pH-Sensitive Nanogel Possessing Reactive PEG Tethered Chains on the Surface

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ABSTRACT: Poly(ethylene glycol) possessing a polymerizable vinylbenzyl group at one end and a carboxylic acid group at the other end was synthesized via the anionic polymerization of ethylene oxide. ^{1}H NMR, SEC, and MALDI-TOF-MS studies confirmed that each poly(ethylene glycol) chain quantitatively possessed vinylbenzyl and carboxyl end groups. Emulsion polymerization of 2-(diethylamino)ethyl methacrylate was carried out to obtain a nanometric-sized gel (nanogel) in the presence of a cross-linking agent such as ethylene dimethacrylate, using the obtained α-vinylbenzyl-*ω*-carboxy-PEG as a stabilizing reagent. The size of the obtained nanogels was controllable in the range between 50 and 680 nm. The nanogel was confirmed to have a PEG shell layer with a carboxylic acid group at the distal end of each PEG strands from the ζ-potential measurement at varying pHs. The pH-sensitive swelling/deswelling behavior of the nanogels was studied by dyanmic light scattering to confirm their volume phase transition at a pH around 7.0. These prepared nanogels are expected to have potential utility in applications such as diagnostics and controlled drug releasing devices.

Introduction

Materials of nanosize dimensions (nanomaterials) often show unique characteristics which are not expected from the bulk properties.[1] Studies on such nanomaterials are receiving great interest and are widely extending into diverse fields of science and technology.[2] Among the many types of nanomaterials, nanospheres are the one with attracting a growing attention, which are utilized not only as conventional paints[3] and pastes[4] but also as diagnostic tools,[5] drug delivery systems,[6] and other biorelated devices.[7]

One of the most important characteristics of the nanosphere is its very large surface area as compared to its volume. The relatively high surface area often changes the characteristics of the materials. For example, the activity of solid catalysts is known to significantly increase with their decreasing size. On the contrary, the large surface area of the nanospheres often causes their unstable dispersion in solution. Once coagulation takes place, the performance of the aggregate, e.g., catalytic activity, significantly decreases. Particularly, when nanospheres are utilized in biochemical fields, the dispersion stability is one of the key issues because of the restricted operational conditions such as high ionic strength and the presence of many proteinous components. Note that biological entities such as serum consists of numerous components including lipids and proteins, which cause the problem of nonspecific adsorption on the nanosphere surface. Thus, the nonfouling characteristics of the nanosphere surface is one of the most important prerequisites for its application in the biomedical field.

To improve the nonfouling characteristics of the nanospheres, we have been focusing on the construction of PEG tethered chains on the nanosphere surface. For example, self-assembled nanospheres (polymeric micelles) made from an amphiphilic block copolymer, poly(ethylene glycol)-*b*-poly(DL-lactide) (PEG/PLA), showed extremely long circulation in bloodstream[8] due to its high dispersion stability along with its nonfouling characteristics. PEG tethered chains constructed on the surface of the gold nanoparticle also improves the dispersion stability in a biorelated environment.[9] Another important feature of PEG-ylated nanospheres used in the biomedical field is to introduce a reactive group at the distal end of PEG in order to install ligand moieties at the free end of the PEG tethered chains. This led us to prepare a heterotelechelic PEG (hetero-PEG), which denotes a PEG having different functional groups at both ends, by novel synthetic routes.[10] The hetero-PEG has been used for the construction of PEG-ylated surfaces possessing a reactive group at the distal end of the PEG.[11]

Among the hetero-PEGs prepared so far, an acetal-PEG-OK was successfully utilized as a macroinitiator for lactide polymerization to form an acetal-PEG/polylactide block copolymer (acetal-PEG/PLA), which forms a polymeric micelle in aqueous media possessing the acetal group on the periphery of the micelles.[12] Note that the acetal group located at the PEG free chain end can readily be converted to an aldehyde group by gentle acid treatment to introduce ligand molecules on the micelle surface. A lactose-installed PEG/PLA micelle was prepared via a reductive amination reaction with aminophenyllactose, which shows an effective molecular recognition. It is anticipated as a new high-performance drug carrier with active targeting characteristics.[13]

As alternative method for the preparation of nanospheres possessing the PEG tethered chain surface is the emulsion polymerization of a hydrophobic monomer

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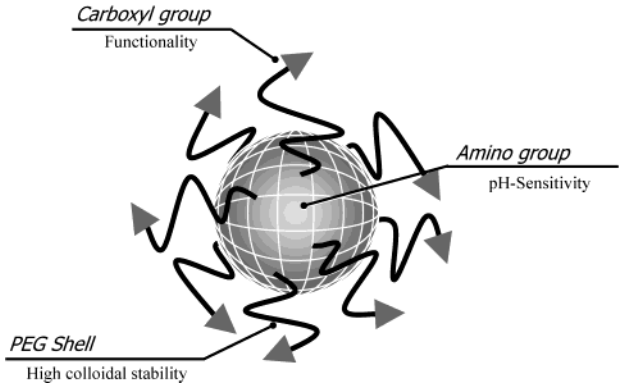

Figure 1. Schematic picture of the functionalized nanogel.

in the presence of PEG macromonomers. Several approaches are indeed available in the literature.[14] These types of approaches reduce the nonspecific adsorption and improve the dispersion stability in aqueous solution of nanospheres. However, when a conventional PEG macromonomer, which generally possesses a methoxy group at one end and a polymerizable double bond at the other chain end, is employed for the emulsion polymerization, the PEG shell layer prevents further delivatization of the nanosphere. To introduce a functionality on the surface of the core−shell nanosphere, we have employed a heterotelechelic PEG, which possessed an acetal group at one end and a methacryloyl group at the other end, for the dispersion polymerization.[15] Thus, the biotin-installed core−shell type nanosphere was prepared.

We would like to report on the further progress in the use of heterotelechelic PEG macromonomers for the preparation of a new class of nanospheres, termed nanogels, showing abrupt changes in the core swelling ratio with pH, retaining the ligand-installation site on the periphery, and achieving a high dispersion stability as well as nonfouling character in a biologcial environment (Figure 1).

Experiments

Materials. Tetrahydrofuran (THF; Kanto) was dried over $LiAlH_4$ and distilled under an argon atmosphere.[16] Acetone (Wako) and ethylene oxide (EO: Sumitomo Seika Chemical) were purified by distillation over CaH_2 and stored under an argon atmosphere. Vinylbenzyl alcohol (VBA) was received as a gift from the Seimi Chemical Co. (Japan) and distilled over CaH_2 under reduced pressure. Pyridine (Aldrich), 2-(*N*,*N*-(diethylamino)ethyl methacrylate (EAMA; Wako), and ethylene glycol dimethacrylate (EDMA; Wako) were distilled over CaH_2 under reduced pressure. Potassium hydride (KH; Wako) was washed with hexane to remove the mineral oil and dried under reduced pressure. Potassium persulfate (KPS; Wako) was recrystallized three times from water and then dried under a vacuum. All other reagents were used as received.

Synthesis of Heterotelechelic PEG Macromonomers. All of the following polymerization procedures were carried out under an argon atmosphere in a glass reactor equipped with a three-way stopcock.

Synthesis of Poly(ethylene glycol) Possessing a Vinylbenzyl Group at One End and a Carboxylic Acid Group at the Other Chain End (VB-PEG-COOK) Using KH as the Metalation Agent. One of the representative procedures for the EO polymerization is described as follows for the preparation of VB-PEG-COOK. A THF solution (8 mL, 16 mmol) of vinylbenzyl alcohol (2 M) was dissolved in 324 mL of freshly distilled dry THF with stirring. To this solution was added a potassium hydride dispersion (8 mL, 24 mmol). After standing for several hours, 318.8 mL (VBA^-K^+: 15 mmol) of the supernatant fluid was moved to another glass reactor, followed by the addition of 17 mL of EO (340.5 mmol) via a cooled syringe. After the mixture was allowed to react for 2 days at room temperature, the end modification reaction was performed by the addition of 0.75 mmol of succinic anhydride and stirred for several hours. The obtained polymer was precipitated into diethyl ether and then finally freeze-dried from benzene.

Synthesis of VB-PEG-COOK Using a Potassium Enolate of Acetone ($Acetone^-K^+$) as the Metalation Agent. A potassium hydride dispersion (5 mL, 15 mmol) was added to 35.3 mL of freshly distilled and dried THF, followed by addition of 735 μL of acetone (10 mmol) and stirred for several hours to form $acetone^-K^+$. A THF solution (0.25 mL, 0.5 mmol) of vinylbenzyl alcohol (2 M) was dissolved in 2 mL of THF solution of the $acetone^-K^+$ (0.25 M) with stirring. The liberated acetone was completely removed by evaporation in vacuo for 1 h. The potassium vinylbenzyl alcoholate thus prepared was dissolved in 20 mL of THF, followed by the addition of 1.1 mL of EO (22.7 mmol) via a cooled syringe. After the polymerization, the obtained polymer was recovered in the same way as described above.

Synthesis of PEG Possessing a Methoxy Group at One End and a Vinylbenzyl Group at the Other Chain End (MeO-PEG-VB). MeOPEGVB was synthesized according to a method described in the literature.[17] 79 μL of 2-methoxyethanol (76 mg, 1.0 mmol) and 3.24 mL of potassium naphthalene (1 mmol, 0.309 mol/L) were dissolved in 30 mL of freshly distilled and dried THF via a syringe. After a few minutes of agitation to form the potassium 2-methoxyethoxide, 2.27 mL of EO (2.0 g, 45.4 mmol) was added via a cooled syringe. After the mixture was allowed to react for 2 days at room temperature, 0.17 mL of vinylbenzyl chloride (0.18 g, 1.2 mmol) was added, and the solution was stirred for another 3 h. The obtained polymer was precipitated in cooled 2-propanol and separated by centrifugation (5000 rpm; 30 min, −10 °C). The polymer was finally freeze-dried from benzene.

Synthesis of pH-Sensitive Nanogel Possessing Heterotelechelic PEG Tethered Chains on the Surface. One of the representative procedures for the preparation of pH-sensitive nanogels possessing heterotelechelic PEG tethered chains on the surface is described. After 150 mg (80 μmol) of VB-PEG-COOK (1.8 K) and 9.1 mg (33.5 μmol) of KPS were loaded into the reactor, vacuum and argon purging cycles were repeated three times. After the deionized water (15 mL) was added to the reactor to dissolve both the PEG and initiator, 600 mg of EAMA (3.24 mmol) and 6.6 mg of EDMA (33.2 μmol) were added. This aqueous suspension was allowed to react at 60 °C for 24 h in a water bath with stirring.

Conjugation of Fluorescent Probe to the PEG Tethered Chain End on the Nanogel. Conjugation of the fluorescent probe, 6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone-3-propylcarboxylic acid hydrazide ($\lambda_{ex}/\lambda_{em}$ = 365 nm/447 nm, Wako, DMEQ-hydrazide), to the carboxylic group adjacent to the PEG chain end on the nanogel surface was carried out via the active ester method using 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide (WSC) as a coupling reagent.[18] A 100 μL aliquot of nanogel solution equivalent to 0.1 μmol of carboxyl groups was added to 778 μL of distilled water, and then 1 μL of a pyridine aqueous solution (1 μM) and 29 μL of the WSC aqueous solution (5.2 μM) were added. After a few minutes, 92 μL of DMEQ-hydrazide aqueous solution (1.6 μM) was added, and then the solution was stirred for 30 min. The unreacted DMEQ-hydrazide and other reagents were removed by ultracentrifugation of nanogel at 900000*g* (*g*: gravity) for 10 min and then dispersed in distilled water. This purification cycle was repeated five times. The fluorescense of the conjugated nanogels were determined using a fluorescence spectrophotometer.

Characterization. Size exclusion chromatography (SEC) measurements were carried out using a TOSOH HLC-8120 equipped with TSK gel columns (TSKgel SuperHZ3000 + HZ2500) and an internal refractive index (RI) detector (TOSOH HLC-8020RI). THF containing 0.5 wt % triethylamine was used as the eluent at a flow rate of 0.35 mL min^{-1} at 40 °C.

Table 1. Results of PEG Macromonomer Synthesis[a]

run	code	[initiater][b]	[monomer][c]	metalation agent	$10^{-3}M_n$[d] calcd[e]	$10^{-3}M_n$[d] obsd[f]	M_w/M_n[g]	yield [%]
0	VBPEGCOOK50	25.4	2.89	K-naph	5.0	5.3	1.14	88
1	VBPEGCOOK18	44.6	1.01	KH	1.0	1.8		80
2	VBPEGCOOK21	23.7	1.08	acetoneK	2.0	2.1	1.06	91
3	VBPEGCOOK29	25.4	1.71	acetoneK	3.0	2.9	1.07	95
4	VBPEGCOOK42	24.0	2.69	acetoneK	5.0	4.2	1.06	96
5	VBPEGCOOK80	24.2	5.50	acetoneK	10.0	8.0	1.08	99
6	MeOPEGVB21	28.1	1.28	K-naph	2.0	2.1	1.07	89

[a] Polymerization for 2 days at room temperature under argon atmosphere. [b] [Initiater] = concentration of vinylbenzyl alkoxide in polymerization solution (μmol/mL). [c] [Monomer] = concentration of ethylene oxide in polymerization solution (mmol/mL). [d] M_n = number-averaged molecular weight. [e] Calcd = 44.05 × [monomer]/[initiater]. [f] Obsd = M_n from SEC measurement, solvent THF, PEG standards. [g] M_w/M_n = polydispersity.

The ^{1}H NMR spectra were monitored by a JEOL EX400 spectrometer at 400 MHz in chloroform-*d* or DMSO-d_6 solutions. The matrix-assisted laser desorption ionization-time-of-flight-mass spectroscopies (MALDI-TOF-MASS) were recorded using a Bruker Reflex II. 2,5-Dihydroxybenzoic acid (DHB) was used as the matrix for the ionization and operated in the reflection mode. Cytochrome C and commercially available PEG oligomers were used for the calibration of the detected ions.

Dynamic light scattering (DLS) measurements were carried out at 25 °C using a light-scattering spectrometer (DLS-7000, Otsuka Electronics Co. Osaka, Japan) equipped with a vertically polarized incident beam at 488 nm supplied by an argon ion laser at scattering angles of 15°−160°. All the DLS measurements were performed at the nanogel concentration above 0.5 mg/mL. Details of the data analysis procedure using a cumulant approach have been described elsewhere.[19] The ζ-potentials of the nanogel surface in 10 mM NaCl solution over a pH range of 3−12 at 25 °C were determined using an electrophoretic light-scattering spectrophotometer (LEZA-600, Otsuka Electronics Co. Osaka, Japan), utilizing a laser Doppler technique, equipped with a helium−neon ion laser (633 nm). Scattering angles of 15°−20° were adopted in the measurements. The ζ-potentials were calculated from the mobility of the nanogels in the electric field at 38−42 V/cm using the Smoluchowski equation. The measurements were performed five times for each sample at 25 °C. Fluorescence spectra were obtained on a Hitachi F-2500 spectrometer with an excitation wavelength of 365 nm. The excitation and emission bandwidths were both 82 nm.

Results and Discussion

As the initial step of the preparation of the core−shell type nanogel depicted in Figure 1, we synthesized a new type of heterotelechelic PEG macromonomer, CH_2═CH-Ph-PEG-COOK. Conventional methods of the PEG macromonomer preparation reported so far are mainly the ω-end modification reaction after the EO polymerization. The ω-end alkoxide was converted to the vinylbenzyl group via a coupling reaction with vinylbenzyl chloride.[20] To prepare the heterotelechelic macromonomers, both the α- and ω-ends should be quantitatively modified. Because numerous types of chemistry for the ω-end modification reactions are available, a quantitative derivatization of the α-chain end to the polymerizable double bond is a key step to obtain the macromonomers having a variety of ω-functionalities. For this objective, 4-vinylbenzyl alcohol (VBA) was chosen as an initiator for the EO polymerization because the vinylbenzyl group should be inert during the EO polymerization by alkoxide. Nevertheless, metalation of the hydroxyl group of VBA to form the alkoxide initiator must be carefully carried out because the metalation agent may react with the double bond of VBA and cause an unexpected side reaction.

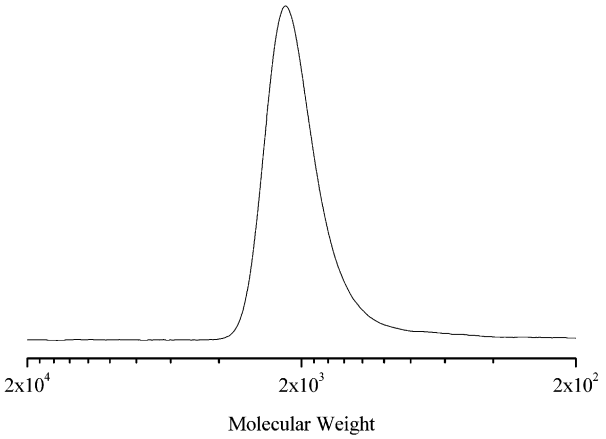

Figure 2. SEC chart of VBPEGCOOK21 (M_w/M_n = 1.06, run 2 in Table 1). Acetone$^-$K$^+$ was used as a metalation agent. Standard PEG samples with known molecular weight were utilized for calibration.

Here, potassium hydride and the potassium enolate of acetone were examined as selective metalation agents of the hydroxyl group of VBA.

The polymerization results are summarized in Table 1. When VBA was metalated by potassium naphthalene, the molecular weight distribution was not appreciably narrow (M_w/M_n = 1.14, run 0 in Table 1). Furthermore, the SEC of the obtained polymer had several shoulders in a higher molecular weight region (data not shown), indicating the occurrence of side reactions. Presumably, vinyl oligomerization took place to some extent when potassium naphthalene is used as a metalation agent for the 4-vinylbenzyl alcohol. The resulting VBA oligomer can also initiate the polymerization of EO to form higher MW products because it possessed several hydroxyl groups as the side chain. Indeed, the functionality of the double bond in the obtained polymer is determined to be fairly low from the ^{1}H NMR analysis.

On the other hand, polymerization smoothly proceeded to form PEG with a narrow molecular weight distribution by the use of potassium hydride as the metalation agent, being consistent with a previous report.[21] The molecular weight of the obtained polymer, however, was rather higher than the expected value calculated from the monomer/initiator ratio, indicating a reduced initiator efficiency. Presumably, the metalation by potassium hydride might not quantitatively proceed since potassium hydride forms a heterogeneous dispersion in THF. Furthermore, a complicated process was required including the decantation of an excess amount of KH after the metalation reaction. Thus, potassium enolate of acetone was examined as the alternative metalation agent. It was simply prepared

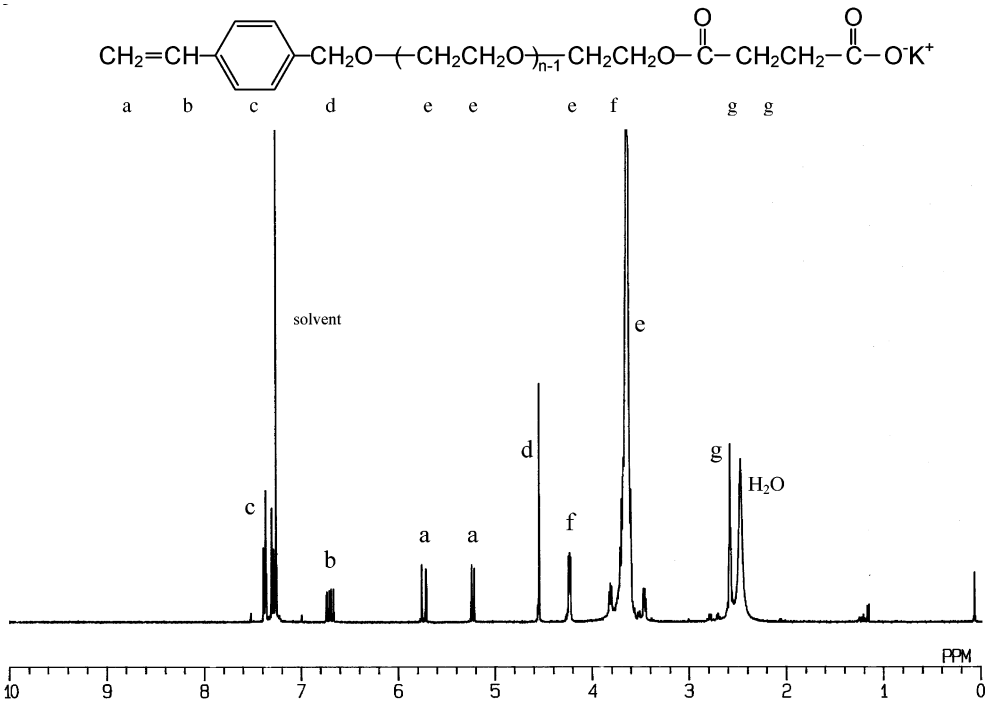

Figure 3. ^{1}H NMR spectrum of the VBPEGCOOK21 (the same sample as in Figure 2); solvent $CDCl_3$, room temperature, accumulation 32 times.

by the addition of potassium hydride to acetone. Note that the enolate anion is not reactive enough to attack the double bond of the vinylbenzyl group, allowing the α-vinyl group to remain intact in the macromonomer structure. A further advantage of this system is that the liberated acetone after the metalation reaction can be readily removed by evaporation under reduced pressure.

As seen in Table 1, VBPEGCOOK from the potassium acetonate system had a consistent molecular weight with a fairly narrow molecular weight distribution. Consequently, potassium acetonate was judged as a suitable metalation agent for the alcohol moiety in VBA. The alkoxide at the ω-end was successfully converted after the EO polymerization to carboxylate via the addition of succinic anhydride.[22] Figure 2 shows a typical size exclusion chromatogram of the obtained polymer (run 2 in Table 1). As can be seen in Figure 2, only a unimodal peak was observed with no shoulder in the high molecular weight region, suggesting no remarkable side reactions. The ^{1}H NMR spectrum of the obtained polymer gave information on the end functionality (Figure 3). Along with the OCH_2 signal based on the PEG main chain at 3.6 ppm, protons based on the vinyl benzyl end group at around 5−8 ppm as well as the succinic end protons at 2.5 ppm (g) were observed. The ^{1}H NMR as well as SEC data indicate that the functionalities of both ends were quantitative.

Furthermore, the MALDI-TOF-MS analysis gave quantitative data on the end-functionality of the macromonomer based on the molecular mass of the individual polymer molecule. Figure 4 shows the MALDI-TOF-MS spectrum of VBPEGCOOK(2.1K), in which the mass of the products appears around 2300 (M_n = 2170; M_w/M_n = 1.02), being in good agreement with the SEC results. The difference in the mass of each signal was roughly estimated as 44, indicating the signals were assignable to the PEG homologues.

In consideration of the MW of the EO monomer and both end groups, the MW of each heterotelechelic PEG should be expressed by the following equation:

$$\underset{\text{MS}}{\text{MW}} = \underset{\text{EO}}{44.053n} + \underset{\text{VB}}{133.170} + \underset{\text{succinate}}{101.082} \quad (1)$$

The detected signals were 39 mass units larger than those calculated from eq 1, probably due to the potassium adduct, which originated from the initiator for the polymerization. For example, the center peak in the expanded spectrum (Figure 4b) showed a mass of 2255.88, which agreed well with 45 mers. Small signals appearing at 16 mass units lower than each large signal are generally known as sodium adduct ions of the products. On the basis of these obtained results, it was confirmed that PEG with a polymerizable vinylbenzyl group at the α-chain end and a carboxylic group at the ω-chain end was quantitatively synthesized.

Preparation of nanosized particle via an emulsion polymerization technique is well established and widely utilized in industrial fields.[23] PEG macromonomers are often used as a stabilizer of monomer droplets during the polymerization.[24] In this case, PEG macromonomers work not only as the stabilizer of the monomer droplet but also as a comonomer to incorporate into the particle. The hetero-PEG macromonomer used in this study, however, possesses a potassium carboxylate end group at one end. In the present system, polymerization of amine-containing monomer in the presence of anionic PEG macromonomer was carried out in order to introduce pH sensitivity of the resulting nanogel. It is often shows a coacervate formation in the copolymerization of opposite charged monomer couples. Actually, when we prepared poly((dimethylamino)ethyl methacrylate)-*g*-PEG-COOK using the same hetero-PEG macromonomers as in this study, the coacervate formation was observed under the certain conditions. The emulsion polymerization was examined if such a monomer couple can be smoothly polymerized to form nanogel with PEG-COOK tethered chains on the surface. 2-(*N*,*N*-Diethylamino)ethyl methacrylate (EAMA) was chosen as a

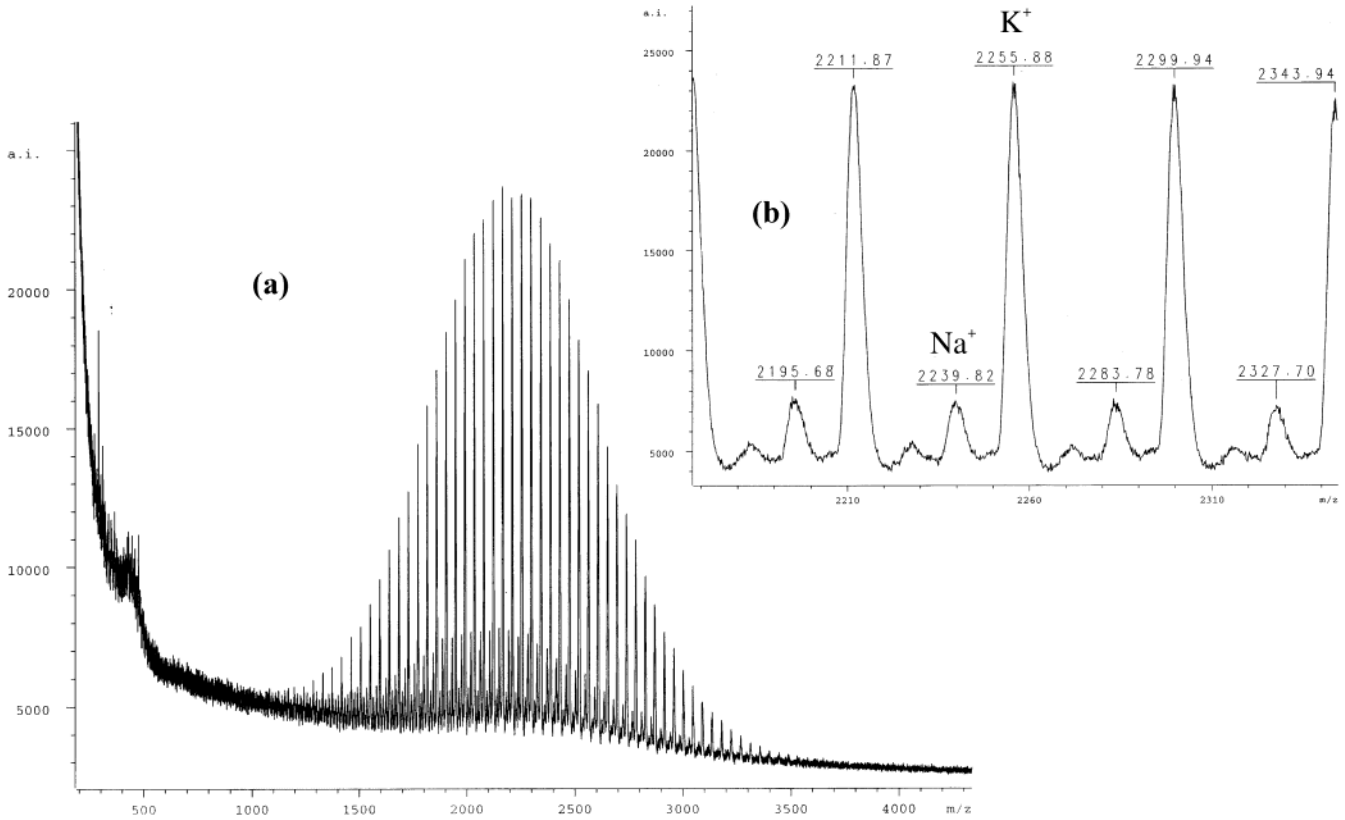

Figure 4. MALDI-TOF mass spectrum of the VBPEGCOOK21 (a) and its expanded spectrum in the region of 2150−2350 (b). The inset shows the two different ionized species, the one with sodium and the other with potassium adducts.

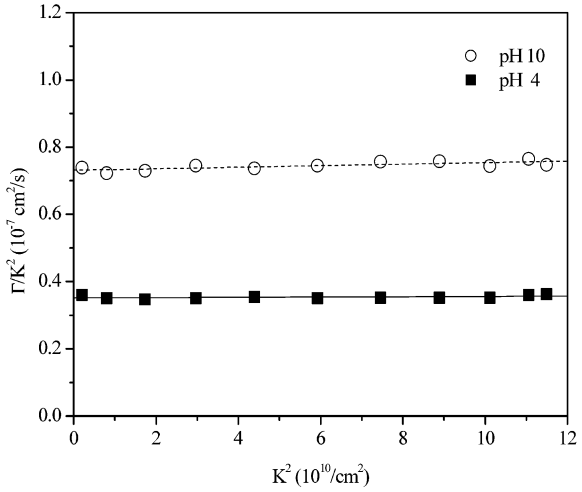

Figure 5. Plots of the K^2-scaled average characteristic line width Γ (Γ/K^2) vs K^2 for the nanogel with PEG/PAMA = 1/4 (1 mol % EDMA cross-linking) at concentration of 0.5 g L^{-1} and temperature of 25 °C at pH 4 (■) and pH 10 (○).

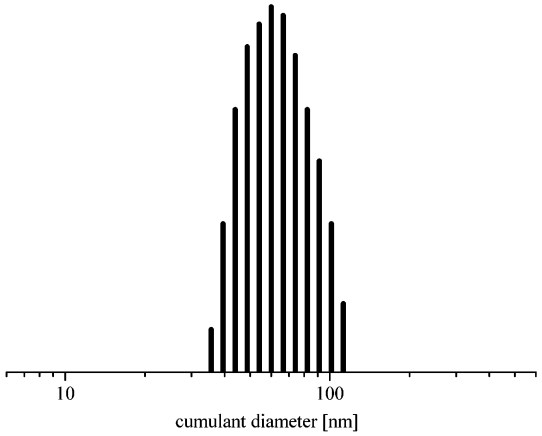

Figure 6. Gamma distribution of the nanogel (PEG/PAMA = 1/4, 1 mol % EDMA cross-linking) analyzed by DLS. Temperature 25 °C, detection angle 90°.

comonomer because it has a hydrophobic nature in the alkaline region and changes the hydrophilicity with pH. The polymerization was conducted with 1 mol % of cross-linking agent (ethylene dimethacrylate).

During the emulsion polymerization in the presence of the heterotelechelic PEG macromonomers, no coacervate was formed and milky liquid was obtained. After the purification of the products, the shape and size of the obtained nanogel were measured by dynamic light scattering measurements. Figure 5 shows the angular dependency of the scaled characteristic line width on the scattering vector, which corresponds to the scattering angle. As can be seen in the figure, no angular dependency of the scaled characteristic line width on the scattering vector was observed. The results indicated that the nanogel is perfectly spherical structure at different pHs 4 and 10, regardless of whether nanogel is swelling state or deswelling state. The gamma distribution of the nanogel showed unimodal distribution; the cumulant diameter and d*w*/d*n* were 62.9 nm and 1.15 as shown in Figure 6 (run 4 in Table 2). The sizes of the obtained nanogels were controllable by the experimental weight ratios of PEG macromonomer vs EAMA, keeping the low size distribution factors as shown in Figure 7. The data obtained by several polymerization conditions are summarized in Table 2. Under the conditions described in this study, nanogels with a diameter in the range between 50 and 680 nm were obtained.

Table 2. Results of the Emulsion Polymerization of EAMA in the Presence of VBPEGCOOK[a]

	VBPEGCOOK21		EAMA		PEG/EAMA	size[b]	d*w*/
run	μmol	mg	mmol	mg	(g/g; mol/mol)	(nm)	d*n*[c]
1	278	500	1.35	250	1/0.5; 1/4.8	49.5	1.50
2	208	375	2.02	375	1/1; 1/9.6	46.5	1.16
3	139	250	2.70	500	1/2; 1/19	55.2	1.14
4	83.3	150	3.24	600	1/4; 1/39	62.9	1.15
5	46.3	83.3	3.60	667	1/8; 1/78	82.3	1.19
6	8.17	14.7	3.97	735	1/50; 1/486	196	1.14
7	4.13	7.4	4.01	743	1/100; 1/971	341	1.21
8	1.04	1.9	4.04	748	1/400; 1/3900	681	1.16

[a] Initiator KPS, cross-linker EDMA, 1 mol % to total monomer, polymerization time 24 h, temperature 60 °C. [b] Size = mean cumulant diameter. [c] d*w*/d*n* = particle size distribution.

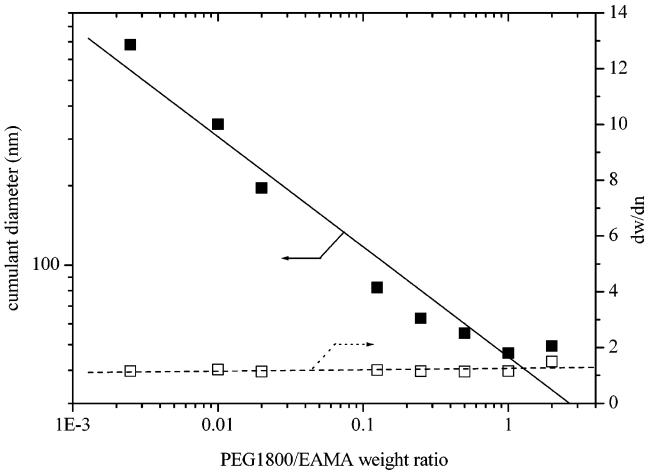

Figure 7. Effects of PEG/EAMA ratio on the particle diameter (■) and the distribution (□) determined by DLS.

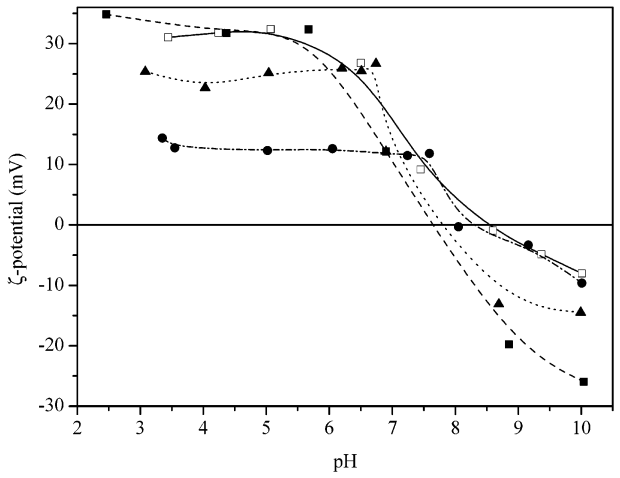

Figure 8. Effects of pH and the length of PEG chain on the ζ-potential of nanogels determined by laser Doppler electrophoresis at 25 °C in 10 mM NaCl aqueous solution. Chain length of PEGCOOK in nanogel was (■) 1800, (▲) 4200, and (●) 8000. The nanogel with MeOPEG (M_n = 2000) tethered chain was used as a control (□).

The obtained nanogel possesses amino groups in the core and carboxylate group at the distal end of the PEG chain; it is interesting to analyze the electrostatic characteristics of the nanogel. Figure 8 shows the ζ-potential of the obtained nanogel by changing the environmental pH. Since the nanogel consists of an amino-containing monomer, the ζ-potential in the acidic region was basically positive. Worth noting is a significant decrease in the ζ-potential at around pH = 7, reflecting the pK_a of PEAMA (pK_a = 7.5).[25]

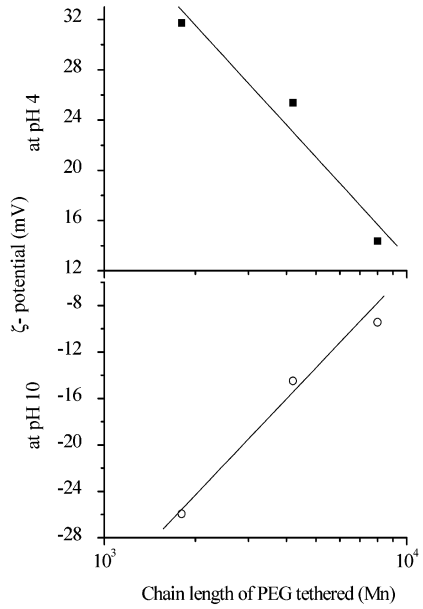

Figure 9. Change in the ζ-potential of nanogel with the chain length of PEG at pH 4 (■) and pH 10 (○).

In Figure 9, the ζ-potentials of several samples at pHs 4 and 10 were depicted vs the MW of PEG. In the acidic region, the ζ-potential of the nanogel possessing the PEG tethered chains of MW = 1.8K showed +32 mV. With the increasing MW of PEG on the nanogel surface, the ζ-potential decreased, indicating that the charge-shielding effect of PEG became more significant with its increasing MW, which is consistent with the previous report.[26] On the contrary, the reverse tendency of the surface charge was observed in the alkaline region. In pH 10, PEAMA should be totally deprotonated, and eventually, the ζ-potential of the nanogel showed a shift in the negative direction. The nanogel with the MeOPEG tethered chain showed a slightly negative ζ-potential value of −6 mV at pH 10. The introduction of a carboxyl group at the distal end of PEG (VBPEGCOOK (1.8K)) made the ζ-potential of the nanogel become more negative at −26 mV due to the contribution of the carboxylate moieties at the PEG chain end. With the increasing MW of the PEG tethered chain, there was a change in the ζ-potential in the positive direction. We reported previously that the average surface area occupied by each PEG molecule is not influenced by the size of the particle obtained by the dispersion polymerization in the presence of PEG macromonomers with the same molecular weight.[15] Wu et al. reported, however, the density of the tethered PEG chain decreased with increasing its molecular weight,[27] which eventually influenced the ζ-potential of the nanogel because that the carboxyl group was adjacent to the end of PEG chain.

To confirm the presence of a carboxylic group at the distal end of the PEG tethered chains on the surface of the nanogel, fluorescent labeling was conducted using the active ester method. The fluorescent labeling was carried out using a water-soluble carbodiimide coupled with DMEQ-hydrazide. After the purification of the conjugated nanogel by centrifugation, a fluorescent signal was clearly observed as seen in Figure 10, which is clear proof that the prepared nanogel possessed carboxylic acids on the periphery to utilize as a conjugation site.

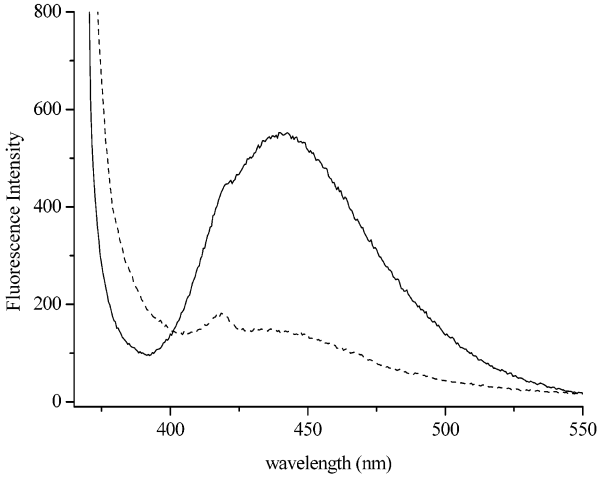

Figure 10. Fluorescence spectrum of the nanogel with (—) and without (···) carboxylic group at the PEG chain end after the fluorescent probe (DMEQ-hydrazide) was reacted with the nanogel via the active ester method.

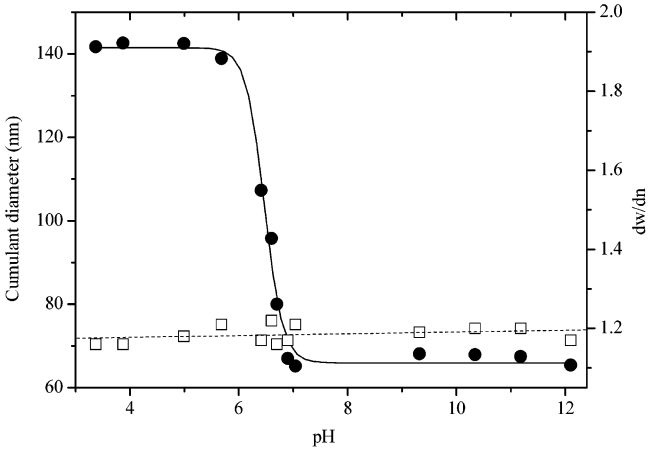

Figure 11. pH dependency of the cumulant diameter (●) and the size distribution (□) of nanogel (run 4, Table 2).

Since the poly(EAMA) gel is known to show a volume phase transition as a function of pH,[28] the size change in the nanogel with pH was monitored by the DLS as shown in Figure 11. The size of the nanogel with VBPEGCOOK(1.8K) obtained in run 1 in Table 1 was ca. 60 nm in the neutral to alkaline region. Of interest, a sharp pH sensitivity was revealed in the pH region of 6−7 for the nanogel, and its size abruptly increased up to 140 nm by decreasing the pH to 6. Protonation of the amino groups in the core triggers the nanogel swelling due to an increase in the ion osmotic pressure along with polymer solvation.[29] Worth noticing is that the narrowly distributed feature of the nanogel was maintained through this volume transition. Also, no coagulation as well as precipitation of the nanogel was observed in over the entire pH change, indicating its high dispersion stability.

The ionic strength (I) effect on the size of the nanogel under acid and alkaline conditions was examined. Under the acid conditions, the hydrodynamic diameter was strongly affected by the ionic strength. As can be seen in Figure 12, the hydrodynamic diameter of the nanogel was decreased with increasing the ionic strength. On the contrary, the hydrodynamic diameter under the alkaline conditions did not affect at all. On the basis of these data, the hydrodynamic diameter was mainly governed the gel osmotic pressure but not the interparticle interaction.

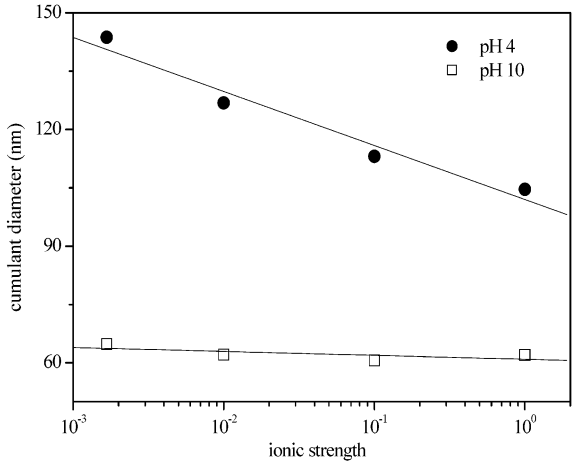

Figure 12. Effect of ionic strength on the cumulant diameter of the nanogel (run 4, Table 2) at pH 4 (●) and at pH 10 (□).

Conclusion

Heterotelechelic PEG macromonomers with a polymerizable vinylbenzyl group at one end and a carboxylic group at the other chain end were quantitatively synthesized using the potassium enolate of acetone as a metalation agent of the initiator, 4-vinylbenzyl alcohol. Using this hetero-PEG macromonomer as a stabilizer, the emulsion polymerization of 2-(*N*,*N*-diethylamino)-ethyl methacrylate was carried out in the presence of a cross-linking agent such as ethylene dimethacrylate. The size of the obtained nanogels was in the range between 50 and 350 nm. The obtained nanogel showed a sharp volume transition in the pH region of 6−7, where the size of the nanogel changed between 60 and 140 nm due to the change in the protonation status of the EAMA groups. From the ζ-potential analysis, the PEG tethered chain located on the nanogel periphery was confirmed to effectively shield the core charge. With the increasing molecular weight of the PEG, the shielding effect increased. Furthermore, the carboxylic acid group located at the distal end of the PEG tethered chains was available as a conjugation site of various ligands. These features indicate the potential utility of nanogels in the diverse fields of functional nanomaterials.

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