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Thermochromic Microgels and Core-Shell Microgels Based on Fluorescence Resonance Energy Transfer

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ABSTRACT: Microgels exhibiting thermochromic behavior based on fluorescence resonance energy transfer (FRET) are prepared. The FRET microgels consist of poly(*N*-isopropylacrylamide) (PNiPAm) networks with fixed fluorescein and rhodamine moieties and exhibits volume phase transition (VPT) at 34–35°C. A critical decrease in their sizes during the VPT enhances the efficiency of FRET between fluorescein as a donor and rhodamine as an acceptor. Therefore, emission from fluorescein (523 nm) and that of rhodamine (579 nm) is dominant at temperatures below and above the VPT temperature, respectively, when fluorescein is excited. We also prepare thermochromic core-shell FRET microgel exhibiting two-step color change. The microgels consist of a PNiPAm core and a poly(*N*-isopropylacrylamide-*co*-*N*,*N*-diethylacrylamide) shell and exhibit dual temperate-responsiveness at 19 and 33°C. The fluorescence spectrum of the microgels also changes in two steps at these temperatures. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 201–205, 2013

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INTRODUCTION

Microgels composed of thermosensitive polymers such as poly(*N*-isopropylacrylamide)[1,2] have extensively studied in the areas of drug delivery[3] and responsive colloidal arrays.[4–6] This is due to critical changes in their sizes and physical properties during reversible volume phase transition (VPT). The cross-linked polymer networks are highly swollen below the phase transition temperature (T_p), but become deswollen during VPT, where dissociation of water molecules from the polymer chains occurs.[7] Thermosensitive gels are also used as materials controlling transmittance and scattering of light. In general, these systems only allow the media to change from a transparent state to an opaque state and gels exhibiting a color change have been desired.

Here, we report microgels exhibiting a thermochromism i.e., displaying different colors at different temperatures. The color change is based on fluorescence resonance energy transfer (FRET).[8] FRET is a radiationless energy transfer mechanism between two chromophores. An excited donor chromophore can transfer energy by a dipole–dipole coupling mechanism to an acceptor chromophore in close proximity (typically < 10 nm). Because the efficiency of FRET (E) decreases with increasing distance (R) between the donor and the acceptor with $E = R_0^6/(R_0^6 + R^6)$, where R_0 is the Förster distance for 50% transfer and typically around 5–10 nm, it is highly sensitive to the distance. Therefore, the phenomenon is often used as a spectroscopic ruler measuring distances at a molecular level.[9] By fixing the donor and acceptor chromophores on polymer networks exhibiting VPT, we can alter the distance between them and the efficiency of FRET (Figure 1). That is, the networks are swollen and the efficiency is low at low temperatures while they are collapsed and the efficiency becomes high at temperatures above T_p. Therefore, emission from donors and acceptors will be dominant at temperatures below and above T_p, respectively. This is our basic strategy to prepare microgels exhibiting the temperature-sensitive color change.

In addition, microgels possessing core-shell architecture have recently attracted keen attention because of their special swelling properties and multistimuli responsiveness. Core-shell microgels consisting of a PNiPAm core and a poly(*N*-isopropylmethacrylamide) shell[10] and a poly(ethylene glycol) ethyl ether methacrylate (PEGEEMA) core and the shell consisted of a copolymer of PEGEEMA and poly(ethylene glycol) methyl ether methacrylate[11] had doubly thermosensitive properties. Temperature- and pH-responsive core-shell microgels were composed of PNiPAm as a core and PNiPAm-acrylic acid as a shell.[12] Here, we prepared core-shell FRET microgels with a PNiPAm core and the shell consisted of the copolymers of *N*-isopropylacrylamide and *N*,*N*-diethylacrylamide, which exhibited double temperature

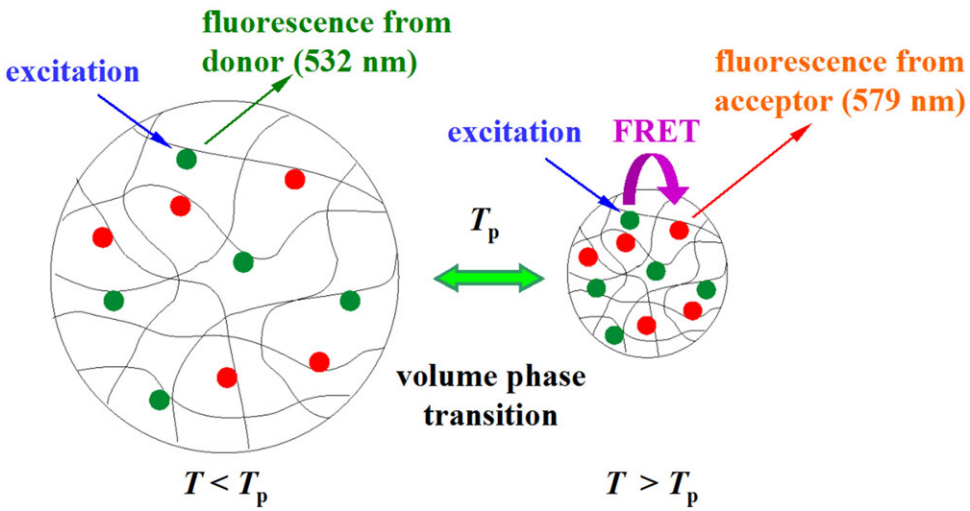

Figure 1. The schematic illustration of FRET-based thermochromism of thermosensitive microgel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

responsiveness. By incorporating the donors and the acceptors of FRET in both core and shell networks, they exhibited two-step color change depending on temperature.

EXPERIMENTAL

Materials

A vinyl monomer derived from fluorescein (Flu) was synthesized by a coupling between fluorescein and acryloylchloride. A vinyl monomer derived from rhodamine B (Rho) was synthesized by a coupling between rhodamine B and *N*-hydroxyethylacrylamide in the presence of *N*,*N*′-dicyclohexylcarbodiimide. The FRET microgels were prepared by radical copolymerization of *N*-isopropylacrylamide (NiPAm, 1.67 g), *N*,*N*′-methylenebis (acrylamide) (BIS, 47 mg) as a crosslinker, 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS, 15 mg) as a ionic monomer and these fluorescent monomers (Flu 10 mg and Rho 10 mg) in water (51.5 mL) at 70°C under N_2 atmosphere with continuous stirring at 500 rpm in the presence of potassium persulfate (KPS, 100 mg) as an initiator and sodium dodecylsulfate (SDS, 0.15 g) as an emulsifier. The microgels were purified via dialysis against water changed 3 times a day for a week before use.

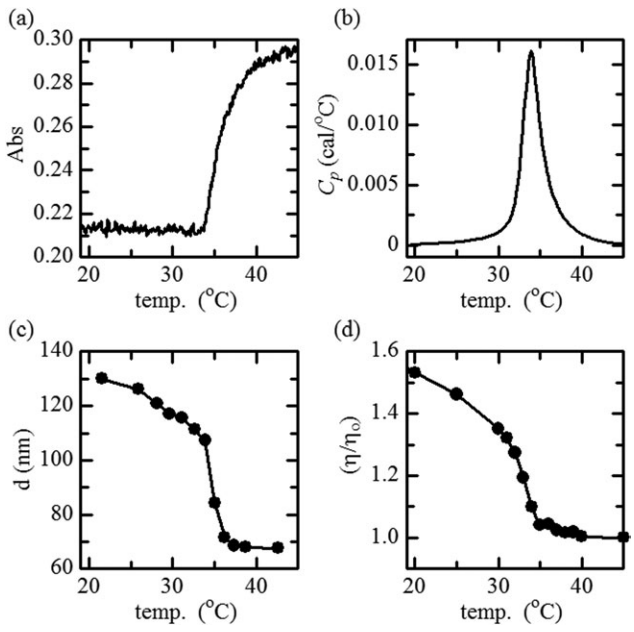

Figure 2. Temperature dependences of (a) turbidity, (b) heat capacity, (c) hydrodynamic diameter, and (d) relative viscosity of the FRET microgels.

To prepare the core-shell FRET microgels, the core was firstly prepared with NiPAm (0.83 g), dEAm (0.95 g), BIS (47 mg), Flu (10 mg), Rho (10 mg), KPS (91 mg), SDS (0.15 g), and water (50 mL). Then, the shell was synthesized with NiPAm (0.55 g), BIS (16 mg), AMPS (5mg), Flu (3 mg), Rho (3 mg), KPS (67 mg), SDS (0.15 g), and water (50 mL) in the presence of 30 mL of the core prepared above.

Measurements

DSC measurements were performed using a Micro Calorimetry System (MicroCal) at a scanning rate of 0.75°C/min. Viscosity of the suspension was measured with Ostwald viscometer. Relative viscosity (η/η_0) was calculated from the ratio of elution time ($\eta/\eta_0 = t/t_0$) of the suspension to that of pure water of the same temperature. Dynamic light scattering (DLS) measurements were carried out using a light-scattering spectrometer (DLS-7000, Otsuka Electronics) equipped with an argon ion laser. Data analysis was performed using a cumulant approach. The turbidity was followed by the absorbance at 500 nm using a spectrophotometer (UV200-100, Hitachi) with a heating rate of about 1°C/min. Fluorescence spectra of the microgels (0.5 wt %) were measured with a FP-777 spectrofluorometer (JASCO) with a heating rate of about 1°C/min. IR spectra were measured at a resolution of 2 cm^{-1} by using a Fourier-transform infrared spectrometer (FTIR-8400, Shimadzu). The microgels dispersed in D_2O were placed between two CaF_2 windows with a 10 μm-thick spacer and its temperature was controlled by using a circulating water bath. IR spectra of the solution were

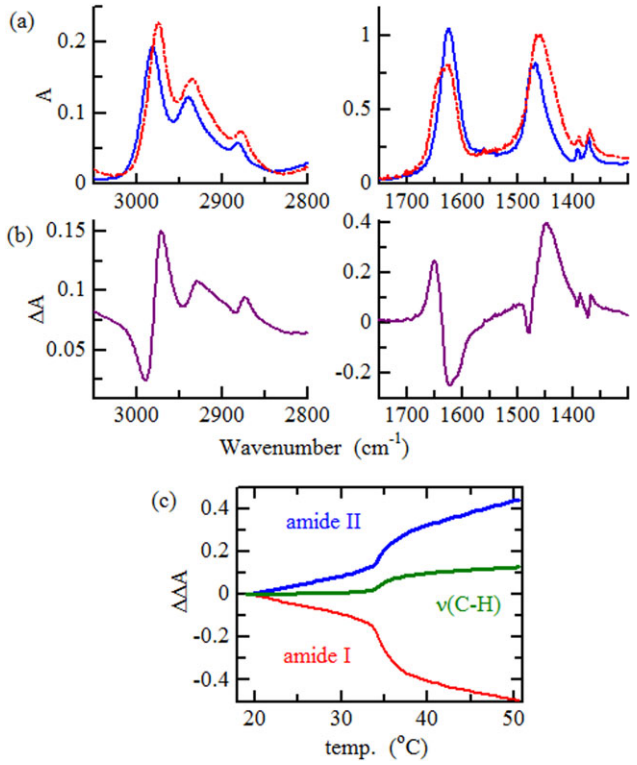

Figure 3. (a) The IR absorption spectra at 19°C (solid line) and 51°C (broken line) and (b) a difference spectrum (A(51°C)–A(19°C)) of the FRET microgels in D_2O. (c) The values of $\Delta s\Delta A(T)$ for the ν(C—H), amide I, and amide II modes are plotted against temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

continuously collected at different temperatures at a heating rate of 0.5°C/min.

RESULTS AND DISCUSSION

Properties of the FRET Microgels

First, VPT of the FRET microgels were characterized by turbidimetry, DSC, DLS, viscosity, and IR measurements. The turbidity at 500 nm and the endothermic peak in the DSC thermogram of aqueous suspension of the microgels indicate T_p exist around 34°C (Figure 2). Dynamic light scattering shows that the average hydrodynamic diameter (D) of the microgels is 130 nm at 21°C and critically decreases around T_p of 34°C upon heating [Figure 2(c)]. The diameter becomes 56 nm at 40°C, which corresponds to the deswelling ratio of 0.43 in diameter and 0.08 in volume as compared with these values at 21°C. Concomitantly relative viscosity of the suspension decreases at the same temperature region [Figure 2(d)]. Figure 3 shows the IR absorption spectrum (A) at 19°C and the difference IR spectrum (ΔA) induced by the VPT. The difference spectrum indicate red shifts of the ν(C—H) bands (3100–2800 cm^{-1}) and amide II band (1470 cm^{-1}) and a blue shift of the amide I band (1625 cm^{-1}). These spectral changes indicate dehydration of the alkyl groups and break of hydrogen bonds between the amide groups and water molecules upon the VPT. Increase in the intensities of the positive and negative peaks of a series of the difference spectra collected at heating can be used to monitor the progress of the dehydration. A difference between the value of ΔA at the positive peak and that at the negative peak of each vibration mode is defined as $\Delta\Delta A$. The values of $\Delta\Delta A$ for ν(C—H), amide I, and amide II modes are plotted against temperature in Figure 3(c). The tangents of the three curves indicate that the hydration changes occur between 34 and 35°C. After all, all experimental data show that the FERT microgels exhibit a sharp VTP in a relatively narrow temperature range (34–35°C).

FRET Measurements

Next, we measured electronic absorption spectra (broken lines) and fluorescence spectra (solid lines) of the fluorescent monomers [Figure 4(a)]. The overlap of the absorption spectrum of Rho and the fluorescence spectrum of Flu shows that Flu and Rho can act as the donor and the acceptor of the FRET, respectively. The fluorescence emission spectra of the microgel particles excited at 497 nm and measured at different temperatures are shown in Figure 4(b). The emission from Flu centered at 523 nm was dominant at low temperatures, although a weak emission from Rho was also observed around 579 nm. The emission from Rho increased and that from Flu decreased on heating. Figure 4(c) shows the intensities at 523 and 579 nm as a function of temperature. The intensities significantly changed in the temperature range of 32–35°C, where the sizes of the microgel particles critically changed. Moreover, the spectral change was fully reversible. A sudden shrink of the gel network during the VPT induced a decrease in the average distance between the donor/acceptor pairs. Thus, the efficiency of FRET was raised and finally Rho emission was enhanced as schematically illustrated in Figure 1. Figure 4(d) is a photograph showing the temperature-dependent color change of the microgel particles. The suspensions in the left and the right cuvettes were kept below and above T_p, respectively, and excited with a blue-light emitting diode. We used two polarizer plates to remove incident and scattered light from fluorescence. A linearly polarized light was selected by passing the light from the source through the first polarizer and depolarized fluorescence was observed through the second polarizer whose plane of polarization is perpendicular to the incident light. Incident and scattered light was absorbed by the second polarizer. The difference of color of the suspensions was significant enough that we could recognize it with the naked eyes.

Control experiments were also performed to confirm that this observation results from intraparticle interaction between Flu and Rho. When we excited at 497 nm, emission from Flu-containing microgel particles was observed at 523 nm. Its intensity slightly decreased during the VPT probably because the suspension became slightly turbid above T_p. With the same excitation wavelength, emission from Rho-containing microgel was weak and spectral change during the VPT was negligibly small. Finally, fluorescence spectra of a suspension of an equal weight of Flu-containing microgel particles and Rho-containing microgel particles were close to the mathematical sum of each component spectrum at any temperatures. A strong emission from Rho could not be observed even at high temperatures meaning

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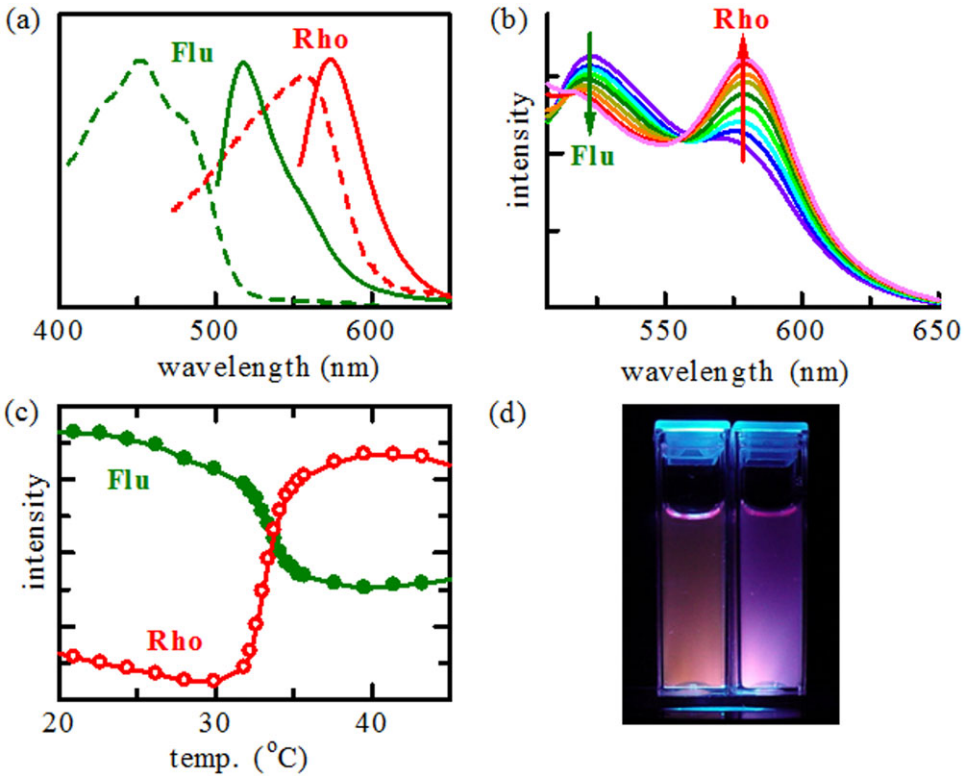

Figure 4. (a) The electronic absorption spectra (broken lines) and fluorescence spectra (solid lines) of fluorescent monomers, Flu and Rho. (b) The fluorescence spectra (excitation: 497 nm) of the FRET microgels at different temperatures (30.1–35.8°C). (c) Fluorescence intensity at 523 and 579 nm of the FRET microgels are plotted against temperature. (d) The photographs of suspensions of the FRET microgels kept below T_p (left) and above T_p (right) and exited by a blue-light-emitting diode. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that no interparticle FRET occurs between the Flu- and Rho-containing particles.

We also investigated the effect of the molar ratio of Rho to Flu on fluorescence spectra. We prepared three samples containing the same amount of Flu and different amount of Rho. They have similar diameters and exhibit similar temperature-responsiveness as observed by DLS and DSC measurements. The intensity of Rho emission below T_p increased with increasing amount of Rho. Because the Förster distance, R_0, for Flu-Rho pair is 5.96 nm,[13] Flu-Rho pairs locating in closer proximity than this value exhibit efficient FRET even in the swollen state of the microgel particles. On the other hand, the magnitude of spectral change during the VPT was reduced with increasing amount of Rho at Rho/Flu > 1.

Two-Step VPT of Core-Shell FRET Microgels

We next tried to prepare FRET microgels, which exhibit a sequential spectral change at two different temperatures. For this purpose we synthesized core-shell type microgel particles via a seed polymerization, in which both the core and the shell contain Flu and Rho and exhibit VPT at different temperatures (Figure 5). To prepare the core, we used NiPAm and *N,N*-diethylacrylamide (dEAm) at a molar ratio of 1 : 1 as thermosensitive

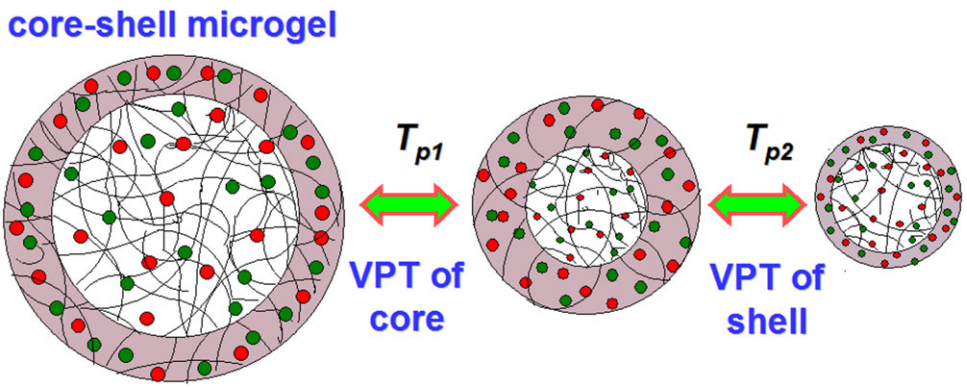

Figure 5. The two-step VPT of core-shell FRET microgel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

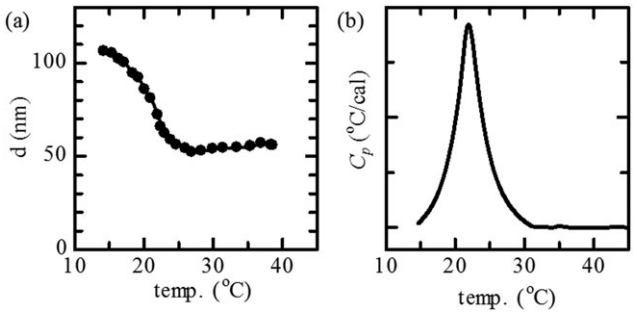

Figure 6. (a) The hydrodynamic diameter and (b) heat capacity of the microgels used as the core of the core-shell FRET microgels are plotted against temperature.

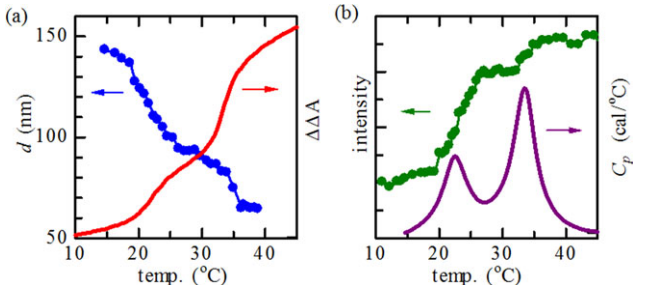

Figure 7. (a) The hydrodynamic diameter (d) and $\Delta\Delta A(T)$ for the ν(C—H) mode of the core-shell FRET microgels are plotted against temperature. (b) The fluorescence intensity at 579 nm and the heat capacity (C_p) of the core-shell FRET microgels are plotted against temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

monomers. It is known that the copolymers of NiPAm and dEAm at any compositions have lower T_p than both of two corresponding homopolymers and that T_p of the copolymer is minimized at NiPAm:dEAm = 1 : 1.[14,15] The microgel, which becomes the core of core-shell microgels later, have T_p around 19°C and their diameter decreased from 106 nm (15°C) to 52 nm (27°C) during the VPT (Figure 6). An endothermic peak was observed in DSC thermogram in the temperature range between 18 and 26°C [Figure 6(b)]. The following seed polymerization with NiPAm results the core-shell FRET microgels. Temperature dependences of the properties of the core-shell FRET microgel are shown in Figure 7. The average hydrodynamic diameter of the microgels is 142 and 65 nm at 15 and 38°C, respectively, and larger than that of the core by 36 and 13 nm at the same temperatures. DSC, DLS, and IR measurements of the core-shell microgels clearly shows that two-step VPT occurred at around 19 and 33°C (Figure 7). Because the core-shell FRET microgels contain Flu and Rho in both core and shell, the intensity of Rho emission is also change in two steps as shown in Figure 7(b).

CONCLUSIONS

The microgels consisting of PNiPAm networks with fixed fluorescein and rhodamine moieties exhibit thermochromic behavior based on FRET at VPT of 34–35°C. The emission from fluorescein (523 nm) and rhodamine (579 nm) is dominant at temperatures below and above the VPT temperature, respectively. The core-shell FRET microgels consisting of a PNiPAm core and a poly(*N*-isopropylacrylamide-*co*-*N*,*N*-diethylacrylamide) shell exhibit dual temperate-responsive spectral changes at 19 and 33°C. In general, fluorescence spectra have a higher signal/noise ratio and can be measured at lower concentrations than absorption spectra, because light intensity of a background is zero in fluorescence spectra. The fluorescence spectra of a single particle of these microgels can be measured by using fluorescence microscopy coupled with spectrometer. These microgels may be useful as a single-particle spectroscopic thermometer.

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