A fluorescence resonance energy transfer probe for sensing pH in aqueous solution

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A B S T R A C T

A fluorescence resonance energy transfer (FRET) sensor, which has a FRET donor and an acceptor attached to each chain end of pH-sensitive polysulfonamide, is synthesized and its pH sensitivity is examined in terms of the FRET efficiency. This polymeric sensor exhibits an instantaneous conformation change from coil to globule at a specific pH, which results in the drastic on-and-off FRET efficiency. To detect a specific pH region, sulfadimethoxine and sulfamethizole are selected among various sulfonamides since their pK_a values are in the physiological pH. For tuning the emission color arising from FRET, 7-hydroxy-4-bromomethyl coumarin and coumarin 343 are used as a FRET donor and an acceptor, respectively, for a blue-to-green FRET sensor, and fluorescence amine isomer I and rhodamine B are used for a green-to-red FRET sensor. Each sensor shows a distinct color change from the emission wavelength of FRET donor to the emission wavelength of FRET acceptor, which well explains their feasibility as a useful optical sensor.

1. Introduction

The growing need for stimuli-sensitive materials explains their wide variety of potential feasibility in biotechnology such as biomedical and biosensing applications [1–10]. Particularly, the development of an alarm-type probe for detecting a specific threshold in such applications may provide a benefit to the pursuit of probing and investigating many important phenomena in biological and environmental applications [11–16]. One of the conceivable means for this purpose is to adopt a concept combining the coil-globule transition of macromolecules at a specific stimulus and the fluorescence resonance energy transfer (FRET). The crucial requirements for the choice of those two concepts are as follows: (1) the conformational change of the polymeric linker from the expanded coil state to the collapsed globule state induced by the external stimuli should be fast enough to exhibit a typical two-state transition, which is appropriate for being an alarm for the specific matter being concerned; (2) the emission wavelength of the FRET acceptor should be largely different from that of the FRET donor, which makes it possible to distinguish optically between two different states [17].

Traditionally, FRET has been used to investigate the statics and dynamics for the conformational changes of macromolecules by attaching fluorophores at specific sites [17–22]. However, most studies have mainly been focused on the conformational change due to external stimuli such as the change of solvent property or temperature. Moreover, the purpose of those studies was to investigate the conformational change of molecule to detect an environmental change being concerned. Among various stimuli caused by environmental or physiological changes, detecting a specific pH in the physiological region has essentially been needed for studying many important programmed functions in biopharmaceutical systems [23–28]. Although there have been several reports about polymers which exhibit the pH-induced FRET change [20,29–34], the primary aims of those studies were to study the pH-induced conformational change of chain molecules.

In our previous papers [13,15], the design and synthesis of a polymeric pH sensor that showed a robust on-and-off characteristic in the FRET efficiency was reported, where the polymeric linker of the FRET sensor comprised a pH-sensitive sulfonamide moiety, and the FRET donor and acceptor were attached to each chain end of the linker. When the pH value of a solution is lower than the pK_a value of polymeric linker in the FRET sensor, the conformation of polysulfonamide linker changes from the expanded coil state to the collapsed globule state, which results in an abrupt on-and-off feature in the FRET efficiency due to a change in the distance between the FRET donor and the acceptor. However, the previous FRET sensor prepared in our group detects only a specific pH region with the color change from blue-to-green induced by FRET.

Since the pK_a value of sulfonamide family depends upon the alkyl group of sulfonamide [35,36], the selection of a specific sulfonamide may enable us to prepare the pH sensor for detecting

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a specific pH as one wishes. It is also possible that the color emitted from FRET can be determined by the choice of a pair of FRET donor and acceptor. For these feasibilities to be realized, two different sulfonamides and two different pairs of FRET donor and acceptor are used in this study to prepare various polymeric pH sensors which have tunable responsive properties. Here, we report the synthesis of those FRET sensors and their pH sensitivity in terms of the FRET efficiency.

2. Experimental

2.1. Materials

All reagents were purchased from Sigma–Aldrich unless noted. Sulfadimethoxine (SD) (Tokyo Chemical Industry), sulfamethizole (SM) (Tokyo Chemical Industry), NaOH (Duksan Pharmaceutical), acetone (Burdick & Jackson), methacryloyl chloride (97%), 7-acetoxy-4-bromomethyl coumarin (Tokyo Chemical Industry), THF (Daejung Chemicals & Metals), conc. HCl (Daejung Chemicals & Metals), methylene chloride (Daejung Chemicals & Metals), magnesium sulfate anhydrous (Daejung Chemicals & Metals), copper(I) bromide (CuBr) (99.999%), N,N-dimethylformamide anhydrous (DMF) (99.8%), methanol (Daejung Chemicals & Metals), dimethylsulfoxide (DMSO) (Tokyo Chemical Industry), 2,2′-(ethylenedioxy)bis(ethylamine) (EDBEA) (98%), triethylamine (99.5%), coumarin 343 (C343), anhydrous dimethylsulfoxide (anhydrous DMSO) (99.9%), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (DEC) (98+%), N-hydroxysuccinimide (NHS) (97%), 4-bromomethyl benzoic acid (BMBA) (97%), fluoresceinamine isomer I (FAII), rhodamine B (RDB) (90%), and pH 10.0 buffer solution (Daejung Chemicals & Metals) were used as received.

2.2. Synthesis of monomers

Sulfadimethoxine methacrylamide (SDM) was synthesized by reacting sulfadimethoxine with methacryloyl chloride at 0 °C for 24 h [37]. Sulfamethizole methacrylamide (SMM) was synthesized by following the same procedure as above except the use of sulfamethizole instead of sulfadimethoxine. ^{1}H NMR spectra (500 MHz, DMSO-d_6) of SDM and SMM are shown in Figs. 1 and 2, respectively.

2.3. Synthesis of hbC-poly(sulfadimethoxine methacrylamide)-coumarin 343 (hbC-PSDM-C343)

For all reactions, a three-neck round bottom was degassed and backfilled with N_2 gas. 7-Hydroxy-4-bromomethyl coumarin (hbC)
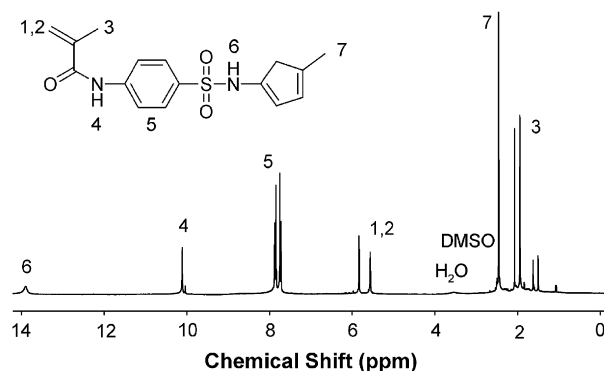

Fig. 2. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of SMM.

was prepared according to the previous report [15] and used as an initiator for atom transfer radical polymerization (ATRP). DMF was degassed for removal of oxygen by three freeze-and-thaw cycles, and distilled water was degassed by boiling for 48 h and bubbling with N_2 gas for 12 h to completely remove dissolved oxygen. SDM (2 g, 5.29×10^{-3} mol) and DMF (5 mL) were put in a three-neck round bottom flask, and 10 mL of an aqueous NaOH (0.211 g, 5.29×10^{-3} mol) solution was sequentially added. After addition of Me$_6$TREN (0.037 mL, 1.33×10^{-4} mol) and copper(I) bromide (0.019 g, 1.32×10^{-4} mol), a solution of hbC (0.034 g, 1.33×10^{-4} mol) in DMF (5 mL) was added. The solution was stirred at 35 °C for 2 h to allow ATRP. For purification of the product, a solution of the crude product was precipitated in 1 N HCl solution. After filtration, the filtered product was washed with methanol at room temperature for 24 h to remove unreacted SDM. The final product (hbC-PSDM) was collected by filtration and then dried in vacuum at 30 °C. Yield: 62.5%; $M_{n,NMR}$: 12 700; ^{1}H NMR spectrum of hbC-PSDM in DMSO-d_6 is shown in Fig. 3. hbC-PSDM (0.470 g) was then placed in a flask and dissolved in DMSO (4.7 mL), followed by adding triethylamine (0.005 mL, 3.66×10^{-5} mol) and EDBEA (0.054 mL, 3.69×10^{-4} mol). After distilled water (0.47 mL) was added, the solution was stirred at 35 °C for 48 h. The product was purified by dialyzing against methanol for 48 h using a cellulose dialysis membrane (molecular weight cut-off: 6000–8000,
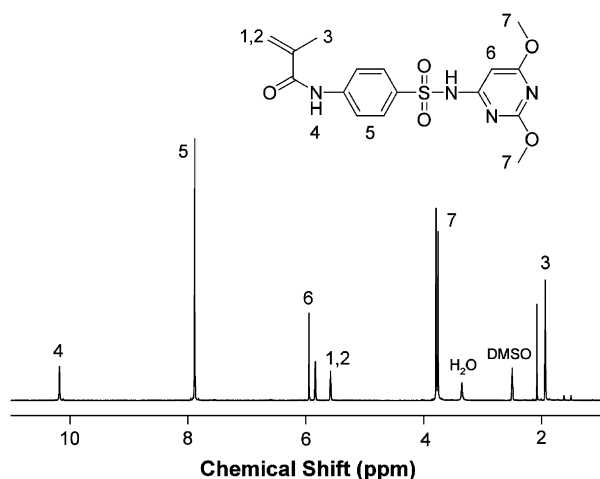

Fig. 1. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of SDM.
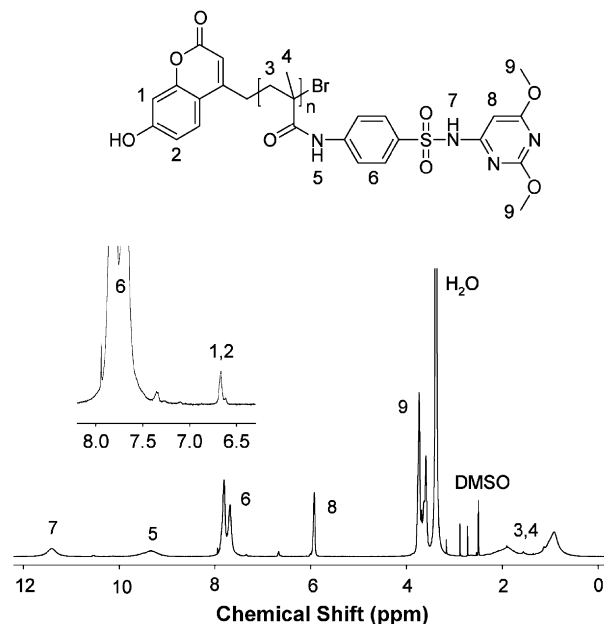

Fig. 3. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of hbC-PSDM.

Membrane Filtration Products, Inc.). The solution was filtered, and the filtered product (hbC-PSDM-NH$_2$) was dried in vacuum at 30 °C. Yield: 89.4%; Nynhidrin test was performed to qualitatively verify the introduction of amine group as shown in Fig. 4.

For preparation of hbC-PSDM-C343, C343 (0.008 g, 2.80 × 10^{-5} mol), DEC (0.016 g, 8.35 × 10^{-5} mol), NHS (0.016 g, 1.39 × 10^{-4} mol), and anhydrous DMSO (3.5 mL) were first put together in a flask, and the mixture was stirred at 35 °C for 12 h for NHS activation. Then a mixture of hbC-PSDM-NH$_2$ (0.35 g) and anhydrous DMSO (3.5 mL) was slowly added to the flask, and the reaction mixture was stirred at 35 °C for 48 h. After filtration, the crude product was purified by dialyzing against DMF for 24 h using dialysis membrane. Further dialysis against methanol was performed for 48 h, and the final solution was filtered and the filtered product was dried in vacuum at 30 °C. Yield: 71.4%; PDI$_{GPC}$: 1.12.

2.4. Synthesis of hbC-poly(sulfamethizole methacrylamide)-coumarin 343 (hbC-PSMM-C343)

hbC-PSMM-C343 was synthesized by following the same procedure as above except using SMM instead of SDM. Briefly, SMM (2 g, 5.92 × 10^{-3} mol), Me$_6$TREN (0.054 mL, 1.95 × 10^{-4} mol), copper(I) bromide (0.019 g, 1.95 × 10^{-4} mol), and hbC (0.050 g, 1.96 × 10^{-4} mol) were used for preparation of hbC-PSMM. Yield: 36.8%; $M_{n,NMR}$: 9000; ^{1}H NMR spectrum of hbC-PSMM in DMSO-d_6 is shown in Fig. 5. After hbC-PSMM (0.600 g) was dissolved in DMSO (4.7 mL), triethylamine (0.010 mL, 7.17 × 10^{-5} mol), EDBEA (0.105 mL, 7.17 × 10^{-4} mol), and distilled water (0.6 mL) were added under stirring at 35 °C for 48 h to yield hbC-PSMM-NH$_2$. Yield: 94.3%; Nynhidrin test was performed to qualitatively verify the introduction of amine group, as shown in Fig. 4. After NHS activation of C343 (0.015 g, 5.26 × 10^{-5} mol) using DEC (0.031 g, 1.62 × 10^{-4} mol) and NHS (0.031 g, 2.69 × 10^{-4} mol) in anhydrous DMSO (4.5 mL), a mixture of hbC-PSMM-NH$_2$ (0.45 g) and anhydrous DMSO (4.5 mL) was slowly added to the activated NHS solution and allowed to react at 35 °C for 48 h. The product was finally purified by dialysis. Yield: 93.3%; PDI$_{GPC}$: 1.14.
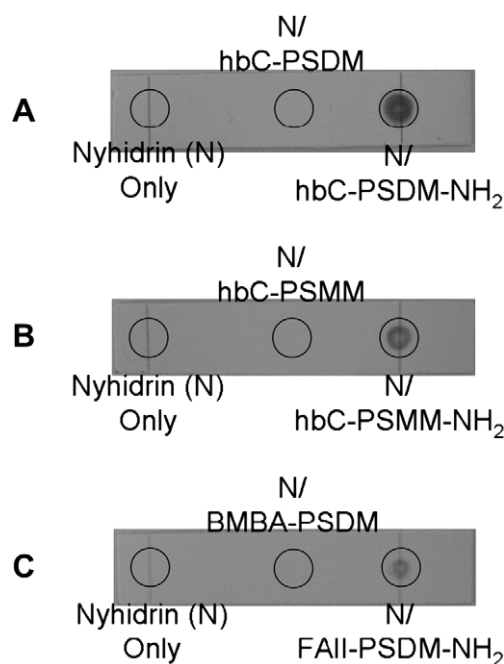

Fig. 4. Results of Nynhidrin test are shown above. It is noted that the original color-version image was converted into the grayscale-version image: (A) hbC-PSDM-NH$_2$; (B) hbC-PSMM-NH$_2$; (C) FAII-PSDM-NH$_2$.
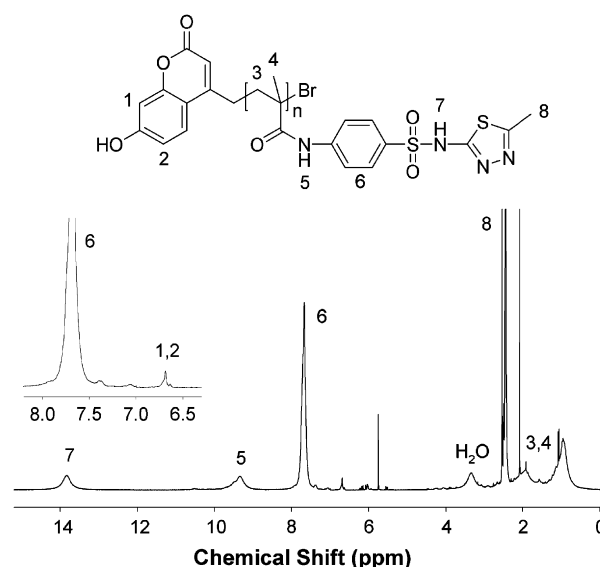

Fig. 5. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of hbC-PSMM.

2.5. Synthesis of fluoresceinamine isomer I-poly(sulfadimethoxine methacrylamide)-rhodamine B (FAII-PSDM-RDB)

The procedure for preparation of FAII-PSDM-RDB is similar to that of hbC-PSDM-C343. A solution of BMBA (0.050 g, 2.33 × 10^{-4} mol) in a mixed solvent of 5 mL of DMF and 5 mL of an aqueous NaOH (0.009 g, 2.25 × 10^{-4} mol) was added to a solution of SDM (2.20 g, 5.81 × 10^{-3} mol), Me$_6$TREN (0.065 mL, 2.34 × 10^{-4} mol), copper(I) bromide (0.034 g, 2.37 × 10^{-4} mol) in 5 mL of DMF and 5 mL of an aqueous NaOH (0.232 g, 5.80 × 10^{-3} mol). The flask was then placed at 35 °C for 2 h to allow ATRP, which yields BMBA-PSDM. Yield: 45.0%; $M_{n,NMR}$: 14 000; ^{1}H NMR spectrum of BMBA-PSDM in DMSO-d_6 is shown in Fig. 6. After NHS activation of BMBA-PSDM (0.5 g) using DEC (0.078 g, 4.07 × 10^{-4} mol) and NHS (0.078 g, 6.78 × 10^{-4} mol) in anhydrous DMSO (5 mL), a mixture of FAII (0.12 g, 3.45 × 10^{-4} mol) and anhydrous DMSO (5 mL) was slowly added to the solution. After the solution is allowed to react at 35 °C for 48 h, the final product (FAII-PDSM) was purified by dialyzing sequentially against DMF and MeOH using dialysis membrane. Yield: 70.0%; ^{1}H NMR spectrum of BMBA-PSDM in DMSO-d_6 is shown in Fig. 7. After FAII-PSDM (0.267 g) was
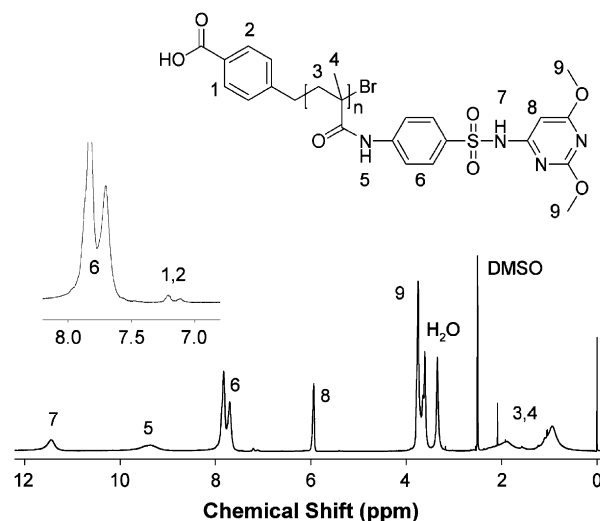

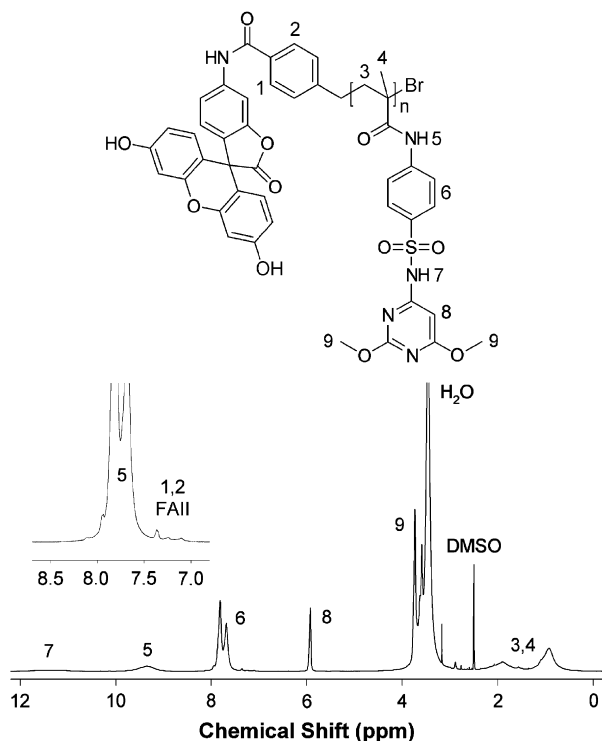**Fig. 6.** ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of BMBA-PSDM.

Fig. 7. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of FAII-PSDM.

Designation	Sulfonamide	Donor	Acceptor
hbC-PSDM-C343	Sulfadimethoxine	hbC[a]	C343[b]
hbC-PSMM-C343	Sulfamethizole	hbC	C343
FAII-PSDM-RDB	Sulfadimethoxine	FAII[c]	RDB[d]

[a] 7-Hydroxy-4-bromomethyl coumarin.
[b] Coumarin 343.
[c] Fluoresceinamine isomer I.
[d] Rhodamine B.

dissolved in DMSO (2.7 mL), triethylamine (0.004 mL, 2.87×10^{-5} mol), EDBEA (0.094 mL, 6.46×10^{-4} mol), and distilled water (0.27 mL) were added to the FAII-PSDM solution and allowed to react at 35 °C for 48 h to produce FAII-PSDM-NH$_2$. Yield: 60.7%; Nynhydrin test was performed to qualitatively verify the introduction of amine group as shown in Fig. 4. After NHS activation of RDB (0.009 g, 1.88×10^{-5} mol) using DEC (0.011 g, 5.74×10^{-5} mol) and NHS (0.011 g, 9.56×10^{-5} mol) in anhydrous DMSO (1.5 mL), a mixture of FAII-PSDM-NH$_2$ (0.15 g) and anhydrous DMSO (1.5 mL) was slowly added to the NHS activated RDB solution. The final product (FAII-PDSM-RDB) was purified by dialyzing sequentially against DMF and MeOH using dialysis membrane. Yield: 52.0%; PDI$_{GPC}$: 1.28.

2.6. Characterization

The chemical structures of materials used in this study were identified by ^{1}H NMR (Avance DPX-300 or Avance 500, Bruker).
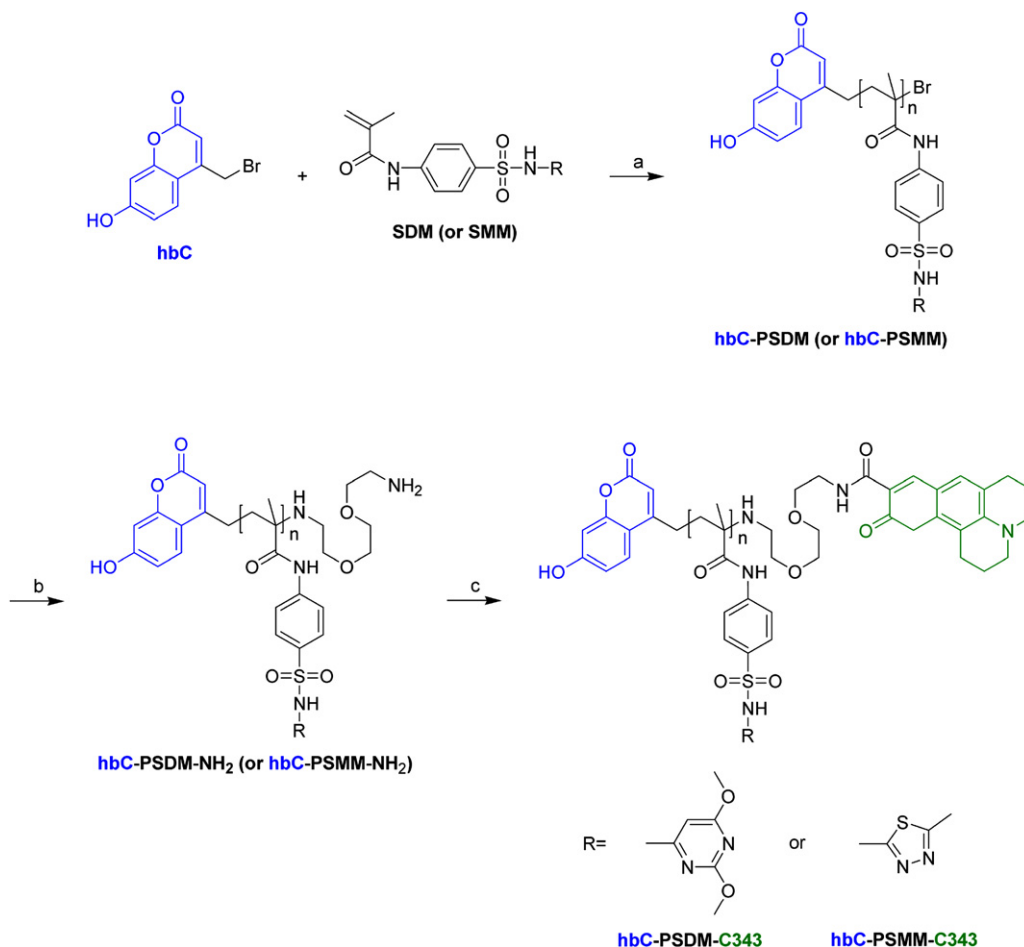
Scheme 1. Overall scheme for synthesis of hbC-PSDM-C343 and hbC-PSMM-C343: a – CuBr, Me$_6$TREN, DMF, NaOH/H$_2$O, 35 °C, 2 h; b – EDBEA, triethylamine, DMSO, H$_2$O, 35 °C, 48 h; c – coumarin 343, NHS, DEC, DMSO, 35 °C, 48 h.

Molecular weight and its distribution were measured by gel permeation chromatography (PL-GPC 50 Integrated GPC System, Polymer Laboratories) equipped with a refractive index detector using DMF as an eluent, where the columns were calibrated against standard poly(methyl methacrylate) samples. All the polymer solutions were prepared by dissolving polymers in pH 10.0 buffer solution, and the pH value was then adjusted by acid–base titration using 0.1 M, 0.05 M, and 0.01 M HCl aqueous solutions. UV–vis absorption and fluorescence spectra were obtained by a UV–visible spectrometer (HP 8452A, Hewlett Packard) and a fluorescence spectrometer (RF 5301, Shimadzu), respectively. The fluorescence color images were obtained using either UV-lamp or X-cite® 120 (EXPO Photonic Solution Inc.). All the polymer solutions were filtered using syringe filter (pore size: 0.20 μm, Minisart®, Satorius) before the absorption/fluorescence spectra and the fluorescent images were obtained.

3. Results and discussion

The sample designations of FRET sensors synthesized in this study are listed in Table 1. Among various sulfonamides, sulfamethizole and sulfadimethoxine are chosen for the moiety of polymeric linker, because pK_a values of these two sulfonamides are in the range of physiological pH. The color emitted from FRET can be tuned by introducing a proper pair of FRET donor and acceptor. In this study, two dye pairs are used for FRET: one is a pair of hbC/C343 for a blue-to-green FRET sensor, and the other is a pair of FAII/RDB for a green-to-red FRET sensor.

The synthetic routes for preparation of the blue-to-green FRET sensors are shown in Scheme 1. It has been known that

sulfonamide-type monomers are not effectively polymerized by conventional ATRP ligands, and therefore a stronger ligand such as Me_6TREN is used for ATRP of SDM and SMM in this study. Since hbC acts as not only an initiator for ATRP but also a FRET donor emitting blue color, the polymeric linker prepared from ATRP has a FRET donor which is linked to the one end of the polymeric linker. It is also noted that such a polymer prepared by ATRP has the *tert*-bromide group at the chain end of polymer. Such bromide group can be transformed into various functional groups via simple nucleophilic displacement reaction. In this study, an amine group is selected as a functional group to be conjugated with the carboxyl acid group of FRET acceptor (C343). One of the important reasons to choose amine group is that the amide linkage formed from the reaction between PSDM (or PSMM) and C343 is not vulnerable to aqueous solution. More specifically, hbC-PSDM is first reacted with EDBEA (diamine-type molecule) to yield hbC-PSDM-NH_2. Although the Nynhydrin test identifies that the amine group is successfully introduced to the chain end of hbC-PSDM, it is not possible to quantitatively estimate the introduction of amine groups, because the proton peaks of EDBEA in NMR overlaps with those peaks of PSDM. Here, it is noted that there is little chance for two polymers to be linked to each other, because an excess amount of diamine-type molecule is added to the reaction solution. Finally, hbC-PSDM-NH_2 is conjugated with the carboxyl acid group of C343.

The synthetic route for preparation of the green-to-red FRET sensor is similar to that of blue-to-green FRET sensor, and the detailed procedure is represented in Scheme 2. The NMR spectra of hbC-PSDM-C343, hbC-PSMM-C343, and FAII-PSDM-RDB are shown in Fig. 8. Although all protons of C343, FAII, or RDB are not assigned in NMR due to significant overlap with the proton peaks of the main
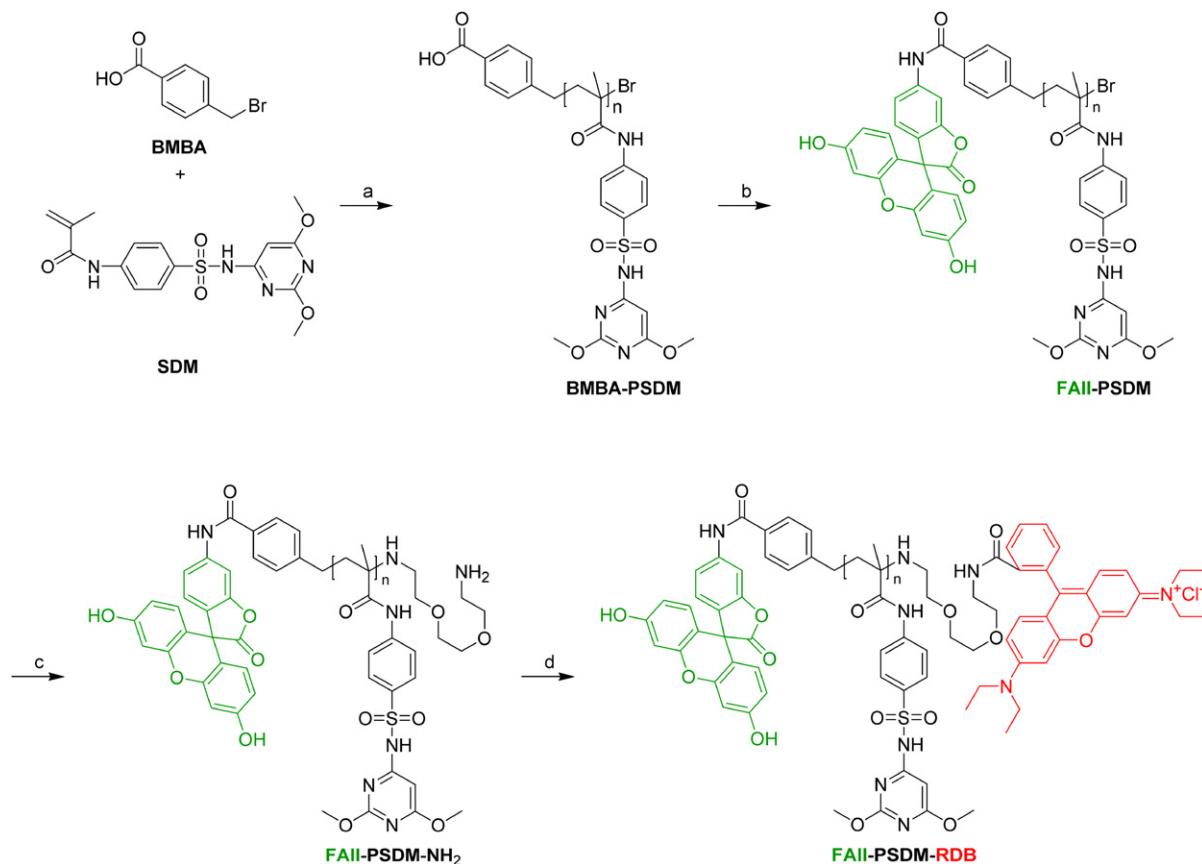
Scheme 2. Overall scheme for synthesis of FAII-PSDM-RDB: a – CuBr, Me_6TREN, DMF, NaOH/H_2O, 35 °C, 2 h; b – FAII, NHS, DEC, DMSO, 35 °C, 48 h; c – EDBEA, triethylamine, DMSO, H_2O, 35 °C, 48 h; d – RDB, NHS, DEC, DMSO, 35 °C, 48 h.
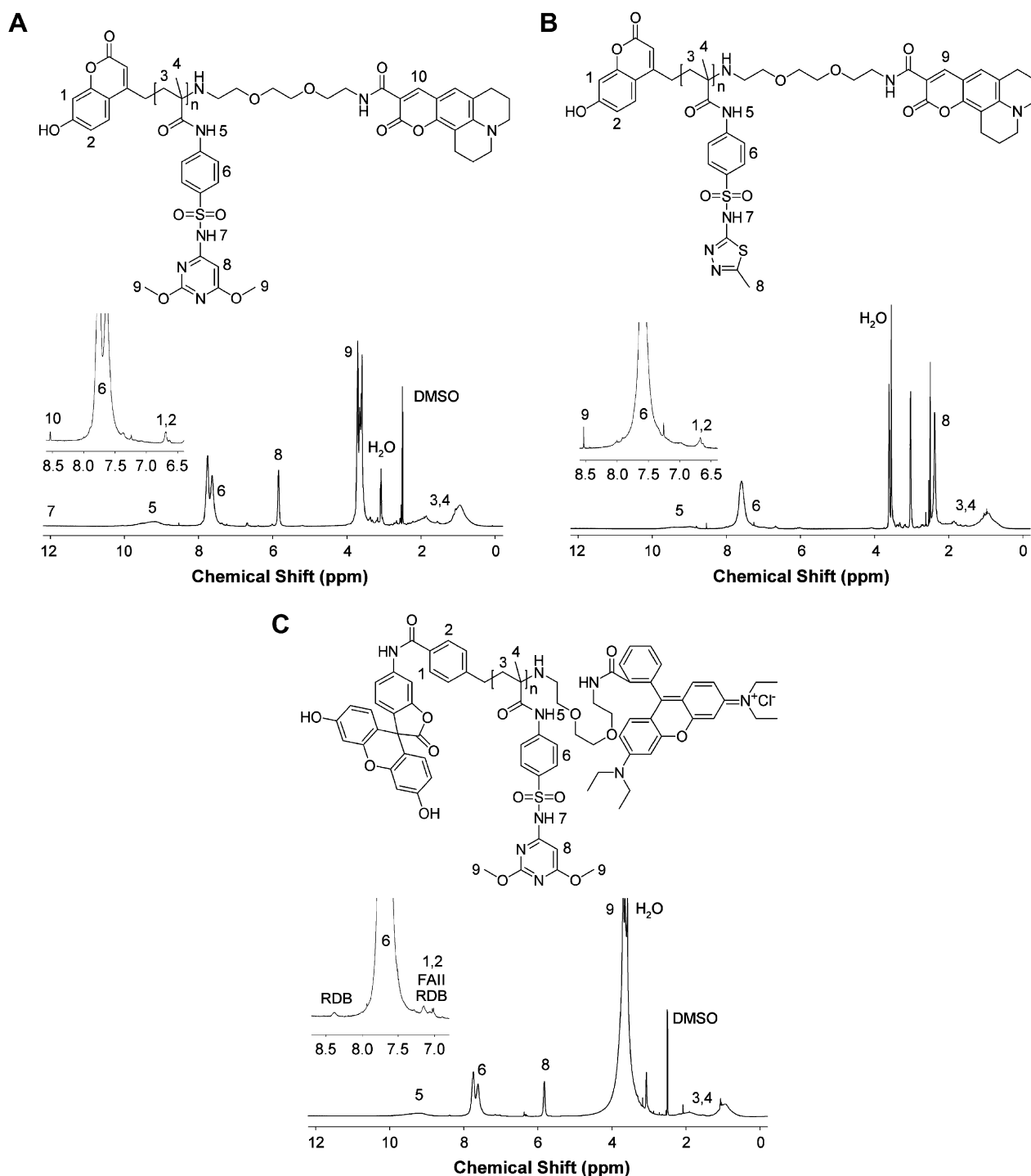
Fig. 8. ^{1}H NMR spectra (500 MHz, DMSO-d_6) of (A) hbC-PSDM-C343; (B) hbC-PSMM-C343 and (C) FAII-PSDM-RDB.

backbone, some NMR peaks due to protons of dye molecules can be distinctly assigned, which confirms that the dye molecules are successfully introduced. Since most peaks of the NMR spectra can be appropriately assigned, as can be seen in Fig. 8, it is concluded that three different kinds of pH-sensitive FRET sensors are successfully synthesized. Fig. 9 shows the GPC traces of hbC-PSDM-C343, hbC-PSMM-C343, and FAII-PSDM-RDB. Each GPC profile shows only one single narrow peak, indicating that two polymers are not linked with each other during amination as discussed above.

Comparison of the absorption and emission spectra of hbC-PSDM and C343 confirm the validity of the choice of hbC and C343

as a FRET donor and a FRET acceptor for the blue-to-green FRET system, respectively, as shown in Fig. 10A. The absorption spectrum of the FRET donor (hbC) does not significantly overlap with that of the FRET acceptor (C343), indicating that it is possible to excite the FRET donor alone at single wavelength. It also shows that the emission spectrum of FRET donor overlaps sufficiently with the absorption spectrum of FRET acceptor. The absorption and emission spectra of FAII and RDB also show that the pair of FAII and RDB is also adequate for the green-to-red FRET system, because the absorption spectrum of FAII does not overlap significantly with that of RDB, while the emission spectrum of FAII overlaps sufficiently with the absorption of RDB, as shown in Fig. 10B. All of these
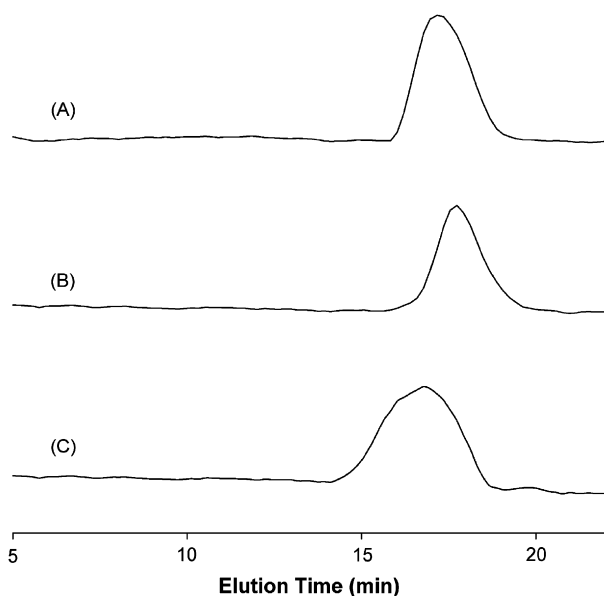
Fig. 9. GPC traces of (A) hbC-PSDM-C343; (B) hbC-PSMM-C343 and (C) FAII-PDSM-RDB.

features satisfy the condition of the overall integral factor for the successful FRET efficiency [17,18].

To examine the feasibility for this FRET sensor to detect various pH ranges, we used two different sulfonamide monomers for preparation of pH-sensitive polymeric linker: hbC-PSDM-C343 and hbC-PSMM-C343. First, we examine the pH sensitivity of hbC-PSDM-C343. When the intensity ratio of 491 nm to 380 nm

representing the quantitative FRET efficiency of hbC-PSDM-C343 is plotted as a function of pH, it reveals that the intensity ratio starts to increase steeply at pH 7.6 as the value of pH is decreased, as can be seen in Fig. 11. This indicates that the energy transfer from FRET donor to acceptor takes place effectively at around pH 7.6. This is easily explained by considering the pH-induced conformational change of PSDM. Since the SDM moiety in the polymeric linker is deprotonated and ionized at pH above its pK_a, it is expected that PSDM becomes water-soluble at pH above its pK_a and thus has an expanded coil conformation. On the other hand, since the PSDM is protonated at pH below its pK_a, it has a globular structure at low pH due to the hydrophobic nature of protonated SDM. This result is well consistent with the previous work, which explains the conformational transition of oligomeric poly(sulfonamide) upon pH change [38]. Consequently, the conformation change from the expanded coil to the globular structure results in a decrease of the distance between FRET donor and acceptor, which induces the FRET effectively. Second, we examine the pH sensitivity of hbC-PSMM-C343, which is expected to show the pH sensitivity at a region different from hbC-PSDM-C343. When the intensity ratio is also plotted against pH, it reveals that the intensity ratio of hbC-PSMM-C343 starts to increase steeply at pH 6.4 as the pH value is decreased. This different transition comes from the fact that the pK_a value of SMM is lower than that of SDM. Therefore, this result shows that the detection of a specific pH is easily accomplished by a proper selection of sulfonamide. It is also noted from Fig. 11 that these FRET sensors show a sharp transition at a narrow region of pH, suggesting that this type of FRET sensor can be used as an alarm-type probe for detecting a specific pH range.

To demonstrate its possibility for an optically detectable sensor, the fluorescence color images of hbC-PSDM-C343 are obtained at two different states of pH 7.6 and 6.8, where the color images are captured by a true-color digital camera without use of amplifiers and filters. The inset in Fig. 11 shows that the solution at pH 6.8 mainly emits the green color corresponding to the emission of the FRET acceptor while the solution at pH 7.6 mainly emits the blue
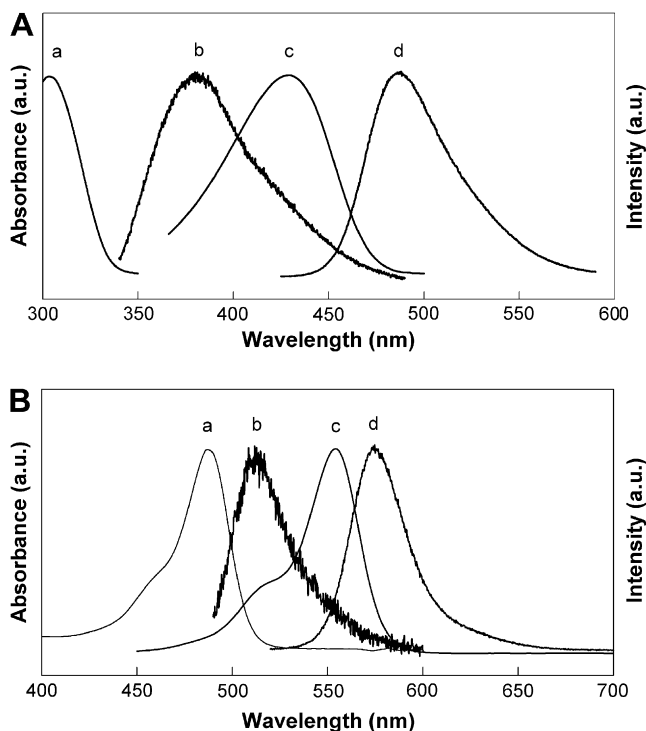

Fig. 10. (A) Normalized absorbance and emission spectra of hbC-PSDM and C343 at pH 8.0: a and c are the absorbance spectrum of hbC-PSDM and C343, respectively; b and d are the emission spectra of hbC-PSDM (excited at 330 nm) and C343 (excited at 400 nm), respectively. (B) Normalized absorbance and emission spectra of FAII and RDB at pH 8.0: a and c are the absorbance spectrum of FAII and RDB, respectively; b and d are the emission spectra of FAII (excited at 480 nm) and RDB (excited at 510 nm), respectively.
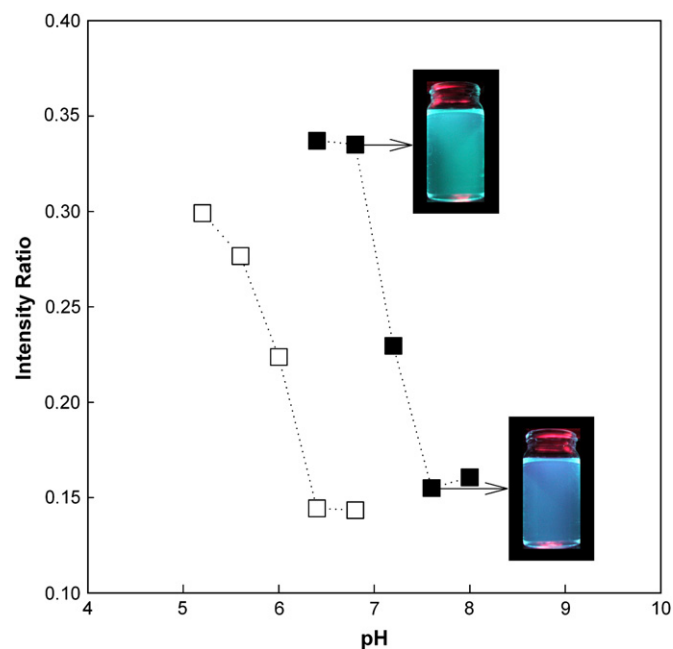

Fig. 11. Plots of the ratio of the intensity at 491 nm (FRET acceptor, C343) to the intensity at 380 nm (FRET donor, hbC) versus pH: hbC-PSDM-C343 (■) and hbC-PSMM-C343 (□). All the solutions are irradiated at the excitation wavelength of 330 nm, and the sample concentrations are below 1.4×10^{-3} g/L for hbC-PSDM-C343 and below 1.6×10^{-3} g/L for hbC-PSMM-C343, respectively.
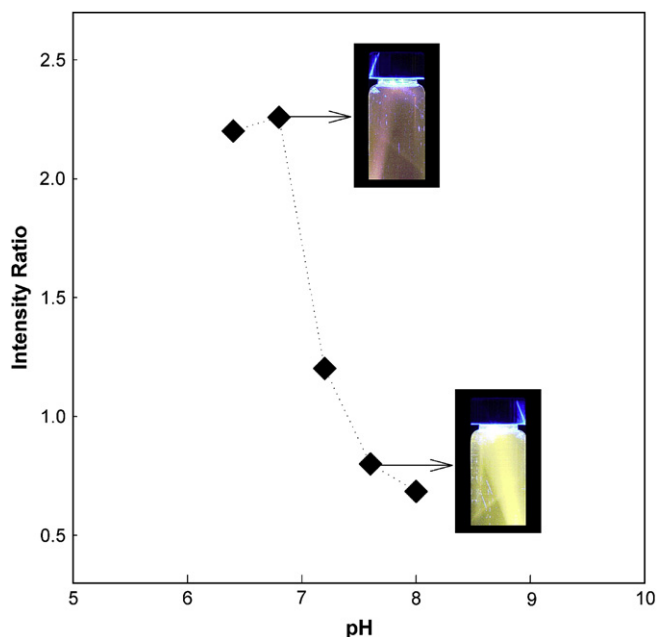
Fig. 12. Plot of the ratio of the intensity at 584 nm (FRET acceptor, RDB) to the intensity at 515 nm (FRET donor, FAII) versus pH. All the solutions are irradiated at the excitation wavelength of 500 nm, and the sample concentrations are below 2.0×10^{-3} g/L.

color corresponding to the emission of FRET donor (for interpretation of the references to colour in text, the reader is referred to the web version of this article). All of these features indicate that the FRET is effectively induced due to the conformational change of PSDM from coil to globule with pH variation.

This type of FRET sensor prepared in this study has another important advantage for practical applications, since the color emitted from this type of FRET sensor can be determined by choosing a proper pair of FRET donor and acceptor. For this advantage to be realized, FAII-PSDM-RDB is synthesized as a representative of the green-to-red FRET sensor, which has a green-emitting FRET donor (FAII) and a red-emitting FRET acceptor (RDB) attached at each chain end of PSDM, respectively. The inset in Fig. 12 shows that this green-to-red FRET sensor mainly emits the green color from the FRET donor at pH 7.6 while the sensor emits mainly the red color from the FRET acceptor at pH 6.8 (for interpretation of the references to colour in text, the reader is referred to the web version of this article). This result strongly suggests that a simple choice of FRET pair determines the FRET-induced emitting color. When the intensity ratio of FRET acceptor to FRET donor is plotted as a function of pH, it also reveals that the transition takes place at a narrow pH range, as shown in Fig. 12.

4. Conclusions

A series of new polymeric pH sensors are synthesized and their FRET behaviors are examined as a function of pH. Since the polymeric linker containing sulfonamide group undergoes the coil–globule transition under pH variation, the distance between the FRET donor and the FRET acceptor attached to each end of the polymeric linker is changed with pH variation, which results in a change of the FRET efficiency with pH. An advantage of this pH sensor is that the sensor can detect at a specific pH as one wishes

simply by choosing a specific sulfonamide among the sulfonamide family showing a wide range of pK_a depending upon the type of substituent. Another advantage of this sensor is that the color emitted from FRET can be determined by choosing a proper pair of FRET donor and acceptor. Since this FRET sensor responds to a small change in pH with well-defined on-and-off behavior, this novel pH-sensitive FRET sensor is expected to be one of the promising candidates for medical and biological applications.

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.polymer.2008.07.044.

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