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Synthesis of water-soluble luminescence polymer and potential application as a bifunctional probe

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At first, an unsaturated monomer bearing luminescence group, acryloylfluorescein was synthesized from fluorescein and acryloyl chloride by simple method and was identified by IR, MS and ^{1}H NMR spectra. The homopolymer of monomer was obtained with solution polymerization and the average molecular weights obtained was not high and the weight average molecular weigh (M_w) was 1.93×10^4. The water-soluble copolymer of acryloylfluorescein and acrylamide, poly(acryloylfluorescein-co-acrylamide), was synthesized with thermal initiator and was characterized by the method of IR, UV-vis and DSC. The luminescence behaviors of monomer and polymers were explored and the pH and temperature dependence of fluorescence of copolymer were investigated in detail. The results showed that copolymer had an excellent linear response between relative fluorescence intensity and temperature in the range of 0-60 °C and had a nonlinear response from pH 4.7 to 9.8 between relative fluorescence intensity and pH. Thus, the copolymer was promising polymeric materials for bifunctional probe for temperature and pH based on fluorescence.

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1. Introduction

Luminescent polymer is a kind of functional polymer with wide research and application. Since the first fluorescent material composed of polymer as a substrate was reported [1], fluorescent polymer has attracted much attention and various new materials have been prepared [2-8]. Recently, more interests have developed in the synthesis of polymeric optical probe for temperature or pH because there is a higher sensitivity to detect temperature and pH using the fluorometric determination and the geometrical design of fluorescent detector is more flexible [9-12]. Moreover, it is convenient for the research of fluorescent micrology and the real-time detecting of dynamic distributing and region change in living cell to detect the temperature and pH using the change of different fluorescent parameters of fluorescent molecule, such as fluorescent intensity and fluorescence lifetime. However, many optical probes are usually obtained by adding functional dyes to polymer supports such as cellulose or glass. As a result, fluorescent molecules easily fall out and their consistency property with substrate is not good in applications, which badly affects the stability of instruments. Compared with them, making fluorescent molecules an integral part of polymers with excellent stability and ability to form very homogeneous films through covalent binding is an excellent project to avoid those limitations, which is an important step in the design of fluorescent materials.

Fluorescein is widely employed as a platform for various florescence probes and fluorescence labels because of its high fluorescence quantum efficiency (Φ_{fl}) in aqueous media and both its excitation wavelength and emission wavelength are in the range of visible region, which is beneficial for its detection [13-15]. Among many fluorescein derivatives, the acrylic derivatives provided a convenience for acryloylfluorescein to homopolymerize or copolymerization with various monomers, such as crylic acid, styrene and acrylamide, through which fluorescein could be attached to macromolecules. Polyacrylamides are amorphous and water-soluble polymers with attractive properties for applications in membrane technology and biomedical devices [16, 17]. Due to the fluorescent characters of fluorescein and the superiority of polyacrylamidse as luminescentless matrices when excited by radiation of wavelengths from 260 to 500 nm, the copolymerization of the dye and polyacrylamides would resulted in the development of new multifunctional materials that find applications in many areas of optics and material science.

In our previous works, different polymeric pH indicators were first synthesized by the reaction of 3-epoxypropoxy fluorescein and polymers under alkaline conditions by a convenience method [18-21]. For this kind of polymeric pH indicators, fluorescein was covalently bound to the surface of polymer. However, we found that it was difficult to control the content of fluorescence group in side-chain of polymer, and the reaction point was in complete disorder because dyes were covalently bound to the surface of polymer.

In this work, we attempted to use the polymerization method to overcome these disadvantages. We synthesized acryloylfluorescein, homopolymer of acryloylfluorescein and copolymer of acryloylfluorescein with acrylamide, respectively. The fluorescence behaviors and the temperature/pH dependence of fluorescence of the soluble copolymer were investigated in detail. This investigation may provide another convenient way to prepared multifunctional polymeric material bearing fluorescein to probe pH and temperature.

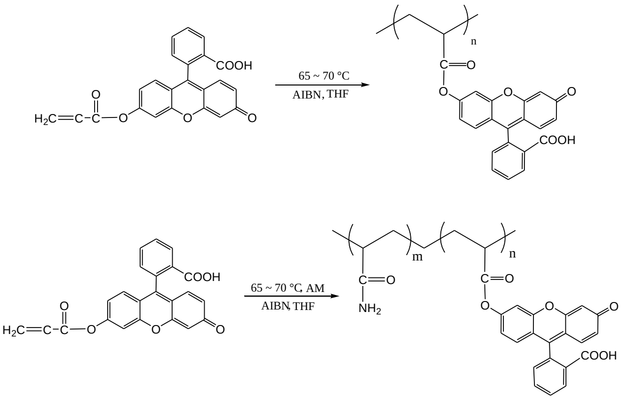
2. Experimental section

2.1 Materials

Fluorescein and acrylamide, purchased from TianJin chemistry reagent company in China, were used after further purification; Triethylamine (TianJin chemistry reagent company) was refluxed with acetic anhydride, then distilled, refluxed with KOH, and again distilled. Azo-bis-isobutyronitrile (AIBN) was recrystallized from ethanol. Organic solvents, such as dichloromethane (CH_2Cl_2) and ethanol were dried and distilled before being used. Other reagents were all analytical pure and the water used in experiment was distilled twice.

2.2 Synthesis of monomer

Acryloylfluorescein was prepared by the reaction of fluorescein and acryloyl chloride in the presence of triethylamine. A mixture of 20 ml of CH_2Cl_2, 3.0 g (9.0×10^{-3} mol) of fluorescein and 5.0 ml of triethylamine was put into 100 ml three-necked round bottom flask equipped with thermometer, a condenser, a dropping funnel, and a magnetic stirring bar. The flask was then maintained at 0 °C, while 1.0 g (1.1×10^{-2} mol) acryloyl chloride dissolved in 20 ml dry CH_2Cl_2 was added with a dropping funnel for 30 min. The reaction mixture was stirred at 25 °C for 24 h. Evaporation removed the solvent and the crude product was finally purified by silicagel chromatography ($CHCl_3$, $CHCl_3$/ethanol 30/1) for monomer. The chromatography furnished about 2.5 g of an orange colored solid that could be dissolved in CH_2Cl_2, acetone, methanol, DMF and other organic solvents. Yield ≈ 72 %. Mp: 116 - 118 °C. IR (KBr), $\tilde{\nu}$ /cm^{-1}: 1768 (C=O, carboxy); 1744 (C=O, ester); 3075, 829 ($C=CH_2$); 3023, 991 (=C-H); 1611, 1583, 1495, 760, 798 (aromatic C=C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 6.042 – 6.078 (m, 1, = CH); 6.278 - 6.533 (m, 2, $=CH_2$), 6.649 – 8.047 (m, 10, benzene ring). MS, m/z calculated for molecular ＝ 386; found: 387（M+1）$^+$. Visible spectra (λ_{max}, nm) 280, 458, 492, 530 nm.



Scheme 1. Preparation of homopolymer and copolymer.

2.3 Thermal polymerization of acryloylfluorescein

The thermo-polymerization was carried out at 65 °C by using AIBN as an initiator (scheme.1). A solution of 0.5 g (1.3×10^{-3} mol) Acryloylfluorescein and 3.5×10^{-3} g (2.1×10^{-5} mol) of AIBN in 10 ml dry tetrahydrofuran (THF) was introduced into a dry polymerization tube. The solution was deoxygenated by purging with purified N_2 gas. The tube was sealed and place in a regulated thermostat bath at 65 °C for 8 h. The solution was allowed to precipitate in excessive methanol and precipitates was collected by filtration and dried under vacuum to constant weigh. The homopolymer obtained was identified by IR and GPC.

2.4 Copolymeration of acryloylfluorescein and acrylamide

The poly (acryloylfluorescein-co-acrylic amide) was prepared by the copolymerization of acryloylfluorescein and acrylamide with AIBN by similar methods as the thermal polymeration of homopolymer (scheme.1). A solution of 3.5×10^{-2} g (1.4×10^{-4} mol) monomer, 0.5 g (7.0×10^{-3} mol) acrylamide and 1.9×10^{-5} mol of AIBN in 10 ml 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 place in a regulated thermostat bath at 65 °C for 10 h. The solution was allowed to precipitate in excessive methanol and precipitates was collected by filtration and dried under vacuum to constant weigh. The copolymer obtained in this way was used for further fluorescent test. The mass concentration of acryloylfluorescein in the copolymer was 7.55 % via the ultraviolet spectrophotometry. The polymer obtained was identified by IR, UV and DSC. T_g = 190 °C. UV spectra (λ_{max}, nm) 280, 455, 528 nm.

2.5 Instrumentation

^{1}H NMR experiments were performed on AM-400 MHz BB Bruker for the monomer in $CDCl_3$. MS was performed on ZAB-HS. IR spectra were recorded on a Nicolet Neus 670 FI-IR spectrophotometer. UV-visible spectra were taken using on Lambda 35 UV/Vis Spectrometer (Perkin Elmer) for measuring the mass concentration of acryloylfluorescein in copolymer. DSC measurements were performed at a heating rate of 10 °C min^{-1} under nitrogen with a Sapphire DSC Differential Scanning Calorimeter (Perkin Elmer). Fluorescent excitation and emission spectra were measured with an LS 55 Luminescence Spectrometer (Perkin Elmer). Average molecular weight was determined by gel permeation chromatography (GPCV2000; Waters, THF). The pH measurements were performed with pH-3B (made in ShangHai, China).

2.6 Fluorescence methods

For fluorescence emission measurements, a 10 ×10 mm quartz cell was used for detection. The temperature effect on the fluorescence yield of copolymer was determined by measuring the variation of the fluorescence intensity, at the peak wavelength, applying a relation 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 and the temperature range of the sample was changed from 274 to 333 K by a digital temperature controller.

Fluorescence intensities against pH were obtained by recording the emission in solutions with different pH at fixed wavelength. During the measurement, a little HCl and NaOH were used to adjust pH to the desired value and samples were carried out 25°C. The pH range of the sample was changed from 4.7 to 9.8 by a digital pH controller.

3. Results and discussion

3.1 Characterization of polymers

3.1.1 Characterization of homopolymer

The homopolymer was characterized by the disappearance of the stretching and bending absorption bands caused by terminal vinyl, such as at 3023 and 829 cm^{-1}, and by the weakening band at 3075 and 991 cm^{-1}.

Molecular weight of homopolymer obtained in this way was not high and the weight average molecular weigh was 1.93×10^{4}. This is probably attributed to the steric hindrance of acryloylfluorescein moiety attached to an acryloyl group.

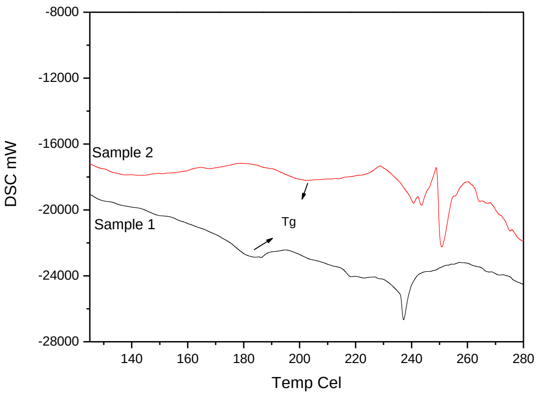


Fig. 1. DSC curves of polyacrylamide (sample1) and copolymer synthesized (sample2).

3.1. 2 Characterization of copolymer

The copolymerization of acryloylfluorescein and acrylamide was also carried out with thermal initiator AIBN. The copolymer was identified by the characteristic absorption band at 3357 (-NH$_2$), 2931, 1450 (-CH$_2$), 1750, 1666 (C=O) and 1497, 763 cm^{-1} (benzene ring), and the disappearance of the stretching and bending absorption bands caused by terminal vinyl, such as at 3075, 996 and 829 cm^{-1} on its IR spectrum. The T_g of copolymer is at near 190 °C and is higher than that of polyacrylamide (175 °C) in Fig. 1, which is due to the rigid structure of acryloylfluorescein which affected the move of main chain of polyacrylamide. The differences of T_m also proved the effect of chromophore.

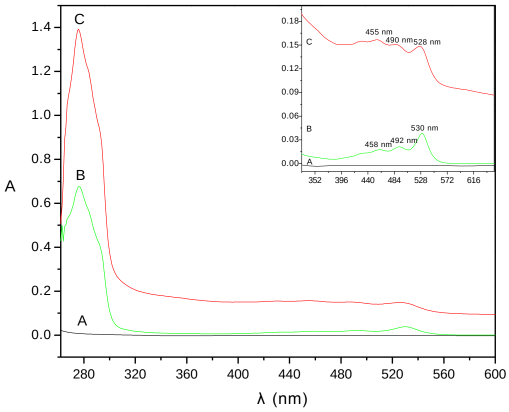


Fig. 2. Absorption spectra of polyacrylamide (A), acryloylfluorescein (B) and copolymer (C) in DMF.

On the UV–Vis spectra in Fig. 2, it was obvious that polyacrylamide was no optical absorption from 260 to 600 nm and the absorption peaks at 280, 455, 490 and 528 nm of copolymer were essentially identical with those observed for the acryloylfluorescein at 280, 458, 492 and 530 nm in the ultraviolet region. Apparently, the absorption of copolymer was because of the chromophore on polyacrylamide and the matrix did not change the optical character of monomer. Therefore, this made it possible to determine the DS of chromophore in the copolymer by using acryloylfluorescein as a standard reference, assuming that the extinction coefficients of polymer and monomer were similar.

3.2 Fluorescent properties of copolymer in solution and film

Thin film of polymer was prepared on glass plates by solvent casting. Absorption and emission spectra for the copolymer in aqueous solution (A) and in film (B) were shown in Fig. 3. Apparently, the fluorescence spectra of polymer resemble the mirror image of the absorption spectrum, which suggested that the emission at 517 nm arose from fluorescein chromophore [22, 23]. Moreover, a new absorption band appeared at short wavelength due to the H-aggregate of chromophore and it displayed typical red-shift of emission maximum in the visible region in film [24, 25]. The broad bands at 522 nm (B) could be ascribed to excimers between the pendant fluorescein moieties [26]. Accordingly, the shorter (in solution) and longer (in film) wavelength bands were identified as the monomer and excimer emissions, respectively.

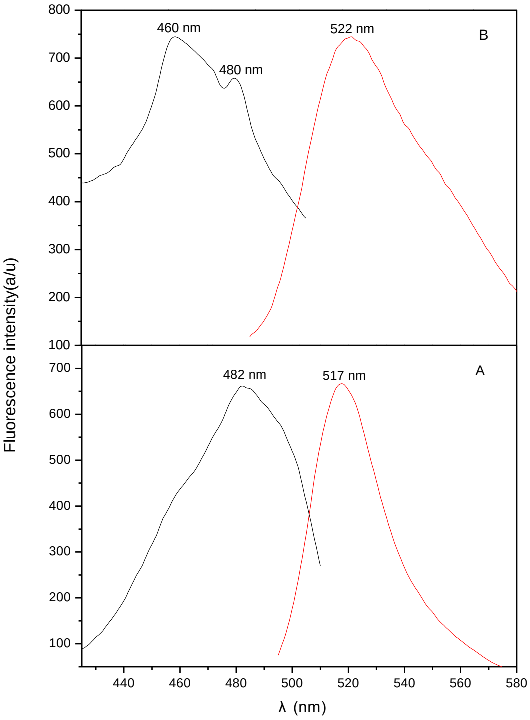


Fig. 3. Absorption spectra and fluorescence spectra of copolymer (A) 5×10^{-3} g l^{-1} in aqueous solution and (B) in film (1.0 %, 0.013 mm).

3.3 Temperature dependence of the fluorescence intensity of copolymer

Fig. 4 showed the fluorescence spectra of copolymer in water (5×10^{-3} g l^{-1}) at several temperatures. These spectra exhibited a red shift of their maximum on heating that is attributed to the inability of solvent reorientation during the lifetime of the electronic excited state (1-5 ns) [27]. The solvent reorientation depends on temperature. At lower T, the ability of solvent reorientation was weak and the state energy difference remains large for the emission transition while at higher T reorientation does occur, which leads to a reduction of the energy difference and a red shift.

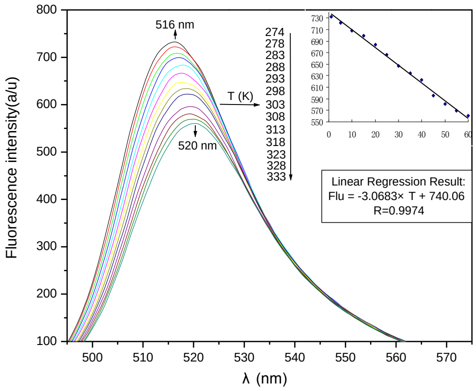


Fig. 4. Fluorescence spectra for 5×10^{-3} g l^{-1} copolymer in water at the different T from 274 to 333 K (λ_{ex} = 485 nm). Inset: the linearity of the fluorescence intensity vs temperature.

It was showed that the maximum fluorescence intensity of polymer decreases on heating in Fig. 4. The temperature dependence of the fluorescence intensity could be discussed in terms of both photophysical and photochemical dye properties and polymer matrix relaxation processes [28].

Take into account the photophysical and photochemical properties of dye, the fluorescence intensity of copolymer was mainly controlled by the radiationless temperature dependent process. Under steady-state conditions and in the absence of either added quenchers or a photochemical process, the fluorescence quantum yield, Φ_{FL}, of a dye molecule in a homogeneous medium, can be described by a general and theoretical equation:

$$\Phi_{FL} = k_{FL}/\{ k_{FL} + k_{IC} + k_{ST} + k_{DM}[FL] + k_{MT}[^3FL]^2 \}. \quad (3)$$

where the rate constants, k, are defined for: fluorescence emission (k_{FL}); radiationless internal conversion (k_{IC}); intersystem crossing (k_{ST}); quenching by collisional and Forster mechanisms (k_{DM}); and triplet-triplet annihilation (k_{MT}), resulting in fluorescence quenching [29]. In a previous study of the fluorescein dyes in aqueous solution, k_{IC} varies between 273 K and 323 K according to [30]:

$$k_{IC} = 2.0 \times 10^{11} \exp [(-5.5\text{kcal/mole})/RT] s^{-1}. \quad (4)$$

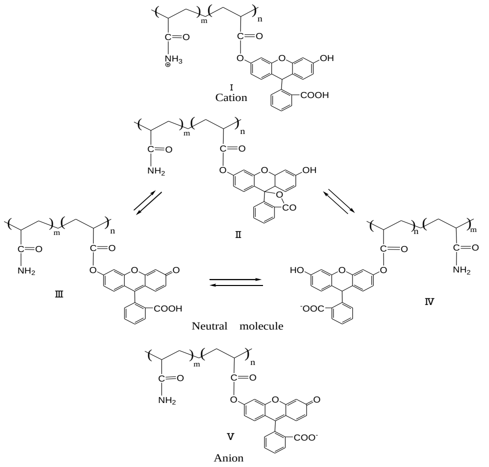
The good linearity observed for the variation of log k_{IC} versus 1/T indicates that there was only one radiationless deactivation channel. Moreover, the collisional probability between molecules, resulting in fluorescence quenching, and the power of intersystem crossing were reinforced with the increasing temperature. At the same time, uniting equ.(3) and (4), we could also easily conclude that the Φ_{FL} of dye molecule decreases on heating.

Polymer relaxation can also influence the fluorescence intensity of dye molecule. Theoretically, the self-quenching process, which was produced by the interaction between neighbor dye molecules or the dimmer configuration, would be reduced if the molecular diffusion induced by the polymer relaxation procession. However, the increasing fluorescence intensity on heating was not observed in our experiment. Maybe, it was because that macromolecular motion was not large enough to separate the molecules forming high order aggregates of acryloylfluorescein because of the high concentration of fluorescein chromophore in copolymer (≈ 7.5 %). Moreover, D. Dibbern-Brunelli et al had concluded that the photobleaching yield of a dye increased with the increase in the mobility of the polymer chains and the hydrogen bonds destroyed in this process can change both the photophysical and photochemical processes of the molecules in the electronically excited state [22]. So, the decreasing fluorescence intensity observed around 274-333 K should be a result of a convolution among photophysical processes dependent on concentration, bimolecular quenching, unimolecular rate processes and photochemical processes decreasing the dye concentration and mobility of polymer chain.

In Fig. 4, the excellent linear relationship was obtained between relative fluorescence intensity and temperature in the range of 274 - 333 K. The linear regression equation of the calibration graph was Flu = 740.06 – 3.0683 T (Flu was relative fluorescence intensity), with a correlation coefficient of linear regression of 0.9974. Obviously, the polymer had promise as an optical indicator for temperature determination and was potential for determining temperature change real time.

3.4 pH dependence of the fluorescence intensity of copolymer

The absorption and fluorescence spectra of copolymer in water (1.5×10^{-3} g l^{-1}) at various intervals of pH (4.7~9.8) are shown in Fig. 5 and Fig. 6, respectively. From Fig. 5, it was apparent that the absorption intensity increased with the increasing of pH, which was due to the various existent form of acryloylfluorescein at different pH. The various states of protonation and tautomeric form of xanthenes are well known [31]. The maximum in the absorption of xanthenes and its derivatives depended on the species present and hence on the pH [32]. For fluorescein, pK_a values of 6.4, 4.3, and 2.1 have been determined for the sequence of protonations of the anion to neutral (zwitterionic and lactone tautomers), and to cationic forms, respectively [33]. The species of copolymer present are shown in scheme 2. The absorption data for copolymer were thus quite similar to those obtained for fluorescein, indicating that the configuration of species of the substituent was similar to those of fluorescein.



Scheme 2. The species of copolymer present at different pH.

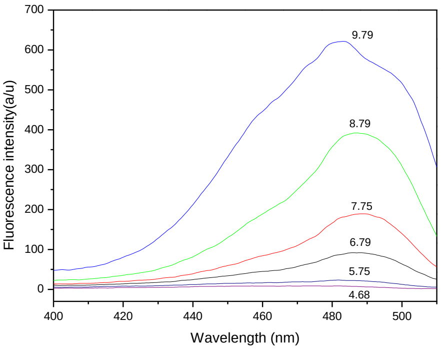


Fig. 5. Absorption spectra for 1.5×10^{-3} g l^{-1} copolymer in aqueous solution at the different pH from 4.7 to 9.8 (λ_{em} = 516 nm).

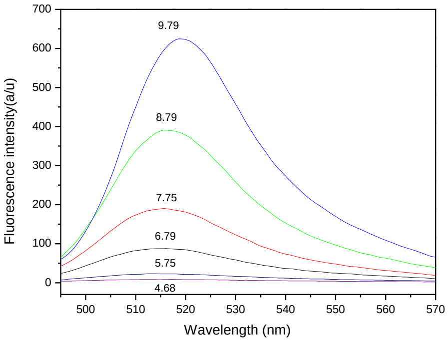


Fig. 6. Fluorescence spectra for 1.5×10^{-3} g l^{-1} copolymer in aqueous solution at the different pH from 4.7 to 9.8 (λ_{ex} = 485 nm).

Excitated at 485 nm, the fluorescence intensity of polymer increased with further basification (Fig. 6). The conjugates showed loss of emission at weak acidic environment, consistent with the earlier findings that the fluorescence of neutral and cationic forms of fluorescein was inefficient [33]. On the contrary, the fluorescence intensity rapidly increased when the pH higher than 7. The result could be interpret that, for the anion form of the acryloylfluorescein, the fluorescence quantum yield was much higher than those of its cation and neutral molecule. It also showed the copolymer largely preserved the well known pH dependence of the fluorescence of fluorescein which was an advantage to probe pH.

4. Conclusions

Acryloylfluorescein was easily synthesized in the work. Its homopolymer was synthesized by thermal initiator and the molecular weight was not high and the weight average molecular weigh was 1.93×10^4. The water-soluble copolymer of acryloylfluorescein and acrylamide was also synthesized by thermal initiator. The mass concentration of fluorescein chromophore in the copolymer was 7.55 % and the T_g of copolymer was higher than that of homopolymer of acrylamide. The pH and temperature dependence of fluorescence of the soluble copolymer were investigated in detail. It was found that copolymer had an excellent linear response between relative fluorescence intensity and temperature in the range of 274-333 K and the linear regression equation of the calibration graph was Flu = 740.06 – 3.0683 T, with a correlation coefficient of linear regression of 0.9974. In addition, the copolymer largely preserved the well known pH dependents of the fluorescence of fluorescein, which results from the fact that the predominance of species of fluorescein changed at different pH. Therefore, the copolymer synthesized is potential to be used as fluorescent and multifunctional material in the future, such as fluorescence temperature sensor, fluorescence pH sensor and water soluble polymer luminescence materials.

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