Preparation and Photophysical Behaviors of Fluorescent Chitosan Bearing Fluorescein: Potential Biomaterial as Temperature/pH Probes

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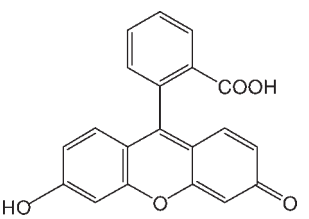
ABSTRACT: A novel method for attaching fluorescein (via its epoxy derivative) to water-soluble chitosan, and the temperature/pH-sensitive qualities of fluorescence were investigated. 3-Epoxypropoxy fluorescein (EPF) was firstly synthesized by the reaction between fluorescein and epichlorohydrin, and then water-soluble chitosan bearing fluorescein (CS-EPF) was prepared via ring-opening reaction with EPF. They were characterized by the methods of ^{1}H NMR, MS, IR, UV–Vis, and luminescence spectra, respectively. The chemiluminescent and photophysical behaviors of EPF and CS-EPF were studied in detail. The results showed that the fluorescent chitosan could still provide temperature and pH sensitivities similar to that of fluorescein, achieving better long-term stability and fast equilibrium response. CS-EPF had an excellent linear response between relative fluorescence intensity and temperature in the range of 0–60°C and two linear relationships between relative fluorescence intensity and pH in 0.0–4.14 and 8.15–13.20, respectively. This investigation may provide a convenient way to prepare low-cost and multifunctional macromolecule biomaterial to probe pH and temperature in biological systems. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 3960–3966, 2007

Key words: 3-epoxypropoxy fluorescein; chitosan; fluorescence; temperature/pH sensitivity

INTRODUCTION

A fluorescent material composed of polymer as a substrate was firstly reported by Wolf and Pressley in 1963.[1] Since then, polymers functionalized by chromophores have attracted much attention and recently various new materials such as poly(vinylcarbazol)[2] and rhodamine 6G-tagged poly (vinyl alcohol) have been prepared.[3] Fwu-Long Mi synthesized a new fluorescence biomacromolecule chitosan–gelatin[4]; Manuela Melucci et al. reported the photoluminescence properties of poly (α-vinyl-ω-alkyloligothiophene) side-chain polymers in solution and in thin film.[5] These polymers have potential applications as photoconductive resins, fluorescent probes, photosensitive crosslinking materials, electroluminescence materials, and photon harvesters.[6–9] For instance, the research of spectral behaviors of dye molecules in polymers would help us to understand the excitonic interaction between chromophores (pigment or dye molecules), which is essential to construct artificial antenna system.[10,11] In addition, Regien et al. indicated that the use of polymer matrices can avoid clustering of the dye molecules to aggregate the chromophores, such as chlorophylls or porphyrins, often displayed in water and organic solvents.[12] These polymers containing chromophores of push–pull type may also have application in nonlinear optics.[13]

Fluorescein (FL) is widely employed as a platform for various fluorescence probes and fluorescence labels because of its high fluorescence quantum efficiency (Φ_{FL}) in aqueous media, and both its excitation and emission wavelengths are in the range of visible region, which is beneficial for its detection.[14–17] The chemical structure of FL (1) is shown below:



The free dye, or dye in derivatized form, is widely used as a tracer in the labeling of proteins.[18] Recent applications employ it to probe the structure of cells or to measure pH at the interface of surfaces at which dye was immobilized.[14,19] The most commonly used derivatives include the carboxy-fluoresceins, the aminofluoresceins, the fluoresceins isothiocyanates, and

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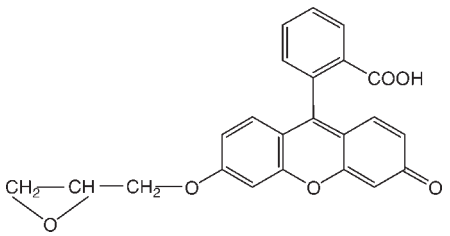
the aminomethylfluoresceins.[20,21] However, there were few reports on the application of epoxyfluorescein. The epoxy group provided a convenient site to react with many nucleophilic groups, such as OH—, NH_2—, COO—, and CN—, through which FL could be easily attached to macromolecules.[22] Moreover, we found that 3-epoxypropoxy fluorescein (EPF) largely preserved the well known temperature and pH dependences of the fluorescence of fluorescein. The chemical structure of EPF (2) is shown below:



Chitosan, a fully or partially deacetylated product of its parent polysaccharide chitin, has attracted significant interest in the broad range of scientific research, including biomedical, agriculture, pH sensitive drug delivery, and environmental protection fields, because of its biocompatibility and bioactivities.[4,23,24] Chitosan is hydrophilic and a large number of its hydroxyl and amino groups provide the sites for numerous attractive chemical modifications. Fluorescent natural polymers (e.g., natural polymers containing various chromophores) for optical study have been gaining attention recently.[25–27] But, to our knowledge, there is no report about the temperature and pH sensors from chitosan via fluorescent techniques.

The target of this article was mainly to successfully synthesize a kind of temperature/pH-sensitive fluorescent macromolecule biomaterial containing FL via chemical reaction and study its photophysical behaviors and temperature/pH-sensitive quality in detail.

MATERIALS AND METHODS

Materials

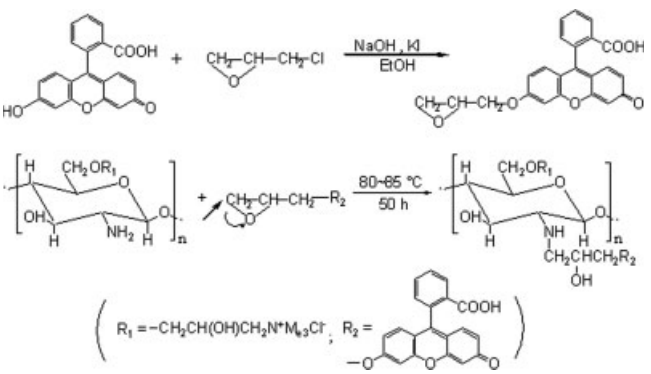
Chitosan (deacetylation degree of 95%), supplied from Yuhuan Chemicals Factory, was used after being dried at 100°C for 24 h. Fluorescein purchased from LanZhou Chemistry and Physics Institute was purified by crystallization from an ethanol solution. Water-soluble chitosan (CS) was prepared by following the literature procedures.[28] Organic solvents, such as dimethyl sulfoxide (DMSO) and ethanol, were distilled before being used. All other chemicals were reagent grade and were used as received. Doubly distilled water was used in all the experiments.

Preparation of EPF

In a 100 mL three-neck round-bottom flask fitted with a condenser, KI (1.66 g, 0.01 mol) and epichlorohydrin (1.0 mL, 0.01 mol), which dispersed in 15 mL anhydrous alcohol, were added and stirred at 50–60°C for 0.5 h. After the addition of fluorescein (0.65 g, 0.002 mol) and NaOH (0.05 g, 0.001 mol) dispersed in alcohol, the reaction mixture was stirred under N_2 atmosphere for 12 h at 60–65°C. The solution was distilled to eliminate alcohol, and the desired product was isolated by sequential column chromatography (50 : 1, $CHCl_3$: C_2H_5OH as eluent, used silica gel 200–300 mesh). The chromatography furnished about 0.2 g of an orange-colored solid that could be dissolved in alcohol, acetone, DMSO, and other organic solvents, as illustrated in Scheme 1. This material reacted with the CS, (Scheme 1). Yield $\approx$ 32%, T_m = 146°C. IR spectra of product (KBr): 1718 (C=O); 1645, 1598, 1514, 1453 (aromatic C=C); 1209, 1070(C—O); ^{1}H NMR (300 MHz, CD_3OD), δ: 1.2–1.4 (s, ring —CH_2—, 2H), 3.9–4.1 (m, epoxy $CHCH_2$, 3H), 4.3(m, —CH_2O, 2H), 6.2–8.2 (m, aromatics, 10H). MS, *m/z* calculated for molecular = 389, $(M + 1)^+$; found, 389, $(M + 1)^+$. Visible spectra (λ_{max}, nm) 385, 456, 475, $\Phi_{epf} \approx 0.28$.

Preparation of water-soluble chitosan bearing fluorescein

The following protocol was based on a procedure described by Eugene et al.[29] CS (0.3 g), dispersed in DMSO (10 mL) for 24 h at 80°C, was added in a 100 mL round-bottomed flash, and EPF (0.3 g) dissolved in DMSO was slowly added dropwise into it under N_2. The reaction mixture was stirred for 50 h at 80–85°C. The bisque solution was allowed to precipitate in excessive alcohol to provide brown deposit matter. After centrifugation, the solid was rinsed with plenty of alcohol to remove free EPF. The powder was then put into a Soxhlet's extractor and extracted with alcohol for at least 12 h to ensure that there was noncovalently bounded EPF in chitosan. The desired product was ultimately synthesized via



Scheme 1 Synthesis of fluorescent chitosan.

vacuum drying as illustrated in Scheme 1. The mass concentration of EPF in CS-EPF was 1.2% via ultraviolet spectrophotometry.

Preparation of CS-EPF films

CS-EPF (0.02 g) was dissolved in distilled water (1.00 mL) to prepare 2.0% aqueous solution and it was poured on level glass plates with a dimension of 3.8 × 1.3 cm, which was kept for 1–2 days at ambient temperature to obtain transparent CS-EPF film. The thickness of film was 0.013 mm and it was stored in ambient atmosphere for fluorescent measure.

Analyses and characterizations

^{1}H NMR experiments were performed on 300 MHz BB Bruker for the EPF in CD_3OD. MS was performed on ZAB-HS. IR spectra were recorded on a Nicolet Neus 670 FTIR spectrophotometer. UV–visible spectra were taken using on Lambda 35 UV/Vis Spectrometer (Perkin-Elmer) for measuring the mass concentration of EPF in CS-EPF. Fluorescent excitation and emission spectra were measured with an LS 55 Luminescence Spectrometer (Perkin–Elmer) in water, and DMSO solutions or in solid film. The pH measurements were performed with pH-3B (made in ShangHai, China).

Fluorescence methods

For fluorescence emission measurements, a 10 × 10 mm^2 quartz cell was used for detection, and samples were excited at the blue edge of the visible absorption band of FL (~ 480 nm). The quantum yield of EPF was determined using the expression:

$$\frac{Q_{F(\text{sample})}}{Q_{F(\text{fluorescein})}} = \frac{I_{(\text{sample})}}{I_{(\text{fluorescein})}} = \frac{E_{(\text{fluorescein})}}{E_{(\text{sample})}} \quad (1)$$

where Q_F = quantum yield ($Q_{F(\text{fluorescein})} = 0.93$),[30,31] I 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 CS-EPF 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, with the temperature range of the sample changed from 273 to 333 K by a digital temperature controller.

Fluorescence intensities against pH were obtained by recording the fluorescence emitted in solutions with different pH at a fixed wavelength. During the measurement, HCl and NaOH were used to adjust pH to the desired value, and samples were carried out at room-temperature. The pH range of the sample was changed from 0.0 to 13.2 by a digital pH controller.

RESULTS AND DISCUSSION

Structural characterizations of CS-EPF

Some alterations were found in FTIR spectrum of CS-EPF compared with that of CS (Fig. 1). The wide absorption band at 3400 cm^{-1}, corresponding to the stretching vibration of NH_2 group and OH group, showed an obvious shift to higher wavenumber (3418 cm^{-1}). New bands at 1734 and 950 cm^{-1}, characteristic of the carboxyl C=O stretching vibration of EPF, and 1028 cm^{-1}, characteristic of the C—N stretching vibration of secondary amine, were found. These results indicated that fluorescein moiety was introduced into CS. However, the absorbed band at 1630 cm^{-1} characteristic of the —NH_2 deformation vibration of CS scarcely changed when compared with the spectrum of unmodified chitosan. It may be interpreted that the loading of the dye in chitosan was not high, and large numbers of —NH_2 still existed on CS-EPF.

The UV spectrum of CS-EPF [Fig. 2(C)] was similar to that of the EPF [Fig. 2(B)] from 400 to 520 nm,

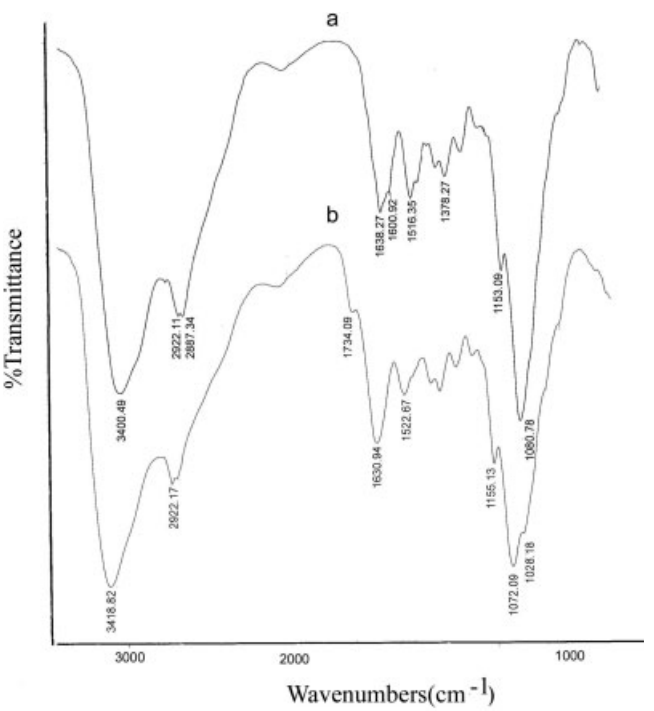


Figure 1 IR spectra of water-soluble chitosan (A) and CS-EPF (B).

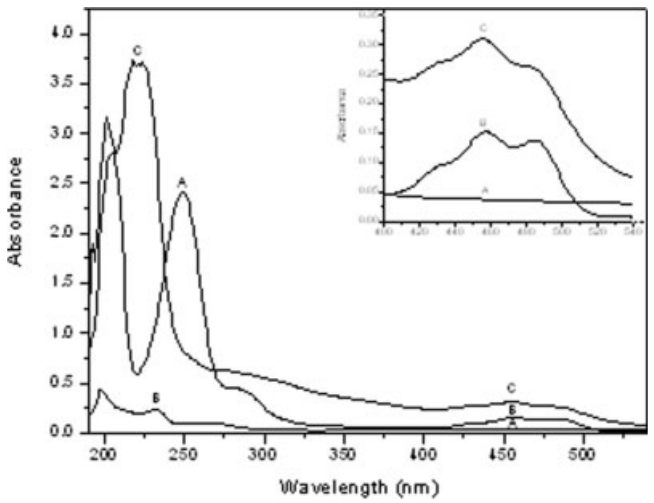
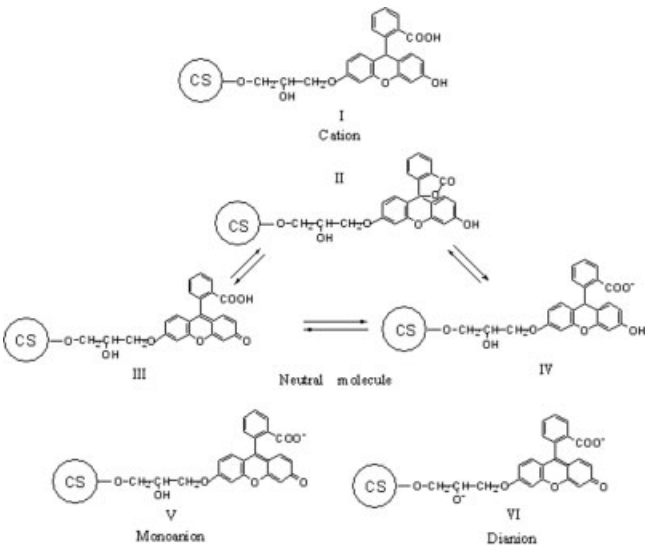


Figure 2 UV spectra of water-soluble chitosan (A), EPF (B), and CS-EPF (C) in aqueous solution. Inset: Absorption spectra in the range of 400–540 nm.



Scheme 2 The possible species of CS-EPF present at different pH (CS: water-soluble chitosan).

but underivatized chitosan had no UV adsorption in this region [Fig. 2(A)]. The λ_{max} of CS-EPF (460 nm) was very close to that of EPF, enabling to determine the degree of substitution (DS) of CS-EPF by using EPF as a standard reference, assuming that the extinction coefficients of CS-EPF and EPF were similar. Moreover, the absorption peak at 255 nm shifted to 225 nm and the peak at 205 weakened sharply after CS modified with EPF. The result showed that fluorophore covalently bound to chitosan might affect the UV adsorption of macromolecule.

Fluorescent properties of EPF in solution and solid state

The quantum efficiency of EPF ($\Phi_{EPF} \approx 0.28$) was lower than that of fluorescein ($\Phi_{FL} \approx 0.93$) because the attachment of the epoxy group to fluorescein resulted in fluorescence quenching. The various states of protonation and tautomeric form of xanthenes are well known.[32] For fluorescein, pK_a values of 6.4, 4.3, and 2.1 have been determined for the sequence of protonations of the dianion to monoanion (Scheme 2), to neutral (zwitterionic and lactone tautomers), and to cationic forms, respectively.[33] Dianion form of fluorescein was weakened, which would lead to the fluorescence quenching, as hydroxyl was replaced by epoxy group in EPF.

Absorption and emission spectra for the EPF in solid state (A) and in aqueous solution (B) are shown in Figure 3. The absorption at 430, 460, and 490 nm and emission at 525 nm revealed that the derivative in aqueous solution, substituted in the hydroxy position of xanthene moiety, displayed a similar absorptivity and emission, when compared with the parent fluorescein ($\lambda_{ex} \approx 490$ nm, $\lambda_{em} \approx 515$ nm). It indicated that the substituent was weakly electronically coupled to xanthene moiety. As shown in Figure 3, it was clear that EPF also exhibited luminescence in solid state, whereas the fluorescein was nonfluorescent in solid state. However, the absorptions at 490 and 530 nm and emission at 570 nm of EPF in solid state were distinctly different with these in aqueous solution. Firstly, EPF in the solid state showed two absorption peaks. Among them, absorption at 490 nm was the same as that of the original xanthene moiety, and absorption at 530 nm was red shifted nearly 45 nm than that in solution. The phenomenon was unexpected and interesting. Previous investigations had indicated that the maximum in

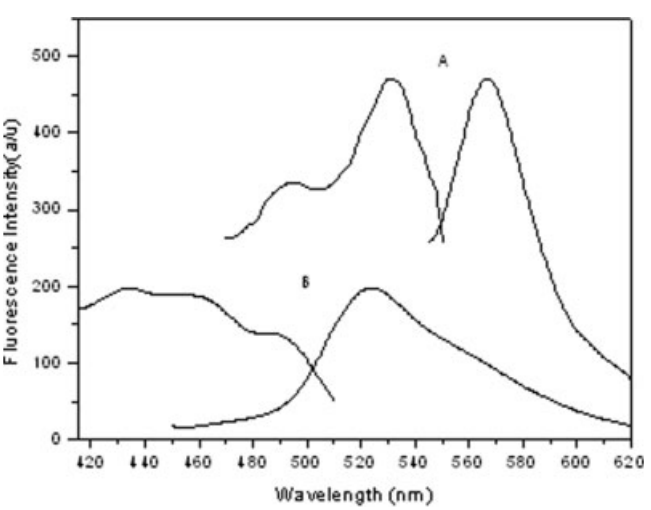


Figure 3 Absorption spectra and fluorescence spectra of EPF in solid state and aqueous solution. A: solid; B: 2.50 $\times 10^{-5}$ mol/L in solution.

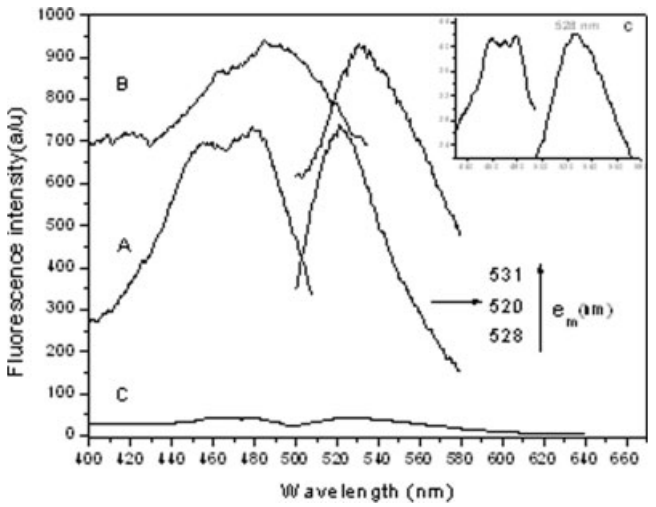


Figure 4 Absorption spectra and fluorescence spectra of CS-EPF, 0.15 g/L in aqueous solutions. A: 2.0 m*M* phosphate buffer (pH = 8.0), B: in solid state, and C: in film. The e_m was defined as emission wavelength. Inset: Absorption spectra and fluorescence spectra of CS-EPF in film.

the absorption of fluorescein and its derivatives depended on the species present.[34] We considered the red shift in the absorption spectrum because of the formation of charge transfer (CT) complex. Recently, Tasuku et al.[35] found that the fluorescein molecule could be understood as a directly linked donor–acceptor system. The two parts are conjugatively uncoupled since they are orthogonal each other, and their fluorescence properties can be modulated by intramolecular photoinduced electron transfer from the benzene moiety to the acceptor fluorophore. The observed spectra [Fig. 3(A)] pointed towards two different ground state species, normal and charge transfer (CT) in solid state. These two different species have site heterogeneity, which resulted in the red shift.[36] In addition, the emission peak in luminescent spectra of the solid powder shifted more than 50 nm to longwave region when compared with that of EPF solution. In solution, the chromophore moieties were separated because of a low concentration in water, and the luminescence spectrum resembled that of the isolated chromophore. Because there was a high concentration of chromophore groups when EPF was in solid state, the EPF moieties may interact with each other and the long wavelength bands in luminescence spectrum may probably be of excimer origin. When excited, the chromophore moiety (A*) may form an excimer (AA*) with a chromophore moiety in the ground state (A), which emitted light at a longer wavelength than the separated chromophore moiety (A*). Perhaps this long wavelength shift may also come from difference in polarization energy of S_0 and S_1 state of the EPF moiety in the solid state. It meant that magnitude of stabilization of S_1 in the solid state was greater than that of S_0 because the polarizability in the former case was higher. Therefore, the decrease of the transition energy $S_0 \leftrightarrow S_1$ caused a long wavelength shift of luminescence maxima.

Fluorescent properties of CS-EPF in solution, solid state, and film

A thin film of CS-EPF was prepared on glass plate by solvent casting. Absorption and emission spectra for the CS-EPF in aqueous solution (A), in solid state (B), and in film (C) were shown in Figure 4. Apparently, the polymer bearing fluorescein derivative EPF showed the characteristic absorption and emission properties of the parent compound [Fig. 3(B)] in aqueous solution. The fluorescence spectra resembled the mirror image of the absorption spectrum, which suggested that the emission at 520 nm arose from the isolated dye molecules. For the spectra of CS-EPF in solid state and film, they displayed typical red-shifts of emission maximum in the visible region. Both of broad bands at 531 nm (B) and at 528 nm (C) could be ascribed to the excimer between the pendant EPF moieties by comparison of band shapes and positions with those of the fluorescence spectrum in solution (A). Accordingly, the shorter (in solution) and longer (in solid state and film) wavelength bands were identified as the monomer and excimer emissions, respectively.

Temperature dependence of the fluorescence intensity of CS-EPF

Figure 5 showed the fluorescence spectra of CS-EPF in water (5.0×10^{-3} g/L) at several temperatures. It is shown in Figure 5 that the maximum fluorescence

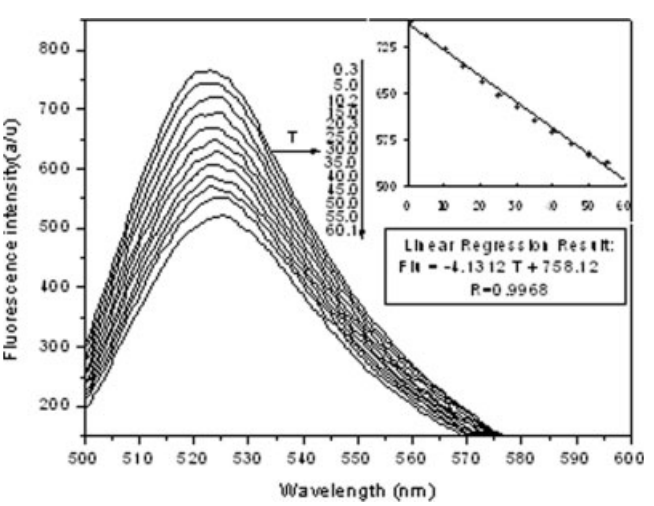


Figure 5 Fluorescence spectra for 0.005 g/L CS-EPF in water at the different *T* from 0.3 to 60.1°C (λ_{ex} = 480 nm). Inset: the linearity of the fluorescence intensity versus pH.

intensity of CS-EPF decreased on heating. The temperature dependence of the fluorescence intensity could be discussed in terms of both photophysical and photochemical dye properties and polymer matrix relaxation processes.[37]

Taking into account the photophysical and photochemical properties of dye, the fluorescence intensity of CS-EPF was mainly controlled by a 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:

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

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.[38] In a previous study of the fluorescein dyes in aqueous solution, k_{IC} varied between 273 and 323 K, according to:[39]

$$k_{IC} = 2.0 \times 10^{11} \exp[(-5.5 \text{ kcal/mol})/RT]\text{s}^{-1}. \quad (3)$$

The good linearity observed for the variation of log k_{IC} versus $1/T$ indicated that there was only one radiationless deactivation channel. Moreover, the probable collision between molecules, resulting in fluorescence quenching, and the power of intersystem crossing were reinforced with the increasing temperature. At the same time, uniting eqs. (2) and (3), we could also easily conclude that the Φ_{FL} of dye molecule decreased upon heating.

Polymer relaxation can also influence the fluorescence intensity of dye molecule. D. Dibbern-Brunelli et al.[30] had concluded that the photobleaching yield of fluorescein dye increased with the increase in the mobility of the polymer chains, and the hydrogen bonds destroyed in this process could change both the photophysical and photochemical processes of the molecules in the electronically excited state.

Moreover, the excellent linear relationship was obtained between relative fluorescence intensity and temperature in the range of 0–60°C. The linear regression equation of the calibration graph was Flu = 758.12–4.1312T (Flu was relative fluorescence intensity), with a correlation coefficient of linear regression of 0.9968. Obviously, CS-EPF was a promising optical indicator for temperature determination and was potential for determining the temperature change real time in biological system.

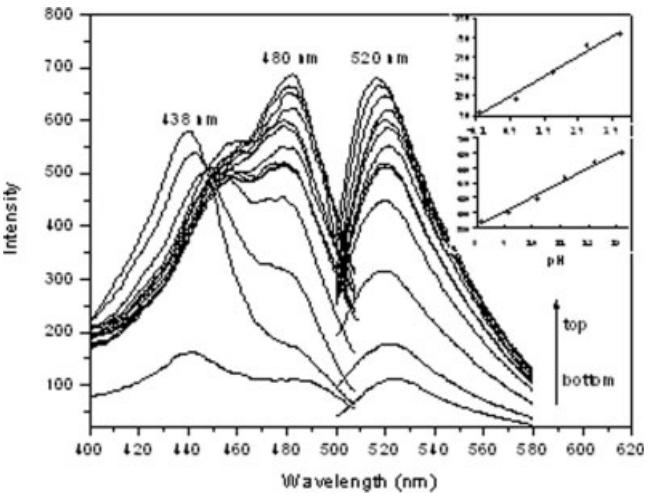


Figure 6 pH sensitivity of CS-EPF in solutions of different pH: 0.00, 1.08, 2.18, 3.16, 4.14, 5.16, 6.15, 7.15, 8.15, 9.15, 10.15, 11.16, 12.20, 13.20 (from bottom to top). Left: absorption spectra; Right: fluorescence spectra. Inset 1: the linearity of the fluorescence intensity versus pH from 0 to 4.14 (linear regression result: Flu = 102.96 pH + 95.094, R = 0.9908); Inset 2: the linearity of the fluorescence intensity versus pH from 8.15 to 13.20 (linear regression result: Flu = 19.535 pH + 425.06, R = 0.9959).

pH dependence of the absorption and fluorescence intensity of CS-EPF

In Figure 6, CS-EPF exhibited two distinct bands at around 440 and 480 nm in acidic and basic media, respectively, which was consistent with earlier findings for fluorescein.[33] Moreover, the fluorescence intensity of CS-EPF increased with further basification and showed nearly loss of emission at very acidic pH when excitated at 480 nm, which was consistent with the earlier findings that the fluorescence of neutral and cationic forms of fluorescein were inefficient.[33] It also indicated that CS-EPF largely preserved the well known pH dependence of the fluorescence of fluorescein, which was an advantage to probe pH. The species of CS-EPF present were shown in Scheme 2. It was worth noting that CS-EPF had two linear responses from pH 0.0–4.14 and 8.15–13.2, respectively. The results showed it might be used as a promising optical transducer for either low or high pH value determinations.

In addition, CS-EPF achieved good long-term stability. Its properties of fluorescence response to temperature and pH were stable for at least 180 days. And the equilibrium response to temperature was within 5 min from 20 to 60°C and the equilibrium response to pH was within 3 min from pH 0 to 12.

CONCLUSIONS

A kind of temperature/pH-responsive fluorescent macromolecular CS-EPF was prepared through the

reaction between synthesized EPF and CS in DMSO. The results presented in this article demonstrated that CS-EPF largely preserved the well known temperature and pH dependences of the fluorescence of fluorescein and could achieve better long-term stability and fast equilibrium response, which can be an advantage to probe temperature and pH. Thus, CS-EPF can be applied as promising for optical temperature sensor of a range (0–60°C) and a pH sensor of a wide pH range (0.0–13.2) based on fluorescence. The product from the proposed method is expected to be used for the preparation of a potential sensor, which is natural, inexpensive and sensitive, for determining temperature and pH changes in biological system simultaneously.

References

1. Wolf, N. E.; Pressley, R. J Appl Phy Lett 1963, 2, 152.
2. Hargreaves, J. S.; Webber, S. E. Macromolecules 1985, 18, 734.
3. Rob, Z.; Florian, K.; Sergei, B. O.; Michel, O. J Phys Chem A 2003, 107, 6770.
4. Fwu-Long, M. Biomacromolecules 2005, 6, 975.
5. Manuela, M.; Giovanna, B.; Massimo, Z.; Mario, B.; Fabio, B. Macromolecules 2004, 37, 5692.
6. Liu, Z. H.; Liu, J. F.; Chen, T. L. J Polym Sci Part A: Polym Chem 2005, 43, 1019.
7. Guan, X. L.; Liu, X. Y.; Su, Z. X.; Liu, P. React Funct Polym 2006, 66, 1227.
8. García-Martínez, J. M.; Laguna, O.; Areso, S.; Collar, E. P. J Appl Polym Sci 1998, 70, 689.
9. Carvell, M.; Ian, D. R.; Small, P. W. Polymer 1998, 39, 393.
10. Nakanishi, S.; Miyawaki, Y.; Nishikawa, M.; Amano, M. J Chem Phys 1994, 100, 3442.
11. Calzaferri, G. P. Indian Acad Sci Chem Sci 1997, 109, 429.
12. Regien, G. S.; Gert van der, Z.; Marc, A. M. J.; Alexander, B. S.; Han. Z.; Frank, J. V. J Phys Chem A 2001, 105, 4235.
13. Guilford, J.; Qian, X. H. J Photochem Photobiol A 1998, 113, 125.
14. Prats, M.; Teissie, J.; Tocanne, J. Nature 1986, 322, 756.
15. Hirano, T.; Kikuchi, K. J Am Chem Soc 2002, 124, 6555.
16. Chen, C. A.; Yeh, R. H.; Lawrence, D. C. J Am Chem Soc 2002, 124, 3840.
17. Setsukinai, S.; Urano, Y. J Biol Chem 2003, 278, 3170.
18. Shapiro, H. M. J Microbiol Methods 2000, 42, 3.
19. Gabriel, B.; Teissie, J. J Am Chem Soc 1991, 113, 8818.
20. Mattingly, P. G. Bioconjugate Chem 1992, 3, 430.
21. Shipchandler, M. T.; Fino, J. R. Anal Biochem 1987, 162, 89.
22. Li, X. H.; Ma, H. M. Chem J Chin Univ 2003, 11, 1984.
23. VandeVord, P. J.; Matthew, H. W. T.; DeSilva, S. P.; Mayton, L.; Wu, B.; Wooley, P. H. J Biomed Mater Res 2002, 59, 585.
24. Qu, X.; Wirsen, A.; Albertsson, A. C. Polymer 2000, 41, 4589.
25. Fang, Y.; Ning, G. H.; Hu, D. D.; Lu, J. R. J Photochem Photobiol A 2000, 135, 141.
26. Roula, B. Q.; Mansoor, M. A. Carbohydr Polym 1999, 38, 99.
27. Kristoffer, T.; Sabina, P. S.; Wei, T.; Lennart, K.; Kjell, M. V. Carbohydr Res 2001, 336, 291.
28. Lin, Y. W.; Xu, C.; Lu, C. H. HeChen HuaXue 2000, 8, 167.
29. Eugene, L.; Michele, O.; Sames, S. Eur Polym J 1991, 27, 311.
30. Dibbern-Bruncllli, D.; Oliverira, M. G. J Photochem Photobiol A 1995, 85, 285.
31. Flemming, G. R.; Knight, A. W. E. J Am Chem Soc 1977, 99, 4306.
32. Neckers, D. C.; Valders-Aguilera, O. M. In Advances in Photochemistry, Vol. 18.; Volman, D.; Hammond, G. S.; Neckers, D. C., Eds.; Wiley: New York, 1993, 315.
33. Sjoback, R.; Nygren, J.; Kubista, M. Spectrochim Acta Part A 1995, 51, L7.
34. Monique, M. M. Chem Phy Lett 1976, 43, 332.
35. Tasuku, U.; Yasuteru, U. J Am Chem Soc 2004, 126, 14079.
36. Mehata, M. S.; Joshil, H. C.; Tripathi, H. B. J Lumin 2001, 93, 275.
37. Martins-Franchetti, S. M.; Altvars, T. D. Z. Polym J 1995, 31, 467.
38. Talhavini, M.; Atvars, T. D. Z. J Photochem Photobiol A 1998, 114, 65.
39. Monique, M. M. Chem Phy Lett 1975, 35, 105.