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Thermoresponsive Copolymer Containing a Coumarin–Spiropyran Conjugate: Reusable Fluorescent Sensor for Cyanide Anion Detection in Water

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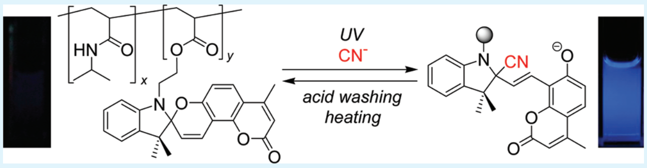
Supporting Information



ABSTRACT: A simple copolymer consisting of *N*-isopropylacrylamide and coumarin-conjugated spiropyran (CS) units, poly(NIPAM-*co*-CS), has been synthesized. This polymer enables selective fluorometric detection of cyanide anion (CN^-) in water at room temperature. The polymer itself shows almost no fluorescence, but shows a strong blue fluorescence in the presence of CN^- under irradiation of UV light. The fluorescence enhancement occurs via a nucleophilic interaction between CN^- and the photoformed merocyanine form of the CS unit, leading to a localization of π-electrons on the coumarin moiety. The polymer enables accurate determination of very low levels of CN^- (>0.5 μM). The polymer can be recovered from water by simple centrifugation at high temperature (>40 °C), due to the heat-induced aggregation of the polymer. In addition, the polymer is regenerated by simple acid treatment, and the resulting polymer is successfully reused for further CN^- sensing without loss of sensitivity.

KEYWORDS: fluorescent sensor, cyanide anion, spiropyran, coumarin, polymer

1. INTRODUCTION

The cyanide anion (CN^-) is extremely hazardous to living organisms.[1] It strongly binds the active site of cytochrome-*c* and inhibits the mitochondrial electron-transport chain, leading to decreased oxidative metabolism and oxygen utilization.[2] The maximum permissive level of cyanide in drinking water is therefore set at 1.9 μM by the World Health Organization (WHO).[3] The industrial use of cyanide salts, however, remains widespread, particularly in gold mining, electroplating, and metallurgy.[4] Accidental release of cyanide into the environment inevitably occurs. There is therefore a strong need for CN^--selective receptors that facilitate quantitative determination of CN^- by a simple assay.

A variety of molecular receptors that show colorimetric or fluorometric response to CN^- have been proposed.[5] Many of these receptors, however, suffer from several problems: they (i) act only in pure organic solvents or solutions containing a large amount of organic solvents;[6] (ii) show poor selectivity to CN^-;[7] and, (iii) show high detection limit.[8] To the best of our knowledge, there are only seven reports of CN^- receptors that work in water with high selectivity and sensitivity.[9] These receptors, however, react with CN^- irreversibly and cannot be reused for further analysis. The design of CN^- receptors that show high selectivity, sensitivity, and reusability is therefore currently the focus of attention.

Earlier, we reported that a coumarin–spiropyran conjugate (**1**, Scheme 1) behaves as a fluorescent CN^- receptor in aqueous media under irradiation of UV light at room temperature.[10] The receptor **1**, when dissolved in a MeCN/water (2/8 v/v) mixture, exists as a colorless spirocyclic (SP) form and shows almost no fluorescence. UV irradiation of the solution leads to an isomerization to the colored merocyanine (MC) form, which is also nonfluorescent. The nucleophilic interaction between CN^- and the positively charged spirocarbon of the MC form produces the **1**–CN^- species. This promotes a localization of π-electrons on the coumarin moiety and results in an appearance of strong coumarin fluorescence. The fluorescence response occurs selectively to CN^- and the receptor enables accurate determination of very low levels of CN^- (>0.5 μM). The receptor **1** is, however, insoluble in total water and the addition of organic solvent is necessary. In addition, tedious separation procedures are necessary to recovery the receptor from the homogeneous solution. For practical applications, the receptor must be improved to have high solubility in water but to be recoverable and regenerable by simple procedures.

Poly-*N*-isopropylacrylamide (polyNIPAM) is a water-soluble thermoresponsive polymer that shows a reversible coil-to-globule phase transition, associated with hydration/dehydration of polymer chain by temperature.[11] PolyNIPAM is soluble in water at low temperature (coil), but a rise in temperature leads to a formation of polymer particles associated with the aggregation of polymer chain (globule). This homogeneous/heterogeneous

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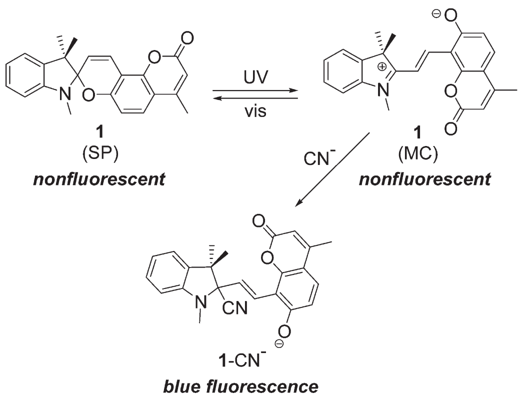
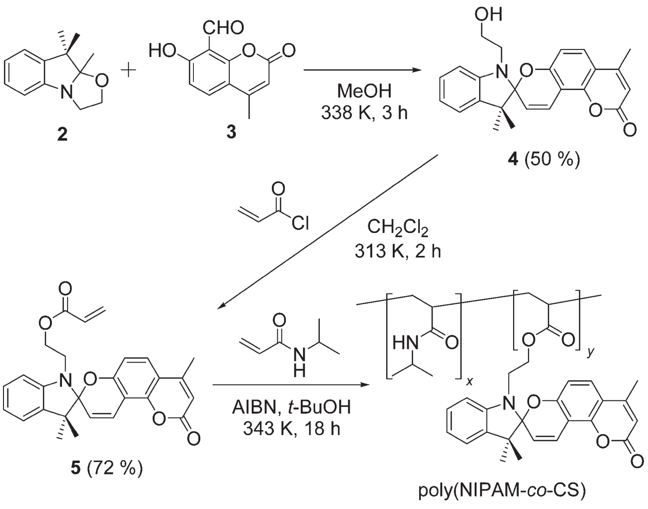
Scheme 1. Structure Change of Receptor 1



Scheme 2. Synthesis of Poly(NIPAM-*co*-CS)



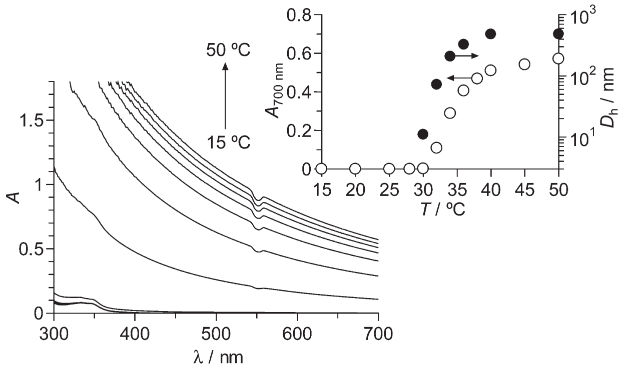


Figure 1. Temperature-dependent change in absorption spectra of poly(NIPAM-*co*-CS) (0.17 g L^{-1}; containing 10 μM CS unit) measured in a buffered water (CHES 100 mM, pH 9.3). (Inset) Change in (white) absorbance at 700 nm and (black) hydrodynamic diameter (D_h) of polymer particles. The detailed D_h data are summarized in Figure S7 in the Supporting Information.

switchable nature suggests that the coumarin−spiropyran unit (**1**), if incorporated into the polyNIPAM matrices, can be dissolved in total water at low temperature and used for fluorescent CN^- sensing. In contrast, at elevated temperature, the polymer aggregation enables the recovery as a precipitate by simple centrifugation.

In the present work, we synthesized a copolymer, poly(NIPAM-*co*-CS) consisting of polyNIPAM and the coumarin−spiropyran (CS) units (Scheme 2), based on the above scenario. The copolymer behaves as a fluorescent CN^- sensor that is recoverable and regenerable by simple procedures. At room temperature, the polymer is soluble in water and enables selective and sensitive fluorescent CN^- sensing, as does the monomer receptor **1**. In contrast, at elevated temperature (>40 °C), the polymer can be recovered by simple centrifugation because of the polymer aggregation. In addition, the polymer can be regenerated by simple acid treatment and the obtained polymer is reusable for further CN^- sensing without loss of sensitivity.

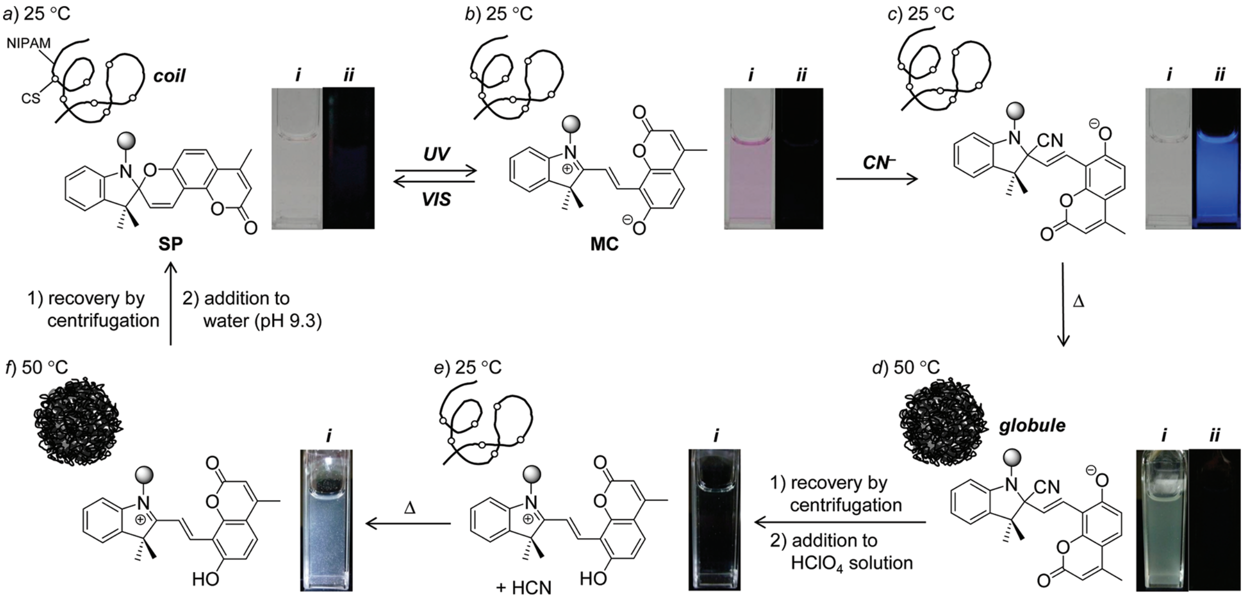
2. RESULTS AND DISCUSSION

2.1. Synthesis and Aggregation Properties of Polymer. The synthesis route of the copolymer, poly(NIPAM-*co*-CS), is summarized in Scheme 2. Condensation of an indole derivative, **2**,[12] and 7-hydroxy-4-methylcoumarin-2-carbardehyde, **3**,[13] affords the OH-modified CS derivative, **4**, with 50% yield. Reaction of **4** with acryloyl chloride gives rise to the acryl-modified CS derivative, **5**, with 72% yield. Copolymerization of a NIPAM monomer and **5** successfully produces poly(NIPAM-*co*-CS) as a pale pink solid. The obtained respective monomer materials were characterized by NMR and MS analysis (see Figures S1−S6 in the Supporting Information). The CS units within the polymer show broad NMR signals, as usually observed for related copolymers.[14] The ratio (x/y) of respective units of poly($NIPAM_x$-*co*-CS_y) was therefore determined by comparison of the absorbance of **1** at 341 nm in THF (25 °C) to be 146/1, according to literature procedure.[15] The molecular weight of the polymer was determined by GPC analysis to be $M_n = 1.98 \times 10^5$ ($M_w/M_n = 3.3$).

The heat-induced aggregation property of the polymer was studied in water. Figure 1 shows the temperature-dependent change in absorption spectra of poly(NIPAM-*co*-CS) in a buffered water (0.17 g L^{-1}; CHES 100 mM, pH 9.3) measured in the dark condition. The spectra scarcely change at <30 °C, but the absorbance at the entire wavelength region increases at >30 °C with a rise in temperature. This indicates that, at <30 °C, the polymer exists as a coil state and is fully dissolved in water, as shown in Scheme 3a. In contrast, at >30 °C, the polymer aggregation produces an insoluble globule state. As shown in the inset of Figure 1 (black circle), a dynamic laser scattering (DLS) analysis of the polymer solution scarcely detects polymer particles at <30 °C (detection limit: 3 nm). However, at >30 °C, the polymer particles form and the size increases with a rise in temperature. These data suggest that the polymer is fully dissolved in water at <30 °C, and the homogeneous solution can be used for fluorescent CN^- sensing at room temperature. It must be noted that the polymer containing low amount of CS unit is necessary for aggregation. As shown in Figure S8 in the Supporting Information, the polymers containing larger amount of CS unit (the NIPAM/CS ratio: 73/1 and 29/1) do not aggregate even at elevated temperature. This is probably because, as usually observed for related polyNIPAM materials,[16] the intra- and interpolymer interactions are weakened by the incorporation of larger amount of other units.

2.2. Photoisomerization Properties of Polymer. Photoisomerization property of poly(NIPAM-*co*-CS) was studied in

Scheme 3. Structure Change of Poly(NIPAM-*co*-CS) during the Fluorometric CN^- Sensing, Recovery, and Regeneration Sequence[a]



[a] i and ii show the visible and fluorescent photographs, respectively.

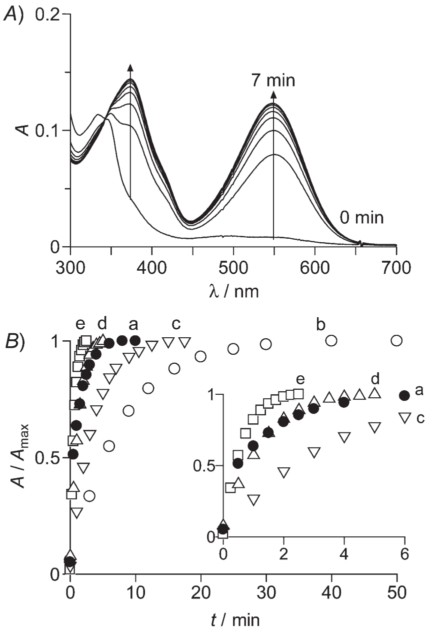


Figure 2. (A) Time-dependent change in absorption spectra of poly(NIPAM-*co*-CS) (0.17 g L^{-1}; containing 10 μM CS unit) measured in water (pH 9.3) under UV irradiation (334 nm) at 25 °C. (B) Change in absorbance of (a) the polymer solution (λ_{abs} = 550 nm), (b) **1** in a MeCN/water mixture (2/8 v/v; pH 9.3, λ_{abs} = 528 nm), (c) **1** in a MeCN/water mixture (5/5 v/v; pH 9.3, λ_{abs} = 540 nm), (d) **1** in MeOH (λ_{abs} = 548 nm), and (e) **1** in MeCN (λ_{abs} = 581 nm). The spectra for samples b−e are summarized in Figure S9 in the Supporting Information.

water. Figure 2A shows the absorption spectra of polymer solution (0.17 g L^{-1}; pH 9.3) measured at 25 °C, where the solution contains 10 μM of the CS units. Without UV irradiation, the polymer shows almost no absorption in the visible region, indicating that the CS unit exists as a SP form, as shown in Scheme 3a. UV irradiation of the solution (334 nm), however, creates a distinctive absorption band centered at 550 nm, assigned to the generation of MC form. As reported,[17] the absorption band of the MC form of spiropyran derivatives red shifts with a decrease in polarity of the media. However, the receptor **1**, when dissolved in a MeCN/water (2/8 v/v) mixture with a lower polarity than water, shows a MC absorption band at a shorter wavelength (528 nm). The long-wavelength MC absorption of the polymer even in water is because polyNIPAM has a less polar nature due to the polymer chain.[18]

The less polar environment of the polymer strongly affects the rate of SP→MC photoisomerization. As shown in Figure 2Bb, in the case of receptor **1** measured in a MeCN/water (2/8 v/v) mixture, the photostationary state is attained by UV irradiation for 30 min, whereas only 7 min is required for the polymer a in water. This is because, as reported,[19] the SP→MC photoisomerization is accelerated in less polar media. As shown in Figure 2Bc−e, the receptor **1**, when dissolved in a MeCN/water (5/5 v/v) mixture, MeOH, and MeCN, attains the photostationary state by 15, 5, and 2 min UV irradiations, respectively, suggesting that less polar media indeed accelerates the SP→MC photoisomerization. The accelerated isomerization in polymer solution is therefore attributable to the less polar environment. The rapid isomerization of the CS unit within the polymer shortens the time required for the generation of MC form and, hence, is advantageous for CN^- sensing. It must also be noted that the MC form of CS units within the polymer reverts to the corresponding SP form upon irradiation of visible light, as does **1**.[10] As shown in Figure S10 in the Supporting Information, an irradiation of 550 nm monochromatic light to the polymer solution containing the MC form promotes complete reversion to the SP form. This indicates that, as shown in parts a and b in Scheme 3, the SP and MC forms are photoisomerized reversibly even in the polymer matrices.

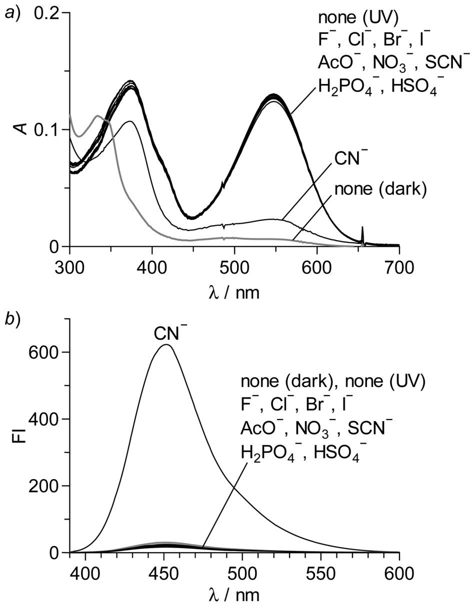


Figure 3. (a) Absorption and (b) fluorescence (λ_{ex} = 376 nm) spectra of poly(NIPAM-*co*-CS) (0.17 g L^{-1}; containing 10 μM CS unit) in water (pH 9.3) measured with 50 equiv. of each respective anion (as n-Bu_4N^+ salts) under UV irradiation (334 nm) at 25 °C. The measurements were carried out as follows: UV light was irradiated to the polymer solution for 10 min. Each anion was added to the solution and irradiated by UV light for 40 min. The gray spectra were obtained without anion in the dark.

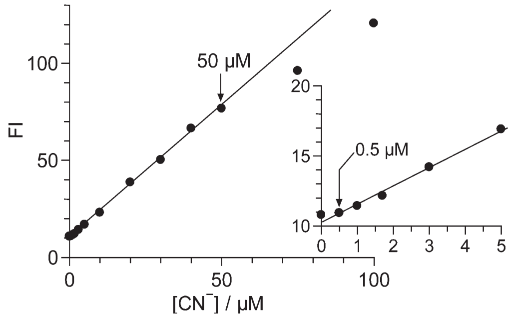


Figure 4. Change in the fluorescence intensity of poly(NIPAM-*co*-CS) (0.087 g L^{-1}; containing 5.1 μM CS unit) with CN^- concentration. The titration was performed in water (pH 9.3) under UV irradiation (334 nm) at 25 °C. The spectra at each CN^- concentration were obtained after UV irradiation of the solution for 40 min. The detailed change in fluorescence spectra is shown in Figure S12 (Supporting Information).

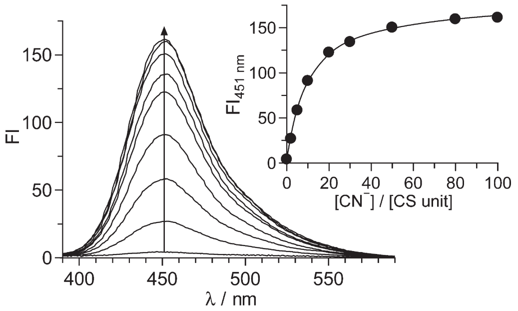


Figure 5. Fluorescence titration (λ_{ex} = 306 nm) of poly(NIPAM-*co*-CS) (0.17 g L^{-1}; containing 10 μM CS unit) with CN^- in water (pH 9.3) under UV irradiation. Each spectrum was obtained after UV irradiation of the solution for 40 min. (Inset) Change in fluorescence intensity at 451 nm. The line is the nonlinear fitting curve obtained assuming 1:1 association between CN^- and the CS unit.

2.3. Fluorometric Sensing. The polymer was applied for CN^- sensing. The polymer was dissolved in water and UV light was irradiated at 25 °C to achieve the photostationary state (10 min). Each respective anion (50 equiv of the CS units) was added to the solution and stirred for 40 min under continued UV irradiation. As shown in Figure 3a, the absorption spectra obtained with anions, except for CN^-, are similar to the MC absorption. In contrast, addition of CN^- leads to a decrease in the 550 nm absorption, leaving the absorption band at 375 nm. These spectral behaviors are similar to those observed for the receptor **1**.[10] This indicates that the MC form of CS unit within the polymer selectively associates with CN^-, as does **1**.

Figure 3b shows the fluorescence spectra (λ_{ex} = 376 nm) of the polymer solution. Without anions, the solution shows almost no fluorescence regardless of the dark or UV irradiation condition. Addition of CN^-, however, creates a strong fluorescence at 400−600 nm and a bright blue fluorescence is observed. In contrast, addition of other anions shows almost no fluorescence. These spectral behaviors are also similar to those observed for **1**.[10] This suggests that the polymer shows selective fluorescence response to CN^-, as does **1**. It is noted that the CN^--induced fluorescence enhancement is unaffected by other anions (see Figure S11 in the Supporting Information), indicating that the polymer enables selective fluorometric detection of CN^- even in the presence of other anions.

The fluorescence quantum yield of the polymer solution after UV irradiation with 50 equiv of CN^- is determined to be Φ_F = 0.55, which is much higher than that of **1**−CN^- species (Φ_F = 0.19) measured in a MeCN/water (2/8 v/v) mixture.[10] The fluorescence quantum yields of **1**−CN^- species measured in a MeCN/water (5/5 v/v) mixture, MeOH, and MeCN are 0.44, 0.47, and 0.49, respectively. This indicates that, as observed for related coumarin derivatives,[20] the fluorescence quantum yield increases with a decrease in polarity of the media. The high fluorescence quantum yield of the polymer solution is due to the less polar environment of polymer. This is consistent with the result for SP→MC photoisomerization (Figure 2).

Quantitative CN^- determination in total water can be carried out with the polymer. Figure 4 shows the change in fluorescent intensity of the polymer solution measured with different amount of CN^-. A linear relationship in the range of 0.5−50 μM CN^- indicates that the polymer enables accurate CN^- determination in this concentration range, which is similar to that for the receptor **1** in a MeCN/water mixture (0.5−35 μM).[10] The results indicate that the polymer enables selective and sensitive CN^- determination in total water.

2.4. Sensing Properties of Polymer. Figure 5 shows the results of fluorescence titration of poly(NIPAM-*co*-CS) with CN^- under UV irradiation at 25 °C. The stepwise addition of CN^- leads to an increase in the 400−600 nm fluorescence. Nonlinear fitting of the data (inset) obtained assuming a 1:1 interaction revealed that the CS unit within the polymer associates with CN^- in a 1:1 stoichiometry, as does **1**.[10] This indicates that the fluorescence enhancement of the polymer upon CN^- addition occurs via the mechanism similar to that of **1**. As shown in Scheme 3c, the 1:1 nucleophilic interaction between CN^- and the positively charged spirocarbon of the MC form of CS unit

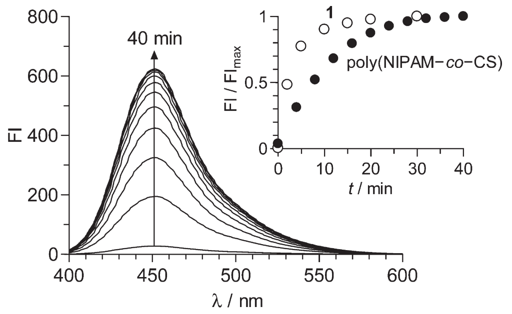


Figure 6. Time-dependent change in fluorescence spectra (λ_{ex} = 376 nm) of poly(NIPAM-*co*-CS) (0.17 g L^{-1}; containing 10 μM CS unit) measured with 50 equiv of CN$^-$ in water (pH 9.3) under UV irradiation (334 nm) at 25 °C. The measurement was carried out as follows: UV light was irradiated to the polymer solution for 10 min. CN$^-$ was added to the solution, and the measurement was started under continued UV irradiation. (Inset) Change in the fluorescence intensity for (black) the polymer solution monitored at 451 nm and (white) **1** (10 μM) in a MeCN/water mixture (2/8 v/v, pH 9.3) monitored at 453 nm.

Table 1. First-Order Rate Constant (k_{CN}) and Activation Energy (E_a) for Interaction between MC Form and CN$^-$ (50 equiv) at Different Temperatures under UV Irradiation[a]

	poly(NIPAM-*co*-CS)		**1**	
T (°C)	k_{CN} (s^{-1})	E_a (kJ mol^{-1})	k_{CN} (s^{-1})	E_a (kJ mol^{-1})
15	1.52×10^{-3}	34.1 ± 1.8	2.24×10^{-3}	28.1 ± 2.9
20	1.95×10^{-3}		2.71×10^{-3}	
25	2.44×10^{-3}		3.30×10^{-3}	

[a] The measurements were carried out on the basis of the absorption analysis. The detailed data are summarized in the Supporting Information (Figures S13 and S14).

within the polymer promotes a localization of π-electrons on the coumarin moiety. This results in an appearance of coumarin fluorescence.

The association constant for 1:1 interaction of the polymer is determined by the nonlinear fitting to be 1.25×10^4 M^{-1}. As reported,[21] the nucleophilic interaction is accelerated in less polar media. The SP→MC photoisomerization results (Figure 2) suggest that the polymer provides a less polar environment as compared to a MeCN/water (2/8 v/v) mixture. However, the association constant between CN$^-$ and **1** (1.60×10^4 M^{-1}) obtained in the MeCN/water mixture[10] is much higher than that for the polymer system. It is well-known that, as reported,[22] the amide groups of polyNIPAM are polarized in water and strongly interact with anions electrostatically. This amide−CN$^-$ interaction probably occurs competitively with the nucleophilic interaction between the CS units and CN$^-$, resulting in lower association constant.

Figure 6 shows the time-dependent change in fluorescence spectra of the polymer solution after an addition of 50 equiv of CN$^-$ under UV irradiation. The fluorescence intensity increases with time and is saturated after 35 min irradiation. In the case of **1**, the intensity is saturated after 20 min irradiation, indicating that the polymer system requires longer time for interaction with CN$^-$. Kinetic measurements were carried out to further clarify the properties of polymer. Table 1 summarizes the results of kinetic absorption analysis for interaction between the MC form and the excess amount of CN$^-$ (50 equiv) at different temperature under UV irradiation. The obtained first-order rate constant (k_{CN}) for interaction of the polymer solution is lower than that for **1** at all tested temperatures: the rate constant for polymer at 25 °C is 2.44×10^{-3} s^{-1}, whereas that for **1** is 3.30×10^{-3} s^{-1}. This again suggests that the interaction between CN$^-$ and the MC form within the polymer is much weaker. The activation energy (E_a) for interaction of the polymer, determined by the Arrhenius plot, is 34.1 kJ mol^{-1}, whereas that for **1** is 28.1 kJ mol^{-1}. The higher activation energy of the polymer system suggests that the nucleophilic interaction between CN$^-$ and the MC form is indeed suppressed, probably due to the electrostatic interaction between the amide groups of polyNIPAM and CN$^-$. These findings indicate that the CS units within the polymer are photoisomerized to the MC forms very rapidly, but the reaction with CN$^-$ occurs slowly.

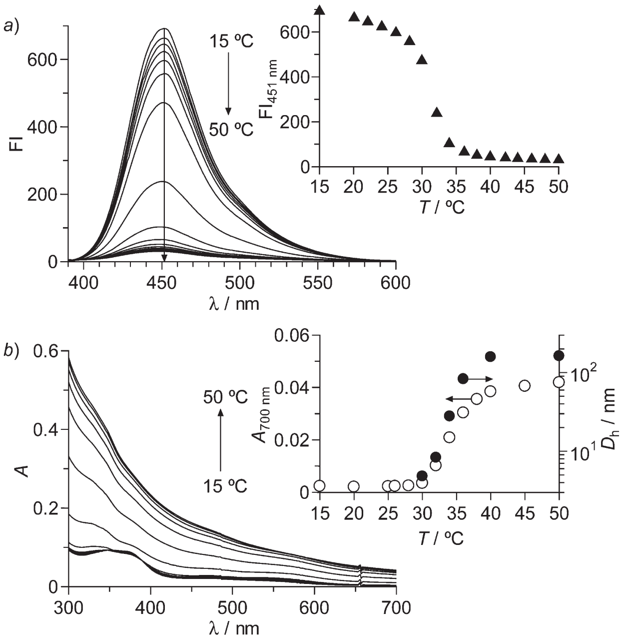


Figure 7. Temperature-dependent change in (a) fluorescence (λ_{ex} = 376 nm) and (b) absorption spectra of poly(NIPAM-*co*-CS) (0.17 g L^{-1}; containing 10 μM CS unit) measured with 50 equiv of CN$^-$ in water (pH 9.3). (Inset) Change in (triangle) fluorescence intensity at 451 nm, (white circle) absorbance at 700 nm and (black circle) D_h of polymer particles. The detailed D_h data are summarized in Figure S7 in the Supporting Information. The measurements were performed after UV irradiation (40 min) of the solution with CN$^-$ at 25 °C.

It is noted that the fluorescent CN$^-$ sensing by the present polymer must be carried out at <30 °C. This is because, at higher temperature (>30 °C), the formation of polymer particles suppresses the incident light absorption of the CS unit within the particles and decreases the fluorescence intensity. This is confirmed by the following experiments. The polymer solution was irradiated by UV light for 40 min with 50 equiv of CN$^-$ at 25 °C, and the fluorescence spectra were measured at different temperatures. As shown in Figure 7a, at <30 °C, the fluorescence intensity is very high. However, at >30 °C, the intensity decreases significantly and almost no fluorescence is observed at >40 °C. Figure 7b shows the absorption spectra of polymer solution measured at different temperatures. As also the case without CN$^-$ (Figure 1), the absorbance increases at >30 °C, associated

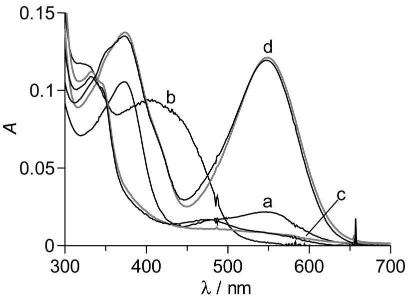


Figure 8. Absorption spectra of poly(NIPAM-*co*-CS). (a) The polymer solution (0.17 g L^{-1}; containing 10 μM CS unit) measured in water (pH 9.3) after UV irradiation with 50 equiv of CN^- for 40 min at 25 °C. (b) The polymer measured in aqueous $HClO_4$ solution (0.1 M) after recovery from sample a by centrifugation. (c) The polymer measured in water (pH 9.3) after recovery from sample b by centrifugation. (d) The polymer measured after UV irradiation of the sample c for 10 min. The gray lines are the spectra obtained with virgin polymer.

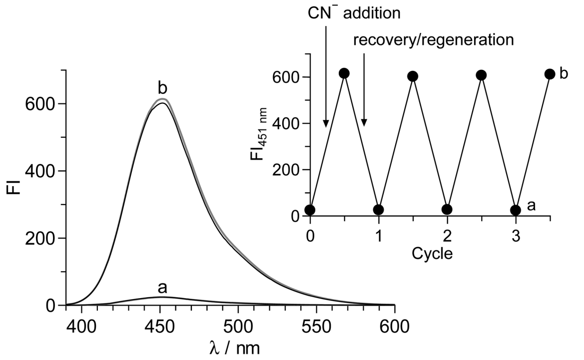


Figure 9. Fluorescence spectra (λ_{ex} = 376 nm) of (gray) virgin and (black) recovered poly(NIPAM-*co*-CS) (0.17 g L^{-1}), measured (a) without and (b) with 50 equiv of CN^- in water (pH 9.3). Spectrum a was obtained after UV irradiation for 10 min at 25 °C, and spectrum b was obtained after UV irradiation for 40 min with CN^-. (Inset) Change in fluorescence intensity during the repeated CN^- sensing and regeneration cycles.

with the formation of polymer particle. This indicates that the polymer aggregates even though the CS units are associated with CN^-. The absorbance profile is consistent with the fluorescence intensity profile (Figure 7a). These data indicate that, at >30 °C, the formation of polymer particles suppresses the incident light absorption of the CS unit within the particles and, hence, quenches the fluorescence. This suggests that the fluorescent CN^- sensing must be carried out at <30 °C.

2.5. Recovery, Regeneration, And Reuse of Polymer. As shown in Figure 7b, the polymer associated with CN^- aggregates at higher temperature (>30 °C). This enables the recovery of polymer from the solution by simple centrifugation. The polymer solution, obtained after UV irradiation for 40 min with 50 equiv of CN^- at 25 °C, was stirred at 50 °C for 5 min in the dark. As shown in Scheme 3d, the solution becomes turbid due to the formation of polymer particles. Centrifugation of the solution for 10 min at 2.5×10^4 rpm successfully produces the precipitate. The removal of supernatant solution followed by drying of the resultant leads to almost complete recovery of the polymer (>96%).

The CN^- contained in the recovered polymer can be removed by simple acid treatment. The recovered polymer was dissolved in aqueous $HClO_4$ solution (0.1 M) and stirred at 25 °C for 1 h in the dark condition. Figure 8b shows the absorption spectra of the solution. The distinctive absorption band at 376 nm for the CN^--associated CS unit (Figure 8a) disappears and new absorption band appears at 400–550 nm, which is assigned to the CN^--free protonated CS unit,[23] as shown in Scheme 3e. The protonation of CN^- ($H^+ + CN^- \leftrightarrow HCN$; $K_a = 6.3 \times 10^{10}$ M^{-1})[24] is much stronger than the interaction between CN^- and the MC form (1.25×10^4 M^{-1}). The H^+ addition to the solution therefore removes CN^- from the CS unit. Subsequent protonation of the phenolate moiety of CS unit thus produces the CN^--free protonated CS unit (Scheme 3e). As shown in Figure S15 in the Supporting Information, the **1**–CN^- species, when treated with $HClO_4$, also shows similar absorption spectrum to that of the polymer system (Figure 8b). These data indicate that simple acid treatment of the polymer successfully removes CN^- from the CS unit. The obtained polymer that contains protonated CS unit also aggregates at >30 °C (see Figure S16 in the Supporting Information). As shown in Scheme 3f, the polymer solution, when heated to 50 °C for 10 min, produces the polymer particles. Centrifugation of the solution for 10 min at 2.5×10^4 rpm leads to almost complete recovery of polymer (>98%).

The recovered polymer was then dissolved in water (pH 9.3). As shown in Figure 8c (black), the absorption spectrum of the solution is similar to that of the virgin polymer (gray). UV irradiation of the polymer solution generates the MC absorption (Figure 8d, black), which is also similar to that of the virgin polymer (gray). Spectra a and b in Figure 9 (black) show the fluorescence spectra of the recovered polymer measured without and with CN^-, respectively. Both spectra are almost the same as those obtained with the virgin polymer (gray). As shown in inset, two more regeneration and reuse cycles show similar spectral changes. These results clearly indicate that the polymer regenerated by acid treatment can be reused for further CN^- analysis without loss of sensitivity.

3. CONCLUSION

The copolymer, poly(NIPAM-*co*-CS), consisting of polyNIPAM unit as a thermoresponsive part and a CS unit as a sensing part was synthesized. The polymer is fully dissolved in water at <30 °C and enables selective fluorescent sensing of CN^-. Accurate CN^- determination in total water in the range of 0.5 μM–50 μM is accomplished. The polymer used for CN^- sensing can be simply recovered by heating of the solution at >40 °C followed by centrifugation, due to the polymer aggregation. The CN^- removal from the recovered polymer can be carried out by simple acid treatment. The resulting polymer is successfully reused for CN^- sensing without loss of sensitivity. The polymer would be a powerful tool for reusable fluorescent CN^- sensor. The strategy for the sensor design presented here based on polyNIPAM might contribute to the development of reusable optical sensors.

4. EXPERIMENTAL SECTION

4.1. Materials. All of the reagents were supplied from Wako, Sigma-Aldrich, and Tokyo Kasei and used without further purification. NIPAM and AIBN were recrystallized from *n*-hexane and MeOH, respectively, prior to use. Water was purified by the Milli-Q system. Other reagents were used without further purification. The compounds **2**[12] and **3**[13] were synthesized according to literature procedure.

*Synthesis of **4***. **2** (45 mg, 0.22 mmol) and **3** (46 mg, 0.23 mmol) were refluxed in MeOH (3 mL) for 3 h and cooled to room temperature. Water (100 mL) was added to the mixture. The formed insoluble solid was recovered by filtration and dried in vacuo, affording **4** as a purple solid (43 mg, 0.11 mmol, 50%). ^{1}H NMR (270 MHz, acetone-d_6, TMS): δ (ppm) = 7.54 (d, J = 8.74 Hz, 1H), 7.38 (d, J = 10.56 Hz, 1H), 7.16−7.09 (m, 2H), 6.81 (t, J = 7.34 Hz, 1H), 6.70−6.65 (m, 2H), 6.14 (d, J = 1.16 Hz, 1H), 6.03 (d, J = 10.55 Hz, 1H), 3.75−3.62 (m, 2H), 3.48−3.22 (m, 2H), 2.43 (d, J = 1.32 Hz, 3H), 1.29 (s, 3H), 1.19 (s, 3H). ^{13}C NMR (68 MHz, CDCl$_3$, TMS): δ (ppm) = 160.42, 156.68, 152.52, 149.66, 146.95, 135.87, 127.53, 125.13, 122.42, 121.61, 119.86, 119.49, 113.14, 111.74, 111.45, 106.99, 106.62, 105.75, 60.96, 52.66, 46.01, 25.89, 20.13, 18.61. EI-MS: calcd for $C_{24}H_{23}NO_4$: 389.16. Found: m/z 389.2 [M$^+$]. ^{1}H, ^{13}C NMR and EI-MS charts are shown in Figures S1−S3 in the Supporting Information.

*Synthesis of **5***. **4** (40 mg, 0.10 mmol) was dissolved in CH_2Cl_2 (30 mL). Acryloyl chloride (10 mg, 0.11 mol) dissolved in CH_2Cl_2 (100 mL) was added dropwise to the solution at 273 K under dry N_2 and stirred at 313 K for 2 h. The resulting solution was washed with 0.1 M HCl and saturated $NaHCO_3$ solutions, dried over Na_2SO_4, and concentrated by evaporation, affording **5** as a yellow solid (32 mg, 72%). ^{1}H NMR (400 MHz, acetone-d_6, TMS): δ = 7.55 (d, J = 8.70 Hz, 1H), 7.42 (d, J = 10.53 Hz, 1H), 7.19−7.13 (m, 2H), 6.85 (t, J = 7.33 Hz, 1H), 6.78 (d, J = 7.78 Hz, 1H), 6.68 (d, J = 8.70 Hz, 1H), 6.33 (dd, J = 17.40, 1.38 Hz, 1H), 6.16−6.09 (m, 2H), 6.03 (d, J = 10.53 Hz, 1H), 5.87 (dd, J = 10.30, 1.60 Hz, 1H), 4.40−4.28 (m, 2H), 3.67−3.45 (m, 2H), 2.43 (d, J = 1.38 Hz, 3H), 1.30 (s, 3H), 1.17 (s, 3H). ^{13}C NMR (68 MHz, CDCl$_3$, TMS): δ (ppm) = 165.61, 160.45, 156.82, 152.53, 146.60, 135.83, 130.70, 128.05, 127.54, 125.11, 122.48, 121.60, 119.66, 119.53, 119.48, 113.01, 111.78, 111.44, 106.86, 106.47, 105.59, 62.49, 52.55, 42.41, 25.84, 19.89, 18.65. EI-MS: calcd for $C_{27}H_{25}NO_5$: 443.17. Found: m/z: 443.2 [M$^+$]. ^{1}H, ^{13}C NMR and EI-MS charts are shown in Figures S4−S6 in the Supporting Information.

Synthesis of Poly(NIPAM-co-CS). **5** (18.1 mg, 0.041 mmol), NIPAM (0.504 g, 4.1 mmol), and AIBN (15 mg, 91 mmol) were dissolved in *t*-BuOH (2 mL). The solution was degassed by two freeze−pump−thaw cycles and stirred at 343 K for 18 h under dry N_2. The polymer formed was concentrated by evaporation and purified by precipitation with methanol (1 mL) and diethyl ether (100 mL). After drying in vacuo, poly(NIPAM-*co*-CS) was obtained as a pale pink solid (0.378 g, 75%). ^{1}H NMR (400 MHz, CDCl$_3$, TMS): δ (ppm) 3.99 (1H, s), 2.46−1.34 (3H, m), 1.13 (6H, s).

4.2. Analysis. Absorption spectra were measured using an UV−visible spectrophotometer (Shimadzu; Multispec-1500) with a temperature controller (S-1700). Fluorescence spectra were measured on a JASCO FP-6500 spectrophotometer with a temperature controller. Light irradiations were carried out with a Xenon lamp (300 W; Asahi Spectra Co. Ltd.; MAX-302) equipped with a 334 nm (light intensity, 69.1 mW m^{-2}) and 550 nm (32.5 mW m^{-2}) band-pass filters. ^{1}H and ^{13}C NMR spectra were obtained by a JEOL JNM-GSX270 Excalibur and JNM-AL400 spectrometer. MS analysis was performed by a JEOL JMS 700 Mass Spectrometer. The size of polymer particles was measured by a dynamic laser scattering spectrometer (LB-500, HORIBA) (detection range, 3 nm−6 μm). Molecular weight of the polymers was determined using a JASCO GPC system equipped with a PU-980 pump and a refractive index detector RI-930, with KF-806 L column (Shodex). The oven temperature was 40 °C, and DMF containing LiBr (0.01 M) was used as the solvent (flow rate, 0.6 mL min^{-1}).

■ ASSOCIATED CONTENT

S Supporting Information. NMR and MS charts; absorption and fluorescence spectra; distribution of hydrodynamic diameter of polymer; kinetic absorption data and Arrhenius plot (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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