Facile Preparation and Potential Application of Water-Soluble Polymeric Temperature/pH Probes Bearing Fluorescein

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ABSTRACT: An unsaturated monomer bearing xanthene groups and allyloxyfluorescein (Al-Flu), was synthesized from fluorescein and allyl bromide by etherification. The structure of the monomer was confirmed by IR and mass spectroscopy and ^{1}H-NMR, and ^{13}C-NMR spectroscopy. With azobisisobutyronitrile as a thermal initiator in tetrahydrofuran under 65–70°C, a copolymer of allyloxyfluorescein and acrylamide [poly(Al-Flu-*co*-AM)] was obtained and was characterized by the methods of IR spectroscopy, ultraviolet–visible spectroscopy, and differential scanning calorimetry. The experimental results show that the fluorescence spectra of water-soluble poly(Al-Flu-*co*-AM) was dependent on the pH and temperature in the solution. Moreover, poly(Al-Flu-*co*-AM) had an excellent linear response between the relative fluorescence intensity and temperature in the range 0–60°C and had a nonlinear response from pH 0.00 to 12.85 between the relative fluorescence intensity and pH. The pH and temperature sensitivities of the fluorescence could be advantageous for it as a multifunctional material to probe pH and temperature. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 1968–1975, 2011

Key words: fluorescence; functionalization of polymers; water-soluble polymers

INTRODUCTION

Recently, more and more interest has developed in the synthesis of stimuli-sensitive polymer materials because of their potential feasibility in biotechnology, such as biomedical and biosensing applications.[1–5] It is noted that temperature- and pH-responsive polymers and polymeric gels have been extensively studied. However, most were polyelectrolytes, and the responsive mechanism was temperature- or pH-induced chain conformation change.[6–8] It is well known that the fluorescence technique is a powerful tool for detection because of its simplicity and high sensitivity. So, fluorescent probe techniques have been widely used in medicine and biology and can give swift responses to changes in ambience, such those in temperature, solvent composition, and pH. For example, highly sensitive fluorophores, such as fluorescein derivatives, are widely used in pH sensors and are commonly dissolved in a polymer matrix such as poly(vinyl alcohol). Unfortunately, leaching of the active sensing ingredients from the matrix dictates the lifetime of the sensors and hampers their applications. Accordingly, a kind of polymeric optical sensor for temperature and pH has been developed and is usually obtained with a functional dye covalently bound to a support matrix, such as cellulose or glass.[9–13] In comparison with the doping type of optical indicators, dyes covalently immobilized on macromolecules do not suffer from leaching and hysteresis, and the effects of swelling can be minimized. Moreover, these kinds of polymeric indicators and materials are more stable, can be more easily immobilized, and can be used repeatedly. Therefore, these polymeric indicators could be very useful in the innovation of conventional thermometers and pH test paper, which may be developed as optical multiresponsive sensors.

As is well known, the fluorescence emission or absorption wavelength of some organic compounds

altered along with pH can be used to indicate the acid–base changes of the object.[14,15] Fluorescein (FL) is widely employed as a platform for various florescence probes and fluorescence labels because of its high-fluorescence quantum efficiency in aqueous media. Moreover, the excitation and emission wavelengths of FL are in the range of the visible region, which is advantageous to for detections.[16–19] Because its simple derivatives were proven to be among the most versatile of chromophores used as fluorescence probes, increasing amounts of FL derivatives have been synthesized.[20,21] Among fluorescein derivatives, allyl derivatives provide convenience for allyloxyfluorescein (Al-Flu) to polymerize with various monomers, such as crylic acid, styrene, and acrylamide (AM), through which fluorescein can be easily attached to macromolecules. Moreover, it was found that the allyl derivative could not weaken the well-known pH dependence of the fluorescence of fluorescein. Herein, it was suitable to adopt this method to prepare fluorescent polymers from synthetic fluorescent monomers.

As a biomaterial, good solubility in water is essential in biotechnology applications. Polyacrylamides (PAMs) are amorphous and water-soluble polymers with attractive properties for applications in membrane technology and biomedical devices.[22,23] Because of the fluorescent characters of Al-Flu and the superiority of PAMs as luminescentless matrices excited by radiation of wavelengths from 260 to 500 nm,[24] the copolymerization of the dye and PAM were adopted to develop new multifunctional materials to be used in many areas of optics and material science.

In our earlier works, different soluble polymeric pH indicators were synthesized by the reaction of functional dyes and polymers by a convenient method.[25–29] However, the results show that it was difficult to control the content of fluorescence groups in the side chain of the polymer, and the reaction point was in complete disorder because the dyes were covalently bound to the surface of polymer. So, we attempted to use the copolymerization method to overcome these disadvantages. We successfully synthesized an unsaturated monomer bearing xanthene groups by esterification and the copolymer of monomer and AM, which may offer potential as a reusable polymer sensor for Fe^{3+} ions in aqueous solution, as discussed in a previous article.[30] Unfortunately, the water-soluble copolymer was not stable and was easily hydrolytic in acid and alkaline solutions because of the ester bonds.

To obtain a stable fluorescence polymer, a new unsaturated monomer bearing xanthene groups and Al-Flu was obtained from fluorescein and allyl bromide by etherification in this study, and the copolymer of Al-Flu with AM was synthesized by a thermal initiator. The temperature/pH dependences of fluorescence of the soluble copolymer were investigated in detail. This investigation may provide another convenient method for preparing multifunctional polymeric materials bearing fluorescein to probe pH and temperature.

EXPERIMENTAL

Materials

Fluorescein was purified by crystallization from an ethanol solution and dried *in vacuo*. Allyl bromide and AM, purchased from TianJin Chemistry Reagent Co. (Tianjin, China), were analytical pure and were used after further purification. Azobisisobutyronitrile (AIBN) was recrystallized from ethanol. Organic solvents (analytical pure), such as *N,N*-dimethylformamide (DMF) and tetrahydrofuran (THF), were dried and distilled before being used. Other reagents were all analytical pure, and doubly distilled water was used in all of the experiments.

Synthesis of the monomer

Al-Flu was prepared by the reaction of fluorescein and allyl bromide in the presence of K_2CO_3. A mixture of 75 mL of DMF, 3.30 g (1.0×10^{-2} mol) of fluorescein, and 2.86 g (2.0×10^{-2} mol) of K_2CO_3 was put into a 250-mL, three-necked, round-bottom flask equipped with thermometer, a dropping funnel, and a magnetic stirring bar. The flask was then maintained at 0°C while 1.70 mL (2.0×10^{-2} mol) of allyl bromide dissolved in 10 mL of dry DMF was added with a dropping funnel for 30 min. The reaction mixture was stirred at 25°C for 12 h. The reaction mixture was then poured into saturated brine (0.1 L), and the aqueous layer was extracted with ether (4 × 30 mL). The organic phases were combined and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure yielded crude product as a yellow powder. The desired product was isolated by sequential column chromatography (2 : 1, petroleum benzene:acetidin as an eluent, with silica gel, 200–300 mesh). The chromatography furnished about 1.1 g of a pale yellow solid that could be dissolved in CH_2Cl_2, acetone, methanol, DMF, and other organic solvents, as illustrated in Scheme 1.

Yield ≈ 30%. mp = 143–145°C. IR (KBr, $\tilde{v}$, cm^{-1}): 1727 (C=O, carboxy); 3052, 820 (=CH$_2$); 1643 (C=C); 3030, 999 (=C—H); 2927, 919 (—CH$_2$—); 1255, 1075 (Ac—O—C); 1595, 1542, 1459, 758 (aromatic C=C). ^{1}H-NMR (400 MHz, CD$_3$COCD$_3$, δ): 4.634–4.765 (m, 2, —CH$_2$—), 5.242–5.453 (m, 2, =CH$_2$), 6.004–6.131 (m, 1, =CH); 6.613–8.009 (m, 10, benzene ring). ^{13}C-NMR (100 MHz, CD$_3$COCD$_3$, δ): 69.02, 83.01, 101.78, 102.66, 110.68, 111.99, 112.41, 112.79, 117.31, 124.25, 124.81, 127.09, 129.32, 129.46,
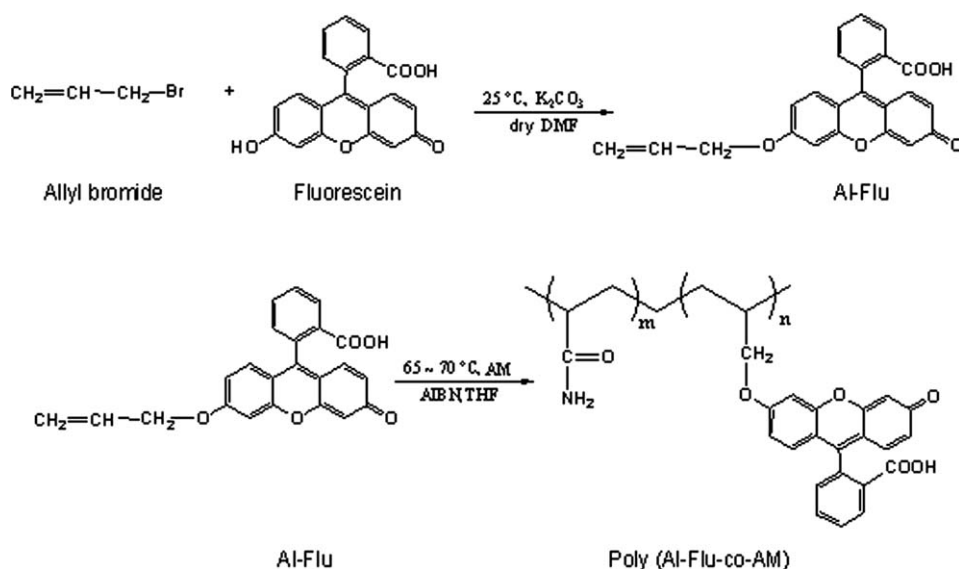
Scheme 1 Preparation of Al-Flu and poly(Al-Flu-*co*-AM).

130.19, 133.47, 135.52, 152.64, 153.27, 159.79, 160.64, 169.02. Mass spectroscopy: m/e calculated for molecular $= 372$; found $m/e = 372.1$ (M^+). Visible spectra (λ_{max} is the wavelength at which the maximum fraction of light is absorbed by a solution, nm): 278, 458, 481.

Copolymerization of Al-Flu and AM

The poly(allyloxyfluorescein-*co*-acrylic amide) [poly (Al-Flu-*co*-AM)] was prepared by the copolymerization of Al-Flu and AM with AIBN. A solution of 0.50 g (1.34×10^{-3} mol) Al-Flu, 0.96 g (1.34×10^{-2} mol) AM, and 0.01 g of AIBN in 30 mL of dry THF was introduced into a dry polymerization tube. The solution was deoxygenated by purging with purified N_2 gas. The tube was sealed and placed in a regulated thermostat bath at 65°C for 10 h. The precipitate was collected by filtration and extracted with THF for at least 12 h in a Soxhlet extractor. Last, the powder was dried *in vacuo* to a constant weight. The poly(Al-Flu-*co*-AM) obtained in this way was used for further fluorescence tests. The degree of substitution of Al-Flu in the copolymer was 5.01% via UV spectrophotometry. The copolymer was identified by IR and UV spectroscopy and differential scanning calorimetry [DSC; glass-transition temperature (T_g) $= 186.1°C$; UV spectra (λ_{max}) $= 278, 458, 482$ nm; viscosity-average molecular weight of the copolymer $= 4.7 \times 10^5$ g/mol].

Measurement

^{1}H-NMR and ^{13}C-NMR experiments were performed on an AM 400-MHz BB Bruker instrument (Bruker Corp., Leipzig, Germany) for the Al-Flu in CD$_3$COCD$_3$. Mass spectroscopy was obtained on a TRACE DSQ (Thermo Electron Corp., State of California). IR spectra were recorded on a Nicolet Neus 670 FT-IR spectrophotometer (Nicolet, State of Wisconsin). Ultraviolet–visible (UV–vis) spectra were taken on a Lambda 35 UV–vis spectrometer (Perkin-Elmer Corp., Fremont) to measure the mass concentration of Al-Flu in the copolymer. Fluorescent excitation and emission spectra were measured with an LS 55 luminescence spectrometer (Perkin-Elmer Corp., Fremont) in solution, solid, and film. The pH measurements were performed with a pH-3B instrument (Xingrui Corp., Shanghai, China).

DSC measurements were performed with a Sapphire differential scanning calorimeter (Perkin-Elmer Corp., Fremont) in a set of three consecutive steps: first, by heating from room temperature to 200°C and, second, by isothermal annealing for 20 min, which was followed by cooling from 200 to 20°C. After the second step, the samples were heated from 40 to 300°C. These scanning processes were performed at a heating rate of 20°C/min for the first two steps and at 10°C/min for the last heating in a dynamic nitrogen atmosphere (30 mL/min). The T_g values quoted are from the second heating run (at 10°C/min) to extrapolate the onset value.

For fluorescence emission measurements, a 10×10 mm^2 quartz cell was used for detection. Determination of the photoluminescence quantum efficiency (Φ) of the obtained poly(Al-Flu-*co*-AM) was carried out according to a conventional method with fluorescein as a standard. The Φ was determined with the following expression:

$$\frac{Q_{F(sample)}}{Q_{F(fluorescein)}} = \frac{I_{(sample)}}{I_{(fluorescein)}} = \frac{E_{(fluorescein)}}{E_{(sample)}}$$
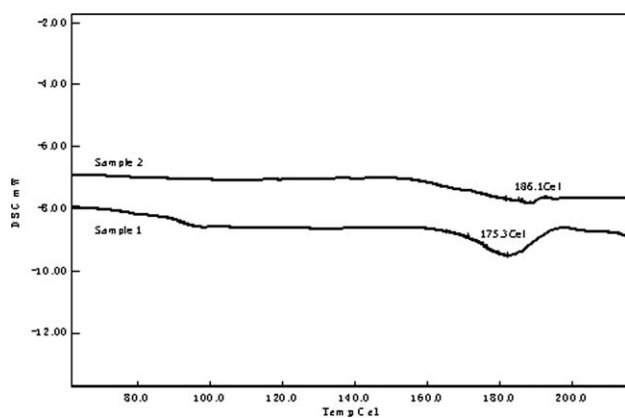
Figure 1 DSC curves of PAM (sample 1) and poly (Al-Flu-*co*-AM) synthesized (sample 2).

where Q_F is the quantum yield [$Q_{F(fluorescein)} = 0.93$], It is the total fluorescence emission intensity over all wavelengths, and E is the molar extinction coefficient determined at 480 nm.

The temperature effect on the fluorescence yield of poly(Al-Flu-*co*-AM) was determined by measurement of the variation of the fluorescence intensity at the peak wavelength, with a relation applied for the variation of quantum yield of the solution with temperature at the excitation wavelength. During the fluorescence measurements, a thermostated fluid was passed through the sample housing to maintain a defined temperature, with the temperature range of the sample changed from 0 to 60°C by a digital temperature controller.

We obtained the fluorescence intensities against pH by recording the emission in solutions with different pHs at a fixed wavelength. During the measurement, HCl and NaOH were used to adjust the pH to the desired value, and samples were carried out 25°C. The pH range of the sample was changed from 0.00 to 12.85 by a digital pH controller.

RESULTS AND DISCUSSION

Characterization of the copolymer

Poly(Al-Flu-*co*-AM) was identified by the characteristic absorption bands at 3404, 1618 (—NH$_2$), 2938, 1451 (—CH$_2$—), 1662 (C═O), and 759 cm^{-1} (benzene ring) and the disappearance of the stretching and bending absorption bands caused by terminal vinyl, such as at 3052, 3030, 999, and 820 cm^{-1} on its IR spectrum.

The comparative result, shown on the DSC curves of poly(Al-Flu-*co*-AM) and PAM in Figure 1, was similar with that of a copolymer that we previously reported.[30] The T_g's of poly(Al-Flu-*co*-AM) and PAM were 186.1 and 175.3°C, respectively. The T_g of the copolymer was higher because of the rigid structure of the xanthene group on the PAM chain, which

affected the movement of the main chain of PAM. The result suggests similarly that Al-Flu was connected to PAM through polymerization.

On the UV–vis spectra of PAM, Al-Flu, and poly(Al-Flu-*co*-AM), shown in Figure 2, a similar result was obtained.[30] According to the absorption data for the PAM, Al-Flu, and poly(Al-Flu-*co*-AM), PAM had no optical absorption from 260 to 600 nm, and the absorption peaks at 278, 458, and 482 nm of poly (Al-Flu-*co*-AM) were essentially identical to those observed for the Al-Flu at 278, 458, and 481 nm in the UV region. Apparently, the absorption of the copolymer was induced by the chromophore on PAM, and the matrix did not change the optical character of Al-Flu. The molar extinction coefficients of Al-Flu and poly(Al-Flu-*co*-AM) were 1.47×10^4 and 1.54×10^4 L mol^{-1} cm^{-1}, respectively, and corresponded to the π→π* transitions.

Fluorescent properties of the monomer and copolymer

Figure 3 shows the absorption and emission spectra of Al-Flu in DMF and in the solid state. The emission length (λ_{em}) of the derivative substituted in the hydroxyl position of the xanthene moiety was found at about 525 nm; this was a similar emission to that of the parent fluorescein ($\lambda_{em} \approx 520$ nm).[31,32] Also, Al-Flu exhibited luminescence in the solid state, whereas the fluorescein was nonfluorescent in the solid state. However, the absorption band at 392 nm and the emission band at 535 nm in the solid state were distinctly different from these in solution. The absorption maximum blueshifted near 55 nm, whereas the emission maximum in the luminescence spectra of the solid powder redshifted near 10 nm
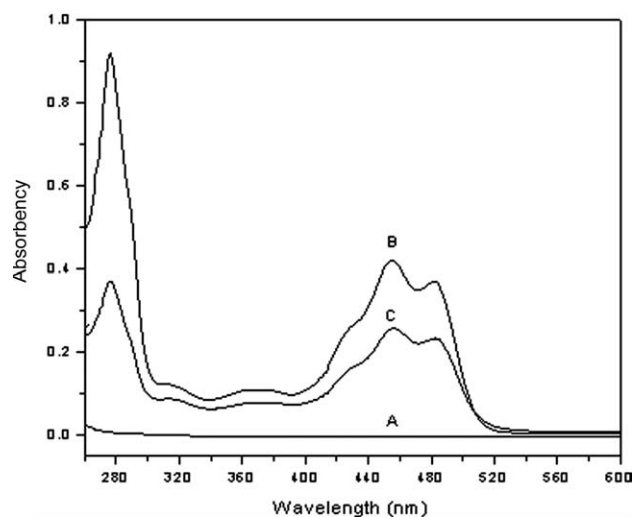

Figure 2 Absorption spectra of (A) PAM, (B) Al-Flu, and (C) poly(Al-Flu-*co*-AM) in mixed solution (DMF/H$_2$O = 1/1).
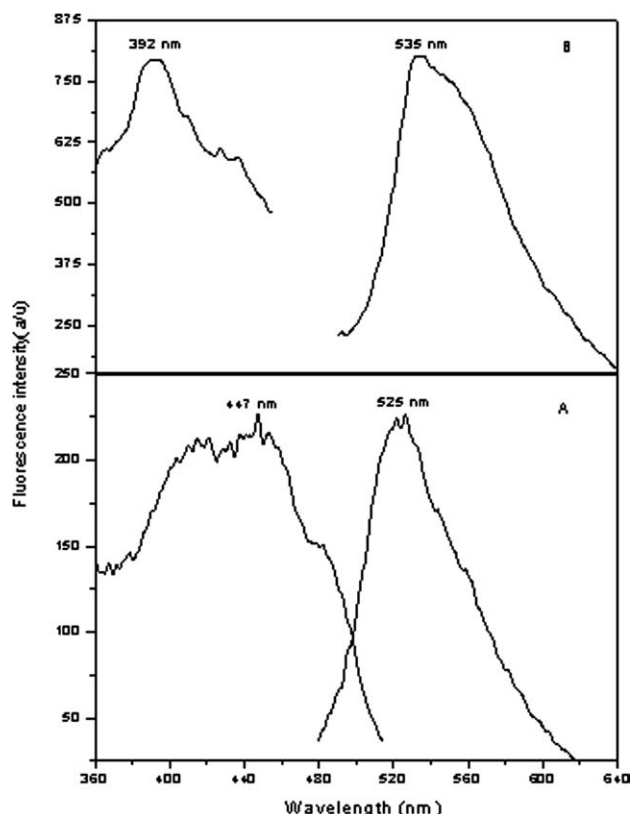
Figure 3 Absorption spectra and fluorescence spectra of Al-Flu: (A) 3×10^{-3} mol/L in DMF and (B) in the solid state.

compared to the spectra of Al-Flu in solution because of the molecular self-association of the dyes.[33,34]

A thin film of poly(Al-Flu-co-AM) was prepared on a glass plate by solvent casting. The absorption and emission spectra for the copolymer in aqueous solution and in the film are shown in Figure 4. Apparently, the fluorescence spectra of poly(Al-Flu-co-AM) resembled the mirror image of the absorption spectrum; this suggested that the emission at 515 nm arose from the fluorescein chromophore. Like in the spectrum of Al-Flu in the solid, a strong absorption band was also found at short wavelength and was due to the H aggregate of the chromophore, and a typical redshift of the emission maximum was also apparent in the visible region in the film.[35] The band at 525 nm could be ascribed to the excimer between the pendant Al-Flu moieties by comparison of its position with that of the fluorescence spectrum in solution. Moreover, the values of Stoke's shift of Al-Flu and poly(Al-Flu-co-AM) were near 80 nm (Fig. 3) and 40 nm (Fig. 4) in solution, respectively. Compared with them, it was evident that the copolymer had the lower Stoke's shift because of the decrease of radiationless transition, such as internal conversion [lowest excited singlet state (S_1) $\rightarrow$ singlet state (S_0)] and intersystem crossing [S_1 $\rightarrow$ lowest triplet state (T_1)]. This showed that the movement of the dye

chromophore in the polymer chain was restricted, which affected the radiationless transition of Al-Flu. In addition, the Φ value for poly(Al-Flu-co-AM) was 0.54.

Temperature dependence of the fluorescence intensity of poly(Al-Flu-co-AM)

The fluorescence spectra of the copolymer in water (1×10^{-2} g/L) at several temperatures are shown in Figure 5. These spectra exhibited a redshift of their maximum on heating, which was attributed to the inability of the solvent to reorient during the lifetime of the electronic excited state (1–5 ns).[36] The solvent reorientation depended on the temperature. At a lower temperature, the ability of the solvent to reorient was weak, and the state energy difference remained large for the emission transition, whereas at higher temperatures, reorientation did occur, which led to a reduction of the energy difference and a redshift.

It was shown that the maximum fluorescence intensity of poly(Al-Flu-co-AM) decreased on heating. The temperature dependence of the fluorescence intensity could be discussed in terms of both photophysical and photochemical dye properties and the polymer matrix relaxation processes.[25–28,37]

As shown in Figure 5, an excellent linear relationship was obtained between the relative fluorescence intensity and temperature in the range 0–60°C. The
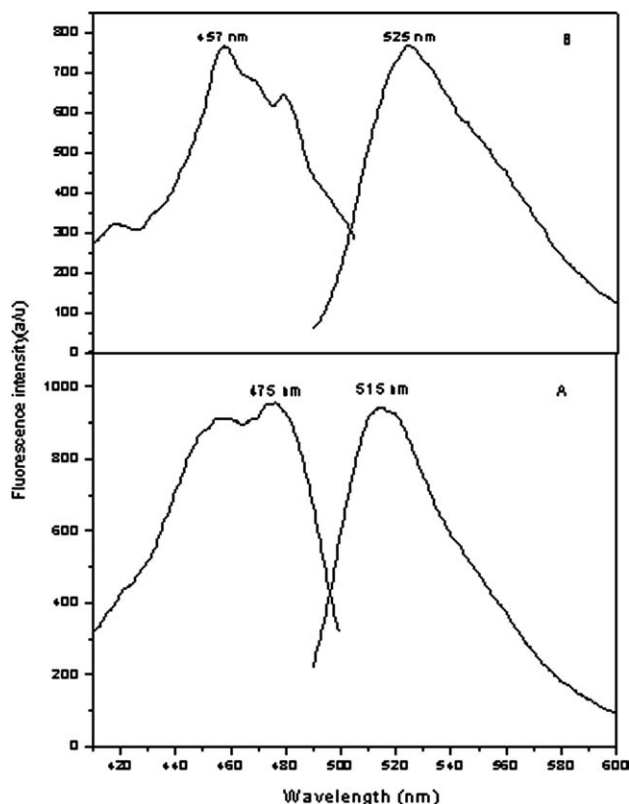

Figure 4 Absorption spectra and fluorescence spectra of poly(Al-Flu-co-AM): (A) 1.2×10^{-2} g/L in aqueous solution and (B) in film (1.0%, 0.015 mm).
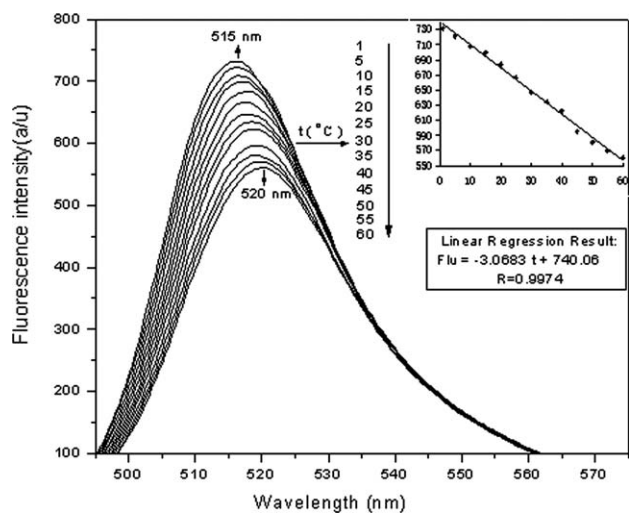
Figure 5 Fluorescence spectra for 1.0×10^{-2} g/L poly(Al-Flu-*co*-AM) in water at the different temperatures from 0 to 60°C ($\lambda_{ex} = 475$ nm). Inset: linearity of the fluorescence intensity versus temperature. R is the correlation coefficient in the linear regresion.

linear regression equation of the calibration graph was Flu = 740.06–3.0683*t* (centigrade temperature) (where Flu is the relative fluorescence intensity), with a correlation coefficient of linear regression of 0.9974. Obviously, poly(Al-Flu-*co*-AM) was a promising optical indicator for temperature determination.

pH dependence of the fluorescence intensity of poly(Al-Flu-*co*-AM)

The various states of protonation and tautomeric form of xanthenes are well known.[38] The maximum absorption of xanthenes and their derivatives depends on the species present and, hence, on the pH.[39] For fluorescein, pK_a values of 6.4, 4.3, and 2.1 were determined for the sequence of protonations of the anion to neutral (zwitterionic and lactone tautomers) and cationic forms, respectively.[40] The species of poly(Al-Flu-*co*-AM) present are shown in Scheme 2. The absorption and fluorescence spectra of poly(Al-Flu-*co*-AM) in water (1.2×10^{-2} g/L) at various intervals of pH are shown in Figures 6 and 7, respectively.
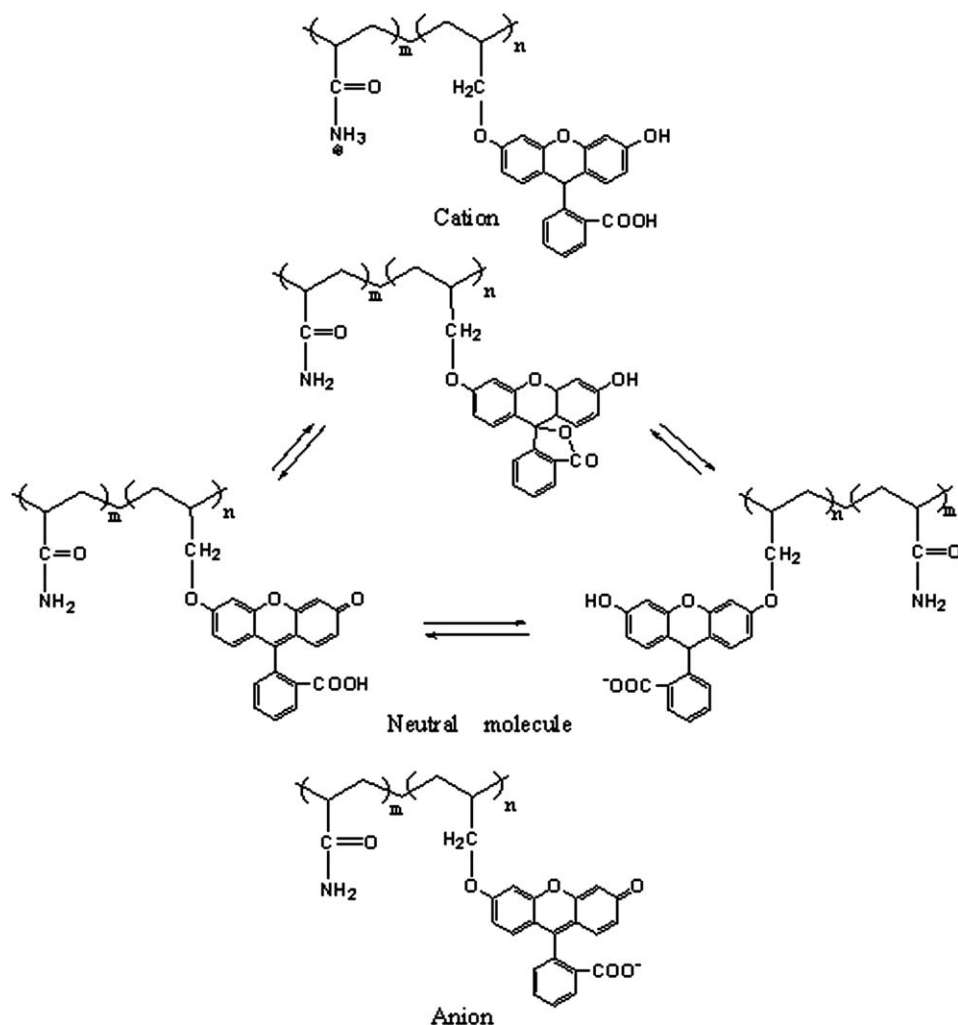
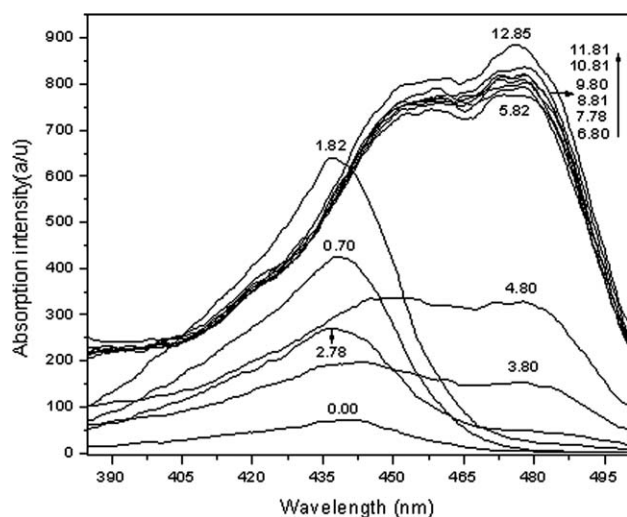**Scheme 2** Species of poly(Al-Flu-*co*-AM) present at different pHs.

Figure 6 Absorption spectra for 1.2×10^{-2} g/L poly(Al-Flu-co-AM) in aqueous solution at the different pHs from 0.00 to 12.85. ($\lambda_{em} = 517$ nm).

From Figure 6, it was apparent that the Al-Flu exhibited two distinct bands around 435 and 480 nm in acidic and basic media, respectively. Consistent with earlier findings for fluorescein,[40] the alteration of the aqueous pH resulted in the transformation of the anion ($\lambda_{max} = 490$ nm) to the neutral form of the conjugates (with the development of two maxima at ca. 455 and 475 nm). Further acidification gave a cationic species ($\lambda_{max} = 440$ nm), for which there was diminished absorption at 440–490 nm. The absorption data for poly(Al-Flu-co-AM) were, thus, quite similar to those obtained for fluorescein; this indicated that the configuration of the species of the substituent was similar to those of fluorescein.
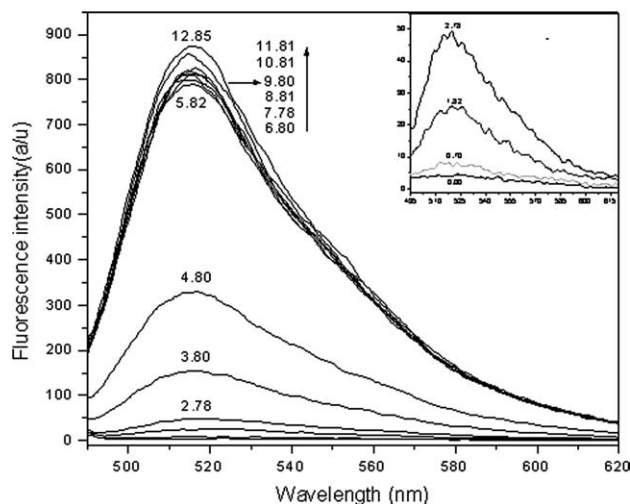

Figure 7 Fluorescence spectra for 1.2×10^{-2} g/L poly(Al-Flu-co-AM) in aqueous solution at different pHs from 0.00 to 12.85. Inset: fluorescence spectra for it at different pHs of 0.00, 0.70, 1.82, and 2.78 [λ_{ex} (excitation wavelength) = 476 nm].
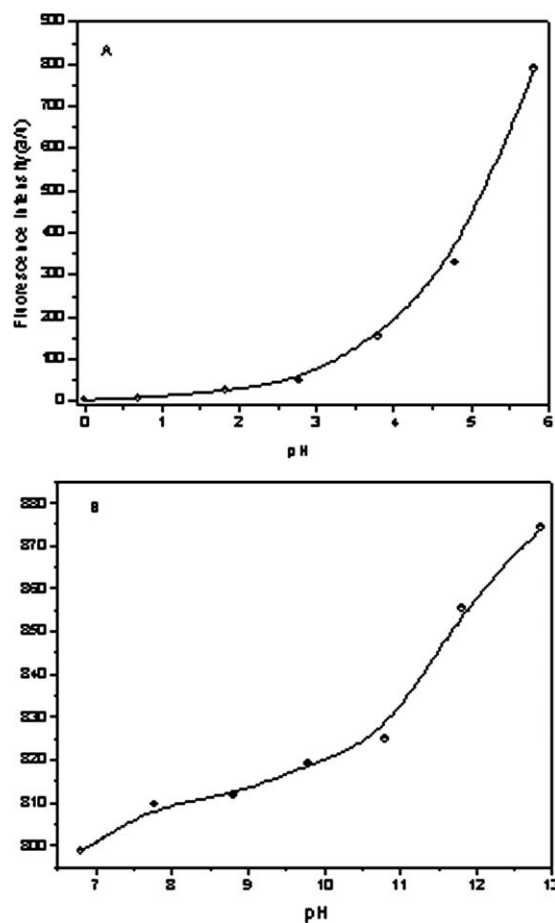
Figure 8 Nonlinearity of the fluorescence intensity of poly(Al-Flu-co-AM) versus pH: (A) nonlinearity of the fluorescence intensity in acidic medium (pH 0.00–5.82) and (B) nonlinearity of the fluorescence intensity in neutral and basic acidic medium (pH 6.80–12.85).

When excited at 476 nm, poly(Al-Flu-co-AM) showed nearly a complete loss of emission at very low pH (Fig. 7); this was consistent with the earlier findings that the fluorescence of cationic forms of fluorescein was inefficient.[40] On the contrary, the fluorescence intensity rapidly increased when the pH was higher than 5 (Fig. 8). The result could be interpreted that for the anion form of the Al-Flu, the fluorescence quantum yield was higher than those of its cation and neutral molecule. It also showed that poly(Al-Flu-co-AM) largely preserved the well-known pH dependence of the fluorescence of fluorescein, which is an advantage for probing pH.

CONCLUSIONS

An allyl monomer bearing fluorescein groups and Al-Flu was easily synthesized in this study, and then, poly(Al-Flu-co-AM) was synthesized by a thermal initiator. The mass concentration of fluorescein chromophore in the water-soluble copolymer was 5.01%, and T_g of poly(Al-Flu-co-AM) (186.1°C) was

higher than that of PAM (175.3°C). The pH and temperature dependence of fluorescence of the soluble poly(Al-Flu-*co*-AM) were investigated in detail. It was found that poly(Al-Flu-*co*-AM) had an excellent linear response between the relative fluorescence intensity and temperature in the range 0–60°C, and the linear regression equation of the calibration graph was Flu = 740.06–3.0683t, with a correlation coefficient of linear regression of 0.9974. In addition, poly(Al-Flu-*co*-AM) largely preserved the well-known pH dependence of the fluorescence of fluorescein, which resulted from the fact that the predominance of species of Al-Flu changed at different pHs. Therefore, the synthesized copolymer bearing Al-Flu could potentially be used as fluorescent and multifunctional material in the future, such as a fluorescence temperature sensor, fluorescence pH sensor, or water-soluble polymer luminescence material.

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