstable, because the coacervate droplets are reversibly formed above T_{PS} and vanish below the T_{PS}. The current bottleneck is difficult to separate of bioactive molecules such as target protein from the coacervation in the fields of bioscience. Thus, stable coacervate droplets, which are microparticle, are required. Stöver’s groups refer to a similar problem on coacervate droplets.[11,20]

Recently, Sugihara et al. reported that poly(4-hydroxybutyl vinyl ether) and the related copolymers were found to undergo phase separation in water at a critical temperature, depending on their composition.[21] The coacervate droplets (sphere) were observed in the micrograph of the opaque solution even at low concentration. These polymers can be synthesized by living cationic polymerization to obtain the copolymers with controlled architecture such as molecular weight, molecular weight distribution (MWD), and sequence of constitutional repeating units along the main chain. In this study, therefore, we examined the precision synthesis of stable hydrogel microspheres by thermoresponsive coacervation from aqueous poly(4-hydroxybutyl vinyl ether).

There are a few examples of hydrogel microspheres by thermoresponsive coacervation.[11,20,22] However, there are no example of precision synthesis of hydrogel microsphere from thermoresponsive coacervation. Thus, the feasibility of the precision synthesis of hydrogel microparticle was first examined by sequential living cationic copolymerization.

Scheme 1 shows a schematic of a synthetic process of hydrogel microsphers. The copolymer, to form thermoresponsive coacervate droplets in water, have evenly photofunctional units

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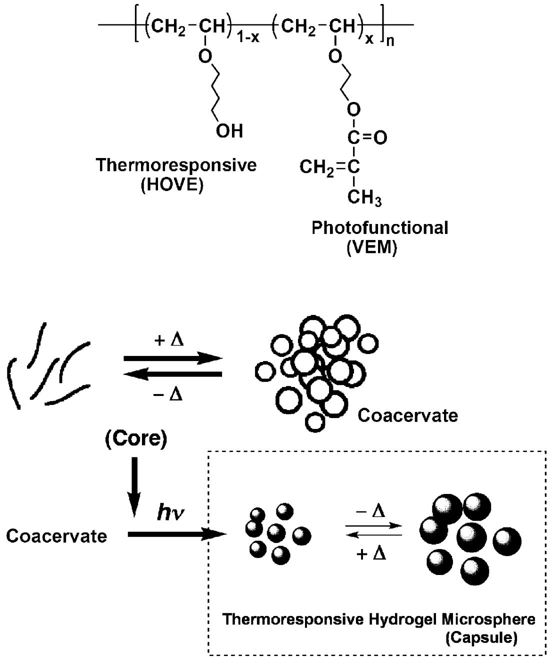
Scheme 1. Synthesis of Thermoresponsive Hydrogel Microspheres (Capsules)



(homogeneous cross-linking point), prepared by sequential living cationic copolymerization. This is the highly random copolymer prepared from both 4-hydroxybutyl vinyl ether (HOBVE) and 2-(vinyloxy)ethyl methacrylate (VEM), that is poly(HOBVE$_{1-x}$-*r*-VEM$_x$). VEM possess both cationically polymerizable vinyl ether and a radically or anionically polymerizable methacryloyl group.[23,24] The cationically obtained poly(VEM) retained the metacryloyl groups intact and readily cross-linked on exposure to heat, light, or X-ray.[24] The initially formed coacervate droplets above T_{PS} in water can be cross-linked by UV (254 nm) irradiating, to form fine and homogeneous hydrogel microspheres. Since the hydrogel microspheres are synthesized by thermoresponsive coacervation, the products are expected to have thermoresponsiveness in water. In addition, the coacervate droplet included in a core allows the synthesis of hydrogel capsule. On the basis of experimental data, we discuss the importance of sequence distribution to prepare the fine hydrogel microspheres or capsules from thermoresponsive coacervation.

Experimental Section

Materials. 4-(*tert*-Butyldimethylsilyloxy)butyl vinyl ether (SiBVE) was prepared from 4-hydroxybutyl vinyl ether (HOBVE, Maruzen Petrochemical) as reported previously.[21] 2-(Vinyloxy)ethyl methacrylate (VEM) was prepared from 2-chloroethyl vinyl ether (46.8 mL, 0.46 mol, Tokyo Kasei) and sodium methacrylate (25 g, 0.23 mol, Aldrich) in dimethyl sulfoxide (113 mL) under reflux with overhead stirring for 4 h in the presence of small amounts of a phase transfer catalyst (tetrabutylammonium iodide) and an inhibitor (4-*tert*-butylcatechol) (yield = 89%; bp = 76 °C/10mmHg).[23,24,26,27] The monomers were distilled twice over calcium hydride and were stored in a brown ampule under dry nitrogen. $Et_{1.5}AlCl_{1.5}$ (Aldrich; 1.82 M solution in toluene) was used as commercially supplied. Toluene was washed by usual methods. The toluene and ethyl acetate were distilled over calcium hydride just before use. Cationogen (1-isobutoxyethyl acetate, IBEA) was prepared from isobutyl vinyl ether and acetic acid and was distilled over calcium hydride under reduced pressure.[28]

Copolymerization Procedures. Polymerization was carried out at 0 °C under a dry nitrogen atmosphere in a glass tube equipped with a three-way stopcock baked at 250 °C for 10 min before use. The reaction was initiated by the addition of $Et_{1.5}AlCl_{1.5}$ solution in toluene into a mixture of a monomer, ethyl acetate, and IBEA in toluene at 0 °C by a dry medical syringe. For copolymerization, e.g., synthesis of poly(SiBVE$_{x-1}$-*co*-VEM$_x$), the monomer is a mixture of SiBVE and VEM as usual. While, for highly random copolymer, a typical example, poly(SiBVE$_{0.75}$-*r*-VEM$_{0.25}$), is as follows. SiBVE (0.66 mL, 0.48 M) and VEM (0.37 mL, 0.48 M) was first polymerized in the presence of ethyl acetate (0.5 mL, 1.0 M) ($[IBEA]_0 = 4.0$ mM; $[Et_{1.5}AlCl_{1.5}]_0 = 20$ mM in toluene). After 2 h, the second monomer, SiBVE (0.76 mL) was added to the reaction mixture. When SiBVE and VEM was polymerized at total 18 h, it was quenched with prechilled methanol containing a small amount of aqueous ammonia solution (0.1 vol %). The quenched reaction mixture was diluted with dichloromethane, then washed with water to remove the initiator residues. The product polymer was recovered from the organic layer by evaporation of the solvents under reduced pressure and vacuum-dried overnight. The conversion of a monomer was determined by gravimetry.

Desilylation of Copolymers.[13,21] A purified copolymer was dissolved in THF (45 mL) at 0 °C, and 3.0 *N* aqueous HCl−EtOH (5 mL) was added. The mixture was stirred at 0 °C for 30 min and then for additional 3 h after EtOH (15 mL) was added into it. The mixtures were neutralized, and water-insoluble residues were filtered off. After removing organic solvents, the polymer was purified by reprecipitation from ethanol into a large excess of hexane, and then dissolved in pure water below estimated T_{PS}. The aqueous copolymer solution was dialyzed against pure water for 3 days using a cellulose tube (SPECTRA/POR, corresponding to a cutoff molecular weight of 6000−8000). These copolymers were recovered by freeze-drying technique (yield ∼ 100%).

Polymer Characterization. The MWD of the copolymers was measured by size exclusion chromatography (SEC) in tetrahydrofuran at 38 °C on two polystyrene gel columns [TSK gel G-MH$_{HR}$-M × 2 (exclusion limit, 4×10^6 (polystyrene, PSt)); 7.8 mm i.d. × 300 mm each; flow rate 1.0 mL/min] connected to a Tosoh CCPS dual pump and a RI-8011 refractive detector. The number-average molecular weight (M_n) and M_w/M_n were calculated from SEC curves on the basis of a PSt calibration. ^1H NMR spectra for structure such as composition of copolymers were recorded on a JEOL JNM-EX500 spectrometer (500 MHz). The theoretical instantaneous composition at a given consumed monomer was calculated from the Skeist integral equation (eq 1)[29] and the analytic function (eq 2)[30] using the following equations:

$$F_{\mathrm{Inst},1} = \frac{f_1^{\,0} - f_1(1-m)}{m} \tag{1}$$

$$m = 1 - \left(\frac{f_1}{f_1^{\,0}}\right)^{r_2/(1-r_2)} \left(\frac{1-f_1}{1-f_1^{\,0}}\right)^{r_1/(1-r_1)} \times \left[\frac{1 - r_2 - (2 - r_1 - r_2)f_1^{\,0}}{1 - r_2 - (2 - r_1 - r_2)f_1}\right]^{(1-r_1r_2)/[(1-r_1)(1-r_2)]} \tag{2}$$

where m is the monomer conversion, the subscript number is a monomer code (1 and 2 correspond to VEM and SiBVE, respectively), $F_{\mathrm{Inst},1}$ is the instantaneous molar fraction in copolymer, r_1 and r_2 are the relative reactivities, and $f_1^{\,0}$ and f_1 are the molar fractions in the feed and at conversion m.

Characterization of Aqueous Copolymer Solutions. Aqueous solutions of the copolymers were prepared by dissolving the polymer in Mill-Q water (18.3 MΩcm) and diluting the sample to those at a desired concentration. The phase separation temperatures (T_{PS}) of the solutions were measured by monitoring the transmittance of a 500 nm light beam through a 1 cm quartz sample cell at a rate of 1.0 °C /min in heating and cooling scans between 5 and 80 °C. The transmittance was recorded on a JASCO V-500 UV/vis spectrometer equipped with a Peltier-type thermostatic cell holder ETC-505. This UV/vis spectrometer was also used to measure adsorption of a dye and the contents of encapsulated of Vitamin E for hydrogel microsphere.

A microscope (BX-51, Olympus Optical) was used for the observation of coacervate droplets. Micro-Raman spectra were measured by a NRS-1000 micro-Raman spectrometer (JASCO) equipped with an Ar laser (GLG2169, Showa Optronics) and CCD detector (DU401FI, Andor, 1024 × 128 pixel). An aqueous copolymer solution was placed between a holed slide glass and a cover glass and put on a metal block thermostated by circulating water bath.

Preparation of Hydrogel Microspheres and Capsules. The product copolymers were dissolved in water (Mill-Q water) at the concentration of 0.5 wt %. As soon as the aqueous solution (with a core for capsule) was transformed into an opaque liquid on heating (i.e., slightly above T_{PS}), UV (254 nm) irradiation for 10 min using mercury lamp (120 W, Eikosha) was carried out in quartz test tube. For microsphere formation, approximate disappearance of the methacryl double bond was confirmed using IR spectra (KBr method) on JASCO FT−IR-260 spectrometer. In preparing the solid KBr samples, dry KBr and the dried uncross-linked or cross-linked sample were ground until homogeneous.

Characterization of Hydrogel Microspheres and Capsules by Dynamic Light Scattering (DLS). (Hydrodynamic) diameter, *d*, and the polydispersity index, PDI, of hydrogel microspheres and capsules was determined by dynamic light scattering (DLS-7000) (Photal, Otsuka Electronics) at 25 to 55 °C. The light source was a He−Ne laser (10 mW, $\lambda_0 = 632.8$ nm), and correlation function for each solution was obtained at 90° in this study. When the temperature was varied, the measurements were performed after the solution reaches the steady-state conditions, typically the solution was left for 10 min at the measurement temperature. The time autocorrelation function was fitted using the cumulant method. In DLS, the second-order correlation function $g_2(\tau)$ can be expressed by eq 3 in the case of a single-exponential decay.

$$g_2(\tau) = B[1 + \beta\exp(-2\Gamma\tau)] = B[\,1 + \beta|g_1(\tau)|^2] \qquad (3)$$

where B is the baseline, β is an optical constant, Γ is the decay rate for the process, τ is time, and $g_1(\tau)$ is the first-order correlation function, which was analyzed by the cumulant method as the following equation

$$g_1(\tau) = \exp[-\Gamma\tau + (\mu_2/2!)\,\tau^2 - (\mu_3/3!)\,\tau^3 + ...] \qquad (4)$$

yielding Γ and the variance μ_2/Γ^2 (PDI). The *Z*-average diffusion coefficient, D, and d can be obtained from Γ using the following equations

$$\Gamma = Dq^2 = D[(4\pi n_0/\lambda_0)\sin(\theta/2)]^2 \qquad (5)$$

$$d = k_BT/(3\pi\eta_0 D) \qquad (6)$$

where q is the absolute value of the scattering vector, n_0 is the refractive index of the solvent, θ is the diffusion angle, k_B is the Boltzmann constant, T is the absolute temperature, and η_0 is the viscosity of the solvent.

Atomic Force Microscopy (AFM) Measurement. The hydrogel microsphere was cast on the silicon wafer and was examined using a SII SPA300 (Seiko Instruments) under ambient conditions. The AFM image was taken with the DFM mode operating by SPI-3800N.

Results and Discussion

Living Cationic Copolymerization of SiBVE and VEM. First, cationic copolymerization of SiBVE and VEM was carried out using IBEA/$Et_{1.5}AlCl_{1.5}$ in toluene in the presence of ethyl acetate at 0 °C. The copolymerization allows both thermally induced coacervation and subsequent cross-linking of the resulting coacervate droplets to form fine hydrogel microspheres or microcapsules by adding a core. Figure 1 shows a typical example of the time−conversion curve for the copolymerization of SiBVE and VEM ($[SiBVE]_0/[VEM]_0 = 0.78/0.02$ M). The conversion in this plot represents the total consumed amount of both monomers. Copolymerization occurred without an induction period and was almost completed in 25 h to afford soluble polymers in quantitative yield. The time−conversion plot shows that both monomers were consumed, neglecting the sequence distribution estimated from cumulative composition in the copolymer by ^{1}H NMR analysis.

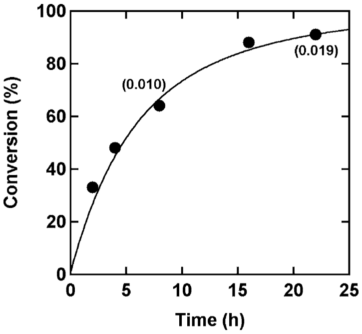


Figure 1. Time−conversion curves for the copolymerization of SiBVE and VEM using IBEA/$Et_{1.5}AlCl_{1.5}$ in toluene in the presence of ethyl acetate at 0 °C: $[SiBVE]_0 = 0.78$ M; $[VEM]_0 = 0.02$ M; $[IBEA]_0 = 4.0$ mM; $[Et_{1.5}AlCl_{1.5}]_0 = 20$ mM; [ethyl acetate] = 1.0 M. The parenthetical values are the cumulative composition (mole fraction) of VEM in the copolymer.

Figure 2 plots the molecular weight and M_w/M_n of copolymers of SiBVE and VEM ($[SiBVE]_0/[VEM]_0 = 0.78/0.02$ M) as a function of total monomer conversion. The M_n increased in direct proportion to the conversion, and the MWDs of the resulting polymers were quite narrow ($M_w/M_n \leq 1.14$).

^{1}H NMR analysis confirmed that only vinyl ether-type polymerization occurred without both polymerization of methacryloyl moieties and deprotection of the silyloxy pendants (peaks *f* and *g*) under those conditions as shown in Figure 3a. However, the content of VEM in copolymer was small at the low conversion. This is due to different reactivities between SiBVE and VEM.

Desilylation of the copolymers was carried out with the addition of aqueous HCl−EtOH in THF at 0 °C. Quantitative desilylation was confirmed by the disappearance of the ^{1}H NMR signals at 0.1 ppm (s, 6H, Si−CH_3) and 0.8 ppm (s, 9H, Si−C−CH_3) of the *tert*-butyldimethylsilyl group as shown in Figure 3b. Furthermore, the MWDs of the product copolymers show monodispersity by aqueous SEC.

Synthesis of Highly Random Copolymers of SiBVE and VEM by Sequential Living Cationic Copolymerization. The relative reactivities for SiBVE and VEM were determined by the Kelen−Tüdös method,[31] giving values of 2.52 ± 0.22 ($\boldsymbol{r}_{SiBVE}$) and 0.10 ± 0.09 ($\boldsymbol{r}_{VEM}$), respectively, indicating that the sequence distribution of the product copolymer is not random. To obtain the homogeneous hydrogel, cross-linking point must be homogeneous. For example, if the product copolymers have the block part of VEM units, they form star polymers by cross-linking.[32,33] Therefore, highly random copolymer called “periodic” copolymer[34] of SiBVE and VEM was prepared with following controlled sequence method for monomer addition. Typical examples are the highly random copolymers whose SiBVE/VEM molar ratio in the copolymer is 4/1. Figure 4a shows the time-consumed monomers curve for the polymerization of a SiBVE/VEM mixture ($[SiBVE]_0 = 0.48$ M, $[VEM]_0 = 0.32$ M, $[IBEA]_0 = 4$ mM, $[Et_{1.5}AlCl_{1.5}]_0 = 20$ mM, [ethyl acetate] = 1.0 M). Polymerization occurred without an induction period. When the conversion was ca. 20% (total consumed monomer: 23 wt %, polym time: 2.0 h), neat SiBVE (initial SiBVE/ addition SiBVE = 33/38 molar ratio) was added

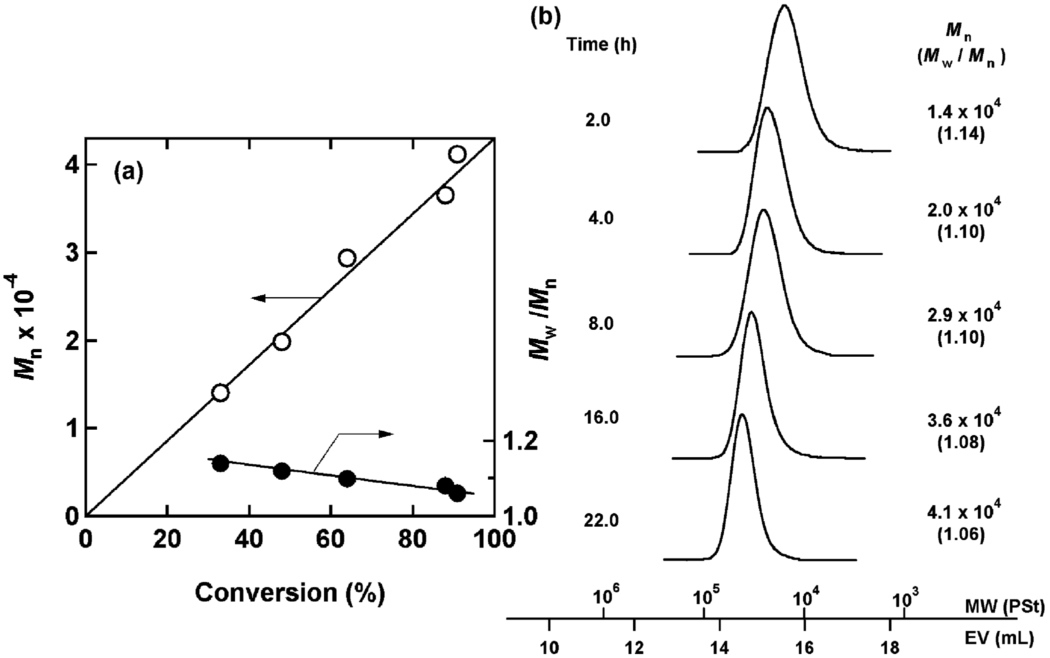


Figure 2. (a) M_n and M_w/M_n of copolymer of SiBVE and VEM obtained using IBEA /$Et_{1.5}AlCl_{1.5}$ in toluene in the presence of ethyl acetate at 0 °C. (b) Molecular weight distribution curves at various polymerization time of the copolymers: see Figure 1 for the reaction condition.

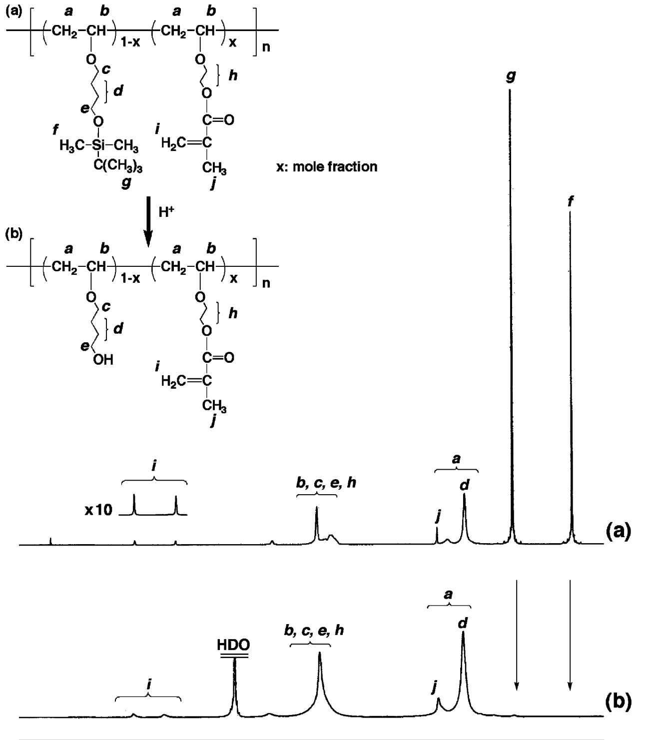


Figure 3. ^{1}H NMR spectra of (a) poly(SiBVE$_{1-x}$-*co*-VEM$_x$) (M_n = 4.1 × 10^4, x = 0.025) in $CDCl_3$ and (b) poly(HOBVE$_{1-x}$-*co*-VEM$_x$) in D_2O at 30 °C, obtained by desilylation.

to the reaction mixture, and all monomers was consumed ca. 20 h to give an apparent "periodic" copolymer. Figure 4b shows the instantaneous composition of VEM ($F_{\mathrm{Inst,\ VEM}}$) at a given consumed monomer, which can be calculated from the residual monomer ratios by ^{1}H NMR analysis. Figure 4b is, in turn, the sequence distribution of the copolymer, the horizontal axis indicates the normalized chain length. The dotted line consisting of curve A and B is the instantaneous composition of VEM obtained by Skeist integral equation (eq 1) and the analytic function (eq 2) from the relative reactivities (r_{VEM} 0.10 and r_{SiBVE} 2.52). The curves A and B were calculated by different mole fraction of VEM (f_1^0), which were 0.4 and 0.28, respectively. The obtained instantaneous copolymer composition is apparent constant against the consumed monomers, indicating that the sequence distribution of the product copolymer can be controlled by this method.

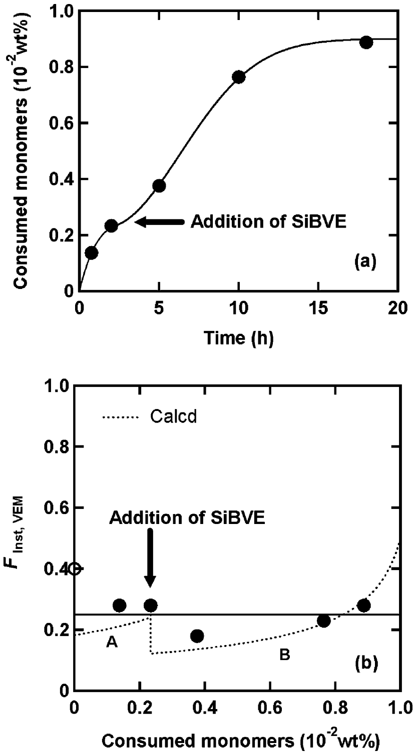


Figure 4. (a) Time-consumed monomers curve and (b) the instantaneous composition of VEM ($F_{\mathrm{Inst,VEM}}$) against the consumed monomers using obtained using IBEA/$Et_{1.5}AlCl_{1.5}$ in toluene in the presence of an added base at 0 °C [(○) initial molar ration of SiBVE and VEM]: $[\mathrm{SiBVE}]_0$ = 0.48 M, $[\mathrm{VEM}]_0$ = 0.32 M, $[\mathrm{IBEA}]_0$ = 4.0 mM; $[Et_{1.5}AlCl_{1.5}]_0$ = 20 mM; [ethyl acetate]$_0$ = 1.0 M, initial SiBVE/addition SiBVE = 33/38 molar ratio.

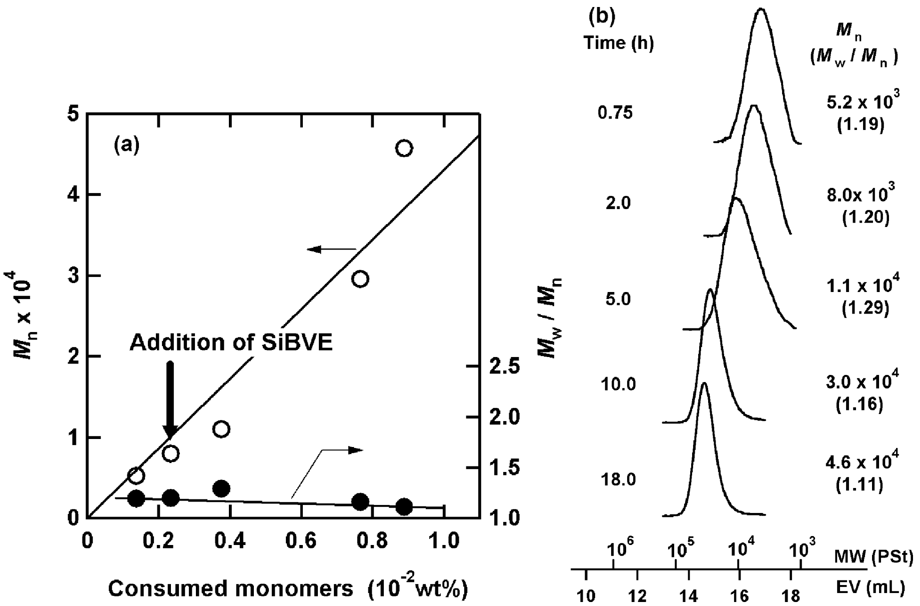


Figure 5. (a) M_n and M_w/M_n, and (b) MWD in the synthesis of poly(SiBVE$_{0.75}$-*r*-VEM$_{0.25}$) obtained using IBEA/Et$_{1.5}$AlCl$_{1.5}$ in toluene in the presence of an added base at 0 °C: see Figure 4 for the reaction condition.

Table 1. Results of Highly Random Copolymerization[a] and the Size of Obtained Hydrogel Microspheres[b]

entry	VEM in copolymer[c] (*x*, mole fraction)	$M_n \times 10^{-4}$[d]	M_w/M_n[d]	diameter (nm)[e]		PDI (μ_2/Γ^2)[e]	
				25 °C	55 °C	25 °C	55 °C
1	0.025	1.4	1.14	462	282	0.09	0.10
2		2.0	1.12	554	242	0.15	0.18
3		2.7	1.10	659	288	0.15	0.11
4		3.7	1.08	701	250	0.11	0.17
5	0.095	1.3	1.17	547	423	0.16	0.18
6		2.0	1.14	556	466	0.16	0.18
7		3.3	1.11	577	444	0.06	0.11
8		3.9	1.12	586	409	0.08	0.10
9	0.25[f]	4.1	1.12	*h*			
10[g]	0.095	3.6	1.08	540	298	0.30	0.20

[a] Polymerization conditions: [IBEA]$_0$ = 4.0 mM, [Et$_{1.5}$AlCl$_{1.5}$]$_0$ = 20 mM, [ethyl acetate] = 1.0 M in toluene at 0 °C. [b] Obtained hydrogel microspheres from 0.50 wt % aqueous solution of poly(HOBVE$_{1-x}$-*r*-VEM$_x$) by UV (254 nm) irradiation using low-pressure mercury lamp (120 W, 10 min). [c] Measured by ^{1}H NMR analysis. [d] Poly(SiBVE$_{1-x}$-*ran*-VEM$_x$) before desilylation measured by SEC (PSt calibration). [e] Measured by DLS (cumulant results). [f] E.g. Polymerization degree of VEM in copolymer (ca. total 200-mer) is approximately 50. [g] Prepared by conventional copolymerization, copolymerization conditions: [SiBVE]$_0$ = 0.724 M, [VEM]$_0$ = 0.076 M, [IBEA]$_0$ = 4.0 mM, [Et$_{1.5}$AlCl$_{1.5}$]$_0$ = 20 mM, [ethyl acetate] = 1.0 M in toluene at 0 °C. [h] Partial macrogelation.

Figure 5 shows the relationship between the consumed monomer (wt %) and the M_n or M_w/M_n ratio of the copolymers. The M_n of the copolymer increases in direct proportion to the consumed monomer and the copolymers exhibits narrow MWDs. The VEM in copolymer, M_n, and M_w/M_n of the prepared samples thus far are summarized in the left side of Table 1.

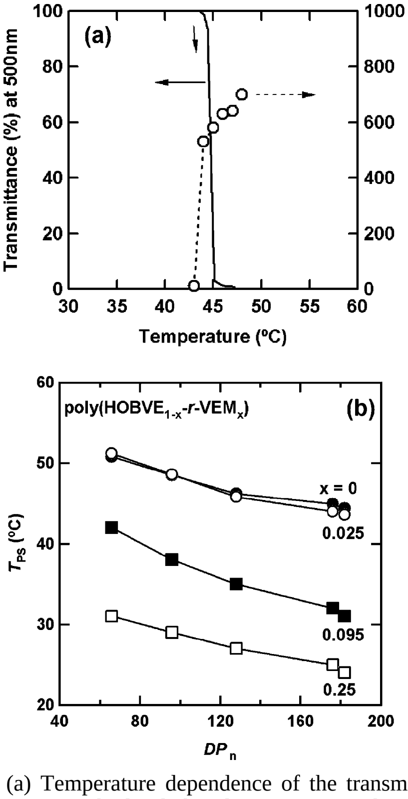


Figure 6. (a) Temperature dependence of the transmittance at 500 nm and the apparent hydrophobic diameter (D_h^{app}) by DLS at 90° of 0.5 wt % aqueous solutions of poly(HOBVE$_{0.975}$-*r*-VEM$_{0.025}$) (corresponding to entry 4), heating rate 1.0 °C/min. (b) Relationships between T_{PS} of poly(HOBVE$_{1-x}$-*r*-VEM$_x$) in water (0.5 wt %) and degree of polymerization (DP$_n$) at various molar ratio of VEM: (●) $x = 0$, (○) 0.02, (■) 0.095, and (□) 0.25.

Thermoresponsive Coacervation of Aqueous Solution of Poly(HOBVE$_{1-x}$-*r*-VEM$_x$). After above-mentioned desilylation, various poly(HOBVE$_{1-x}$-*r*-VEM$_x$) were obtained. All the product copolymers (x = 0.025, 0.095, and 0.25) were soluble in water around room temperature. Upon warming a 0.5 wt % aqueous solution of the product copolymers, they transformed into an opaque liquid like aqueous HOBVE homopolymer, to be observed the coacervate droplets in micrograph. The transition was monitored by the transmittance at 500 nm and the apparent hydrophobic diameter by DLS at 90° upon heating as shown in Figure 6a. For poly(HOBVE$_{0.975}$-*r*-VEM$_{0.025}$) (corresponding to entry 4), the transmittance decreased and the apparent hydrophobic diameter increased when the solution was warmed to ca. 50 °C near T_{PS} of poly(HOBVE). Figure 6b plots the T_{PS}, determined from the 50% transmittance at 500 nm of a 0.5 wt % aqueous solution of poly(HOBVE$_{1-x}$-*r*-VEM$_x$), against the degree of polymerization (DP$_n$). The T_{PS} of poly(HOBVE$_{1-x}$-*r*-VEM$_x$) decreased with increasing the DP$_n$ and/or the number of VEM units. This is due to hydrophobic effects

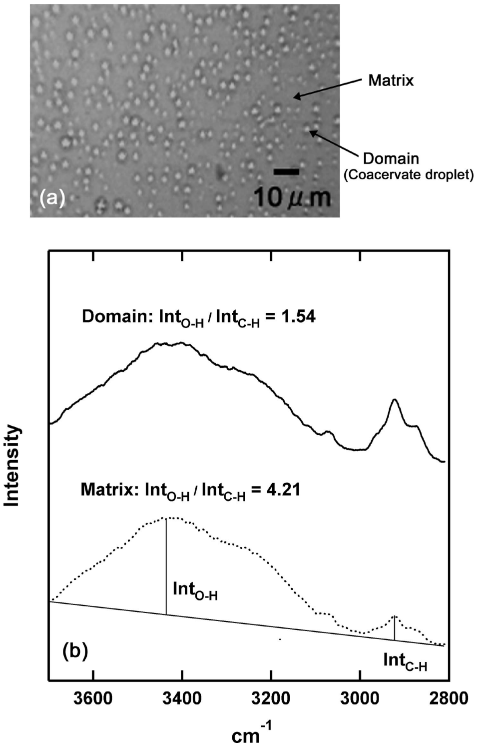


Figure 7. (a) Optical micrograph of the coacervate droplets from poly(HOBVE$_{0.975}$-*r*-VEM$_{0.025}$) (entry 4). (b) Micro-Raman spectra measured at the points in the domain and matrix phases of the micrograph in part a.

of VEM unit, because VEM is insoluble in water at every temperature. Hydrogel microspheres are, thus, synthesized from coacervation slightly above the measured T_{PS}.

The typical optical micrograph of the coacervate droplets from poly(HOBVE$_{0.975}$-*r*-VEM$_{0.025}$) (entry 4) is shown in Figure 7a. Micro-Raman spectra measured at the points in the domain, i.e., the coacervate droplet, and matrix phases of the system are shown in Figure 7b. The O−H stretching vibration broad band observed between around 3400 cm^{-1} indicates the presence of H_2O and OH groups of the copolymers. The C−H stretching vibration band observed between 2800 and 3000 cm^{-1} indicates the presence of methylene groups of the copolymers. From these intensity of O−H and C−H (Int$_{O-H}$ and Int$_{C-H}$),[35] the droplet is rich in polymer, comprised of both concentration polymers and waters. Almost thermoresponsive (LCST-type) copolymer solutions show the characteristically sharp liquid−solid phase transition such as poly(*N*-isopropylacrylamide).[7,8] However, this is the thermoresponsive copolymer exhibiting coacervation accompanied by the liquid−liquid-phase separations though unlike precipitation. Thus, the initially formed coacervate droplets including in photofunctional unit can be cross-linked by UV (254 nm) irradiating in water to form fine hydrogel microspheres.

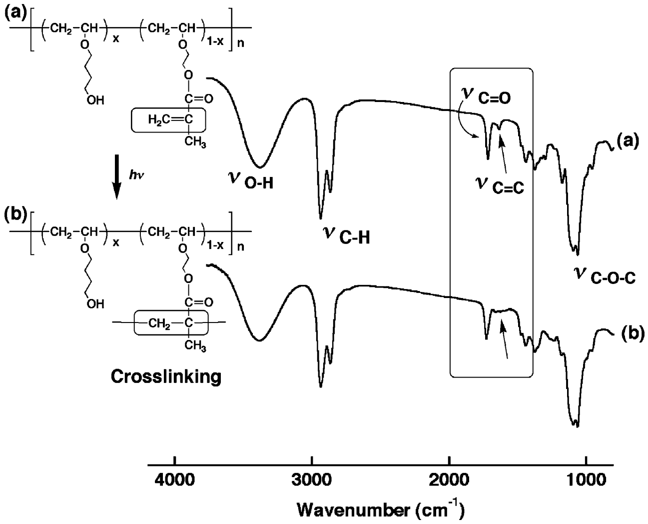


Figure 8. Cross-linking of poly(HOBVE$_{0.905}$-*r*-VEM$_{0.095}$) (corresponding to entry 8) monitored by IR (KBr method) spectroscopy: (a) before and (b) after UV (254 nm) irradiation.

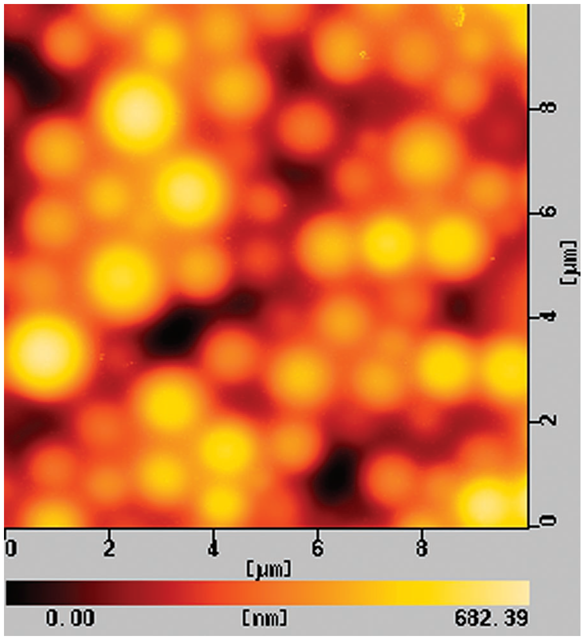


Figure 9. AFM image of the hydrogel microspheres (entry 4) on a silicon wafer surface (top view). Image areas are approximately 10 × 10 μm^2.

Cross-Linking of Thermoresponsive Coacervate Droplets from Aqueous Poly(HOBVE$_{1-x}$-*r*-VEM$_x$). Irrespective of inherently unstable coacervate droplets, they can be cross-linked to form colloidally stable hydrogel microspheres. Coacervation and cross-linking were carried out at temperatures slightly above the corresponding T_{PS}. Under these conditions, the thermally induced poly(HOBVE$_{1-x}$-*r*-VEM$_x$) coacervate droplets were irradiated by UV (254 nm) due to the UV-induced cross-linking polymerization of the methacryloyl moieties in the copolymers. Figure 8 compares typical IR spectra by KBr method of dried poly(HOBVE$_{0.905}$-*r*-VEM$_{0.095}$) (corresponding to entry 8) and dried microsphere after irradiating UV (254 nm) for 10 min. Quantitative cross-linking was confirmed by the approximate disappeared of C═C of a methacryloyl group stretching band was seen at 1636 cm^{-1} in Figure 8b, and remained another peaks. The conversion (>90%) was estimated by the absorbance or the transmittance at the wavelength.

After the cross-linked sample was drop on silicon wafer and dried below the corresponding T_{PS}, the AFM image (DFM mode) was observed. Figure 9 presents a typical AFM micrograph of cross-linked sample prepared from 0.50 wt % poly(HOBVE$_{0.975}$ -*r*-VEM$_{0.025}$) (corresponding to entry 4) around room temperature. Obviously, the cross-linked samples are microspheres, which were stable during workup. The photograph of Figure 10a shows the state of (a) 0.50 wt % poly(HOBVE$_{0.975}$ -*r*-VEM$_{0.025}$) in water at room temperature, (b) the sample at 55 °C, and (c) the sample at 25 °C after irradiation of UV.

Since obtained cross-linked samples estimated by AFM were sphere, their hydrodynamic diameters can be determined by

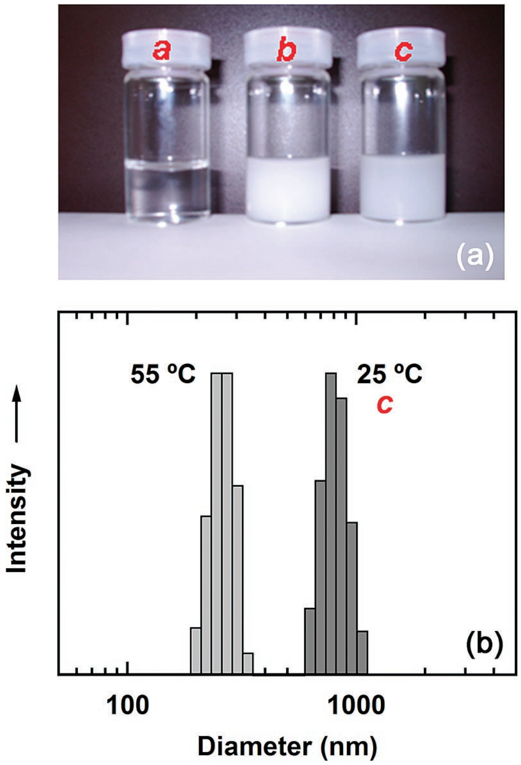


Figure 10. (a) Photograph of the state of (red a) 0.50 wt % poly(HOBVE$_{0.975}$ -r-VEM$_{0.025}$) in water at room temperature, (red b) the sample at 55 °C, and (red c) the hydrogel microsphere sample at 25 °C after irradiation of UV. (b) Size distribution by DLS of the hydrogel microsphere at 25 °C and 55 °C (corresponding to entry 4).

dynamic light scattering at 90°. Figure 10b shows the typical diameters (size distribution) by DLS of microspheres (entry 4) at 25 and 55 °C. These temperatures are below and above $T_{\rm PS}$ of poly(HOBVE). The diameters and PDIs (μ_2/Γ^2) by cumulant analysis (eq 3−6) at 25 and 55 °C are summarized in the right side of Table 1. In the case of small amount of VEM (entry 1−4), the diameter of microsphere is ca. 250 nm above $T_{\rm PS}$ of poly(HOBVE), irrespective of the molecular weight. However, at lower temperature of $T_{\rm PS}$ of poly(HOBVE), swelling microsphere were observed and the diameters depended on molecular weight. The diameter between 25 and 55 °C was in reasonably good agreement with the value of the original coacervate droplet in water shown in Figure 6a. This indicates that the size of shrink hydrogel microsphere was affected by that of coacervate droplet, regardless of the molecular weight of the copolymers. Furthermore, the size of swelling one was affected by molecular weight of the copolymers. The size of microspheres is also close to AFM results. In the case of higher amount of VEM (entry 5−8), the same swelling-shrink behavior was observed. However, the degree of swelling was low, rigid microspheres could be obtained. Since entry 9 is the highest amount of VEM, the visible macrogelation occurred and diameter could not be measured. Interestingly, all the diameters of obtained hydrogel microsphere, except for entry 10 obtained by conventional copolymerization, were comparatively equality of size (PDI ∼ 0.1). It is likely that comparative equality of size of hydrogel microspheres can be obtained from coacervation formed by copolymer having uniform length and periodic sequence of cross-linking point. In addition, the obtained hydrogel microspheres well-dispersed in water without a dispersant such as a sodium dodecyl sulfate. The reason may be due to the effect of hydroxy groups which are at outer microspheres in water.

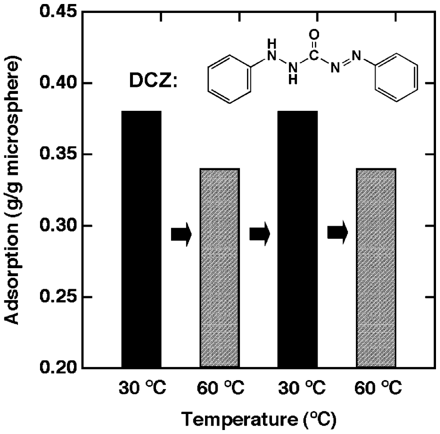


Figure 11. Temperature dependence of adsorption of DCZ (g) per hydrogel micrspheres (g) (entry 4). (By calculation at 283 nm of maximum absorption wavelength of DCZ).

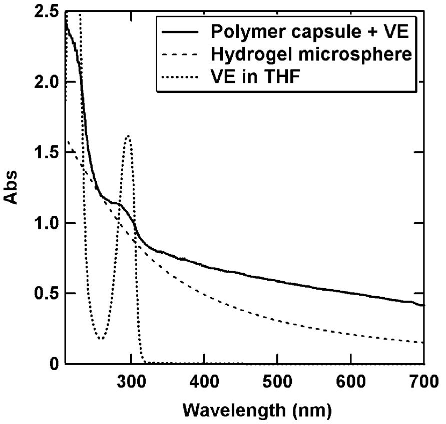


Figure 12. UV−vis absorption spectra of hydrogel microsphere with VE (i.e., hydrogel capsule, thick line) and without VE (broken line). The copolymer is corresponding to 0.01 wt % aqueous poly(HOBVE$_{0.975}$-r-VEM$_{0.025}$) solution (entry 4) before cross-linking. The dotted line is absorption of VE in tetrahydrofuran (2.1×10^{-4} g/mL).

Adsorption of a Dye in the Thermoresponsive Hydrogel Microsphere. The coacervate droplet was known to be condensation phase from water, various hydrophobic substances can be concentrated.[10] As one of the adsorption examples, the repeatability of a dye adsorption/desorption by cycling the heating/cooling process was demonstrated. A hydrophobic dye, 1, 5-diphenylcarbazone (DCZ, the structure is inset in Figure 11), was adsorbed in 0.01 wt % aqueus solution of hydrogel microsphere (entry 4) at different temperatures. The concentration of the dye employed was enough excess (0.1 g in 10 mL of 0.01 wt % hydrogel microsphere solution) and then the mixed solution was stirred for 1 h at 30 °C. Next, the aqueous solution containing a dye was put in the dialytic tube and filtrated (glass filter; pore size = 10−16 μm), the nonabsorbate was removed, and then the absorption spectra were obtained by UV−vis spectrometer. Figure 11 shows the adsorption of DCZ in hydrogel micrspheres measured above and below the transition temperature, repeatedly, calculated at 283 nm of maximum absorption wavelength of DCZ. This figure clearly shows that the amount of hydrophobic dye adsorbed was reproducibly changed by simply controlling the temperature. For the mechanism of adsorption/desorption of dye, it is conceivable that the swelling/shrunken hydrogel microsphere affects adsorption/desorption of dye, respectively. Because the maximum amounts of DCZ were present in hydrogel microsphere solution at 30 °C, hydrogel microsphere shrunk at 60 °C, and then have decreasing of sorbable volume, thus desorption of dye is occurred at high temperature.

Encapsulation of Vitamin E in the Thermoresponsive Hydrogel Microsphere. α -Tocopherol (Vitamin E, VE, 2.0 g), which is a medicine for a chilblains, frostbite or cold disease, was dissolved in ethanol (0.79 mL) because of the insolubility of VE in water.[36] The 0.5 μL of VE ethanol solution was added to the 10 mL of 0.01 wt % aqueous solution of poly(HOBVE$_{0.975}$-*r*-VEM$_{0.025}$) (corresponding to entry 4) and cross-linked at 50 °C by irradiation of UV (254 nm). After dialysis, the opaque solution including in VE was obtained. By DLS analysis, the diameters of obtained microsphere are 910 nm at 30 °C and 298 nm at 50 °C, respectively, indicating formation of thermoresponsive hydrogel microsphere. Figure 12 shows the UV−vis absorption spectra of obtained hydrogel microsphere with and without VE. In the hydrogel microsphere with VE, an absorption peak of VE at 292 nm was observed. The content of capsulated VE was calculated by differential UV spectrum (difference between hydrogel capsule and hydrogel microsphere) at 292 nm. The analytical curve was calculated by VE in tetrahydrofuran. The content was 92% for addition VE to the random copolymer. Thus, VE capsule could be quantitatively synthesized from thermoresponsive coacervate droplet.

Conclusion

Highly random copolymers, poly(HOBVE$_{1-x}$-*ran*-VEM$_x$)s, have been prepared by sequential living cationic copolymerization. When the aqueous solution was heated, coacervate droplets was formed. The initially formed coacervate droplets slightly above phase separation temperature was cross-linked by UV irradiating in water, to form fine hydrogel microspheres. By DLS and AFM, comparative equality of size of hydrogel microspheres was obtained. The obtained hydrogel microspheres show the thermoresponsiveness and adsorption/desorption effect on a dye. Furthermore, the VE capsule could be quantitatively synthesized. To the best of the author's knowledge, this is the first example of hydrogel microspheres from thermoresposnive coacervation formed by a well-defined random copolymer.

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(33) We preliminarily prepared the block copolymer of HOBVE$_{195}$-*b*-VEM$_5$ by sequential living cationic polymerization. The polymerization was carried out under the following conditions: $[\mathrm{SiBVE}]_0 = 0.724$ M, $[\mathrm{IBEA}]_0 = 4.0$ mM, $[\mathrm{Et_{1.5}AlCl_{1.5}}]_0 = 20$ mM, and $[\text{ethyl acetate}]_0 = 1.0$ M in toluene at 0 °C. After SiBVE was polymerized (20 h), the second monomer VEM (VEM/SiBVE = 5/195) was added. The polymerization was quenched after 24 h. After desilyation and purification, the product, HOBVE$_{195}$-*b*-VEM$_5$, was obtained. The cross-linked sample (0.5 wt % aqueous, irradiation slightly above T_{PS}) was microgel (or star) polymer, because poly(VEM) segment is hydrophobic. The apparent diameter was smaller than the same composition of hydrogel microsphere, which was ca. 40 nm by DLS at 90°.
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