

Synthesis and characterization of acrylate copolymer containing fluorescein functional group

Guodong Hui, Weiyun Huang, Yunzhao Song, Deben Chen, and Anyong Zhong[†]

College of Chemistry, Sichuan University, Chengdu 610064, P. R. China
(Received 13 March 2013 • accepted 14 May 2013)

Abstract—We report a novel method to fabricate fluorescent polymer (F-CPA) based on the esterification between acrylate copolymer (CPA) and fluorescein using N, N-dicyclohexylcarbodiimide (DCC)/4-dimethylaminopyridine (DMAP) as catalyst. The resulting copolymer was characterized by Fourier transform-infrared spectroscopy (FT-IR), gel permeation chromatography (GPC), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), ultraviolet-visible spectroscopy (UV-Vis) and fluorescence spectroscopy. In addition, the influences of concentration, solvents, pH and metal cations (Cu^{2+} , Fe^{3+} and Zn^{2+}) on the fluorescent behaviors of F-CPA are discussed in detail. All those observations suggest that the synthesized F-CPA is an excellent luminescent macromolecular material with simple synthesis method and excellent solubility. Moreover, its sensitive fluorescence response behaviors to solvents, pH and metal cations make it to become a polymer-based probe.

Key words: Fluorescein, Acrylate Copolymer, DCC/DMAP, Fluorescent Polymer, Probe

INTRODUCTION

Fluorescent polymers have attracted much research attention because they are play an important role in medicine [1], biology [2-4] and the environment [5-9]. Highly sensitive fluorescent polymers are gaining increased acceptance due to their high sensitivity and testability. The sensitivity definition is associated with a change in the fluorescence intensity, a shift in the emission wavelength, or a change in the fluorescence lifetime, so highly sensitive fluorescent polymers can be potentially used as probes. Polymer probes have several advantages over small molecule probes [10], such as better stability, reusability and film-forming ability. In this regard, fluorescent polymers have been of particular interest since their behaviors could be simply inspected by fluorescence analytical, which has turned to be one of the most effective and reliable analytical methods. Nowadays, most of the research associated with fluorescent polymers is focused on conjugated polymers with fluorescent units in the main chain [11-14] or as pendant groups [15]. However, less attention has been paid to non-conjugated polymer probes, which have showed great potential for transition metal ions detection. In general, there are two main approaches for preparation of fluorescent polymers: (i) polymerizing of a monomer containing a fluorescent chromophore [16-18], but it is cumbersome to synthesize and purify a new fluorescent monomer; (ii) incorporating fluorescent dyes or organic molecules with reactive groups contained polymer matrixes [19-22], which has been approved as an efficient method to produce the non-conjugated polymer probes. The incorporation of fluorescent dyes into the polymer matrix is expected to improve their performance and efficiency.

Fluorescein, one of the most commonly used fluorescent dyes, is widely employed as a platform for synthesis of various flores-

cence probes and fluorescence labels because of its high fluorescence quantum efficiency in aqueous media, and more importantly, both of its excitation and emission spectra are in the range of visible region, which is beneficial for its detection [23,24]. Fluorescein and its derivatives have been proved to be the most versatile chromophores used as fluorescence probes. Recently, more and more fluorescein derivatives of small molecule fluorescence probes have been synthesized, and used in a number of bioresponsive applications, ranging from drug delivery to genomics [25-29]. However, there were few reports on the highly sensitive fluorescent polymers which were composed by acrylate copolymer and fluorescein. It makes great sense to study luminescent materials.

Acrylate copolymers are widely used as polymer matrixes because of their low cost, simple synthetic method, good solubility and excellent film-forming ability. Acrylate copolymers are formed by polymerization of various monomers, which can be adjusted by the content of monomers to improve their film-forming ability, transparency, glass transition temperature and functional groups. With the purpose of connecting carboxy group of fluorescein, there must be a reactive group in the polymer matrix, such as hydroxyl or amino-group. In our case, an acrylate copolymer (CPA), synthesized with methyl methacrylate (MMA), n-butyl acrylate (BA) and 2-hydroxyethyl acrylate (HEA) was selected as the main chain since there was an active hydroxyl group, which could react with the carboxy group of fluorescein.

In this study, we demonstrate a method to fabricate a fluorescent polymer (F-CPA) via the esterification reaction between carboxy groups of fluorescein and hydroxyl groups of acrylate copolymer (CPA), using DCC/DMAP as double catalyst in DMSO medium. The optical properties of the obtained F-CPA was studied in detail, and we further investigated the influences of solvents, pH and metal cations (Cu^{2+} , Fe^{3+} and Zn^{2+}) on the fluorescent behaviors of F-CPA. All those observations suggest that F-CPA can be used as a fluorescent polymer probe, and it provides a new way for the preparation of fluorescent polymer.

[†]To whom correspondence should be addressed.
E-mail: zhongany@sina.com.cn

EXPERIMENTAL

1. Materials

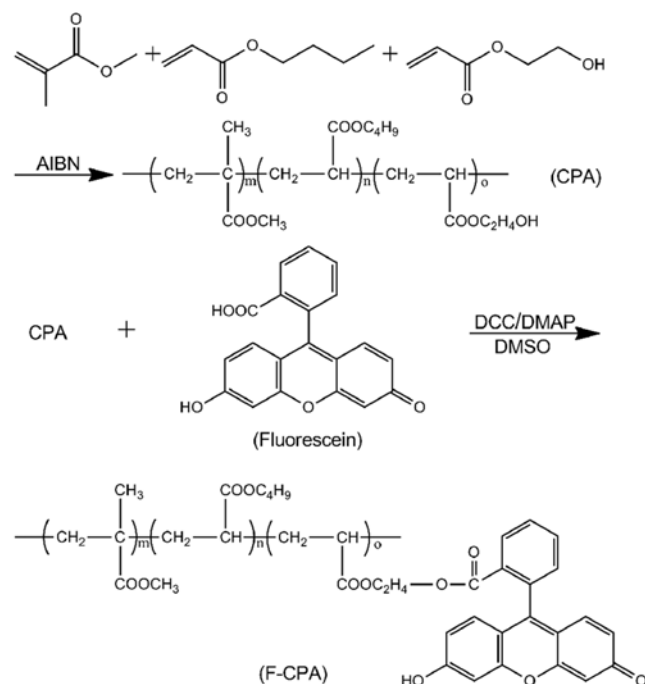
Methyl methacrylate (MMA), 2-hydroxyethyl acrylate (HEA) and n-butyl acrylate (BA) were purchased from Beijing East Chemical Industry Factory (Beijing, China), distilled before use. Azobisisobutyronitrile (AIBN), fluorescein, N, N-dicyclohexyl carbodiimide (DCC), 4-dimethylaminopyridine (DMAP), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{Fe}_2(\text{SO}_4)_3$ were purchased from Chengdu Kelong Chemical and Technology Reagents (Chengdu, China), and were used without further purification. Tetrahydrofuran (THF), acetonitrile, chloroform, dimethylsulfoxide (DMSO), acetone and other chemical reagents were of analytical grade and were used without further purification.

2. Synthesis of Acrylate Copolymer (CPA)

The procedure was carried out in a three-necked flask equipped with a stirrer, a reflux condenser and a constant-pressure dropping funnel. A total of 70 mL of anhydrous toluene was added in the flask as solvent; 14 g of MMA, 4 g of BA, 2 g of HEA and 0.15 g of AIBN were dissolved in 30 mL of anhydrous toluene and slowly added in the system using constant-pressure dropping funnel. The polymerization reaction was carried out at 85 °C for 6 h to obtain the transparent CPA, which has good film-forming property. The transparent CPA was collected by filtration (isolation yield 86%). The molecular structure is given in Scheme 1.

3. Synthesis of Fluorescent Polymer (F-CPA)

CPA (3.5 g), fluorescein (1.2 g) and absolute DMSO (120 mL) were put into a 250 mL flask which was equipped with a stirrer, a condenser and a drying tube. Anhydrous DCC (0.65 g) and DMAP (0.18 g) were fed into the mixture after the solids were dissolved. (nDMAP : nDCC : nFluorescein = 0.05 : 1 : 1) [30]. Then the system was submerged in a water bath carefully controlled at 35 °C. The resulting mixture was refluxed for 24 h, cooled and then filtrated.



Scheme 1. The synthetic route of CPA and F-CPA.

August, 2013

Distilled water (100 mL) was added into the filtrated stock solution with continuous stirring. Some yellow precipitates were formed. The rough products were purified by means of dissolution/deposition in acetone/petroleum ether by turns. The resultant yellow product (F-CPA) was collected by filtration. The dissolution and precipitation procedure were repeated to obtain the product (isolation yield 70%). The molecular structure is given in Scheme 1.

4. Characterization

The Fourier transform infrared (FT-IR) spectra were obtained with a Nicolet 1700SX spectrometer (all samples grounded in KBr). The molecular weights and distribution of the polymers were measured by gel permeation chromatography (GPC, Agilent 1100 chromatograph) at 35 °C, while THF was used as the eluent at a flow rate of 1.0 mL/min and standard polystyrene as the reference. Differential scanning calorimetry (DSC) was performed under nitrogen using a Perkin-Elmer 200PC at a heating rate of 10 °C/min. Thermogravimetric analysis (TGA) was performed under nitrogen with a TG209F3 thermal analyzer. The sample was heated at 10 °C/min, heating rate from 30 to 450 °C. The UV-Vis absorption measurements were performed on a TU-1901 UV spectrometer (Beijing Puxi Universal Devices Ltd., Co.). Photoluminescence spectra of the polymers were recorded on a Hitachi F-7000 fluorescence spectrophotometer.

RESULTS AND DISCUSSION

1. Characterization of CPA and F-CPA

DCC/DMAP were used as catalyst for the synthesis of F-CPA, so that the esterification reaction could take place in mild condi-

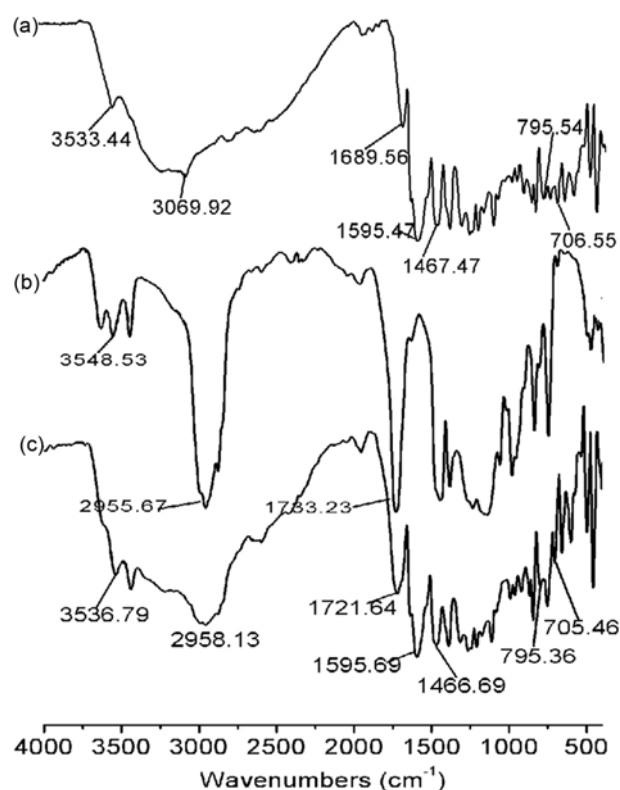


Fig. 1. The FT-IR spectra of fluorescein (curve (a)), CPA (curve (b)) and F-CPA (curve (c)) were collected on KBr.

Table 1. The GPC results of CPA and F-CPA

	$\overline{M}_n (\times 10^4) (\text{g} \cdot \text{mol}^{-1})$	$\overline{M}_w (\times 10^4) (\text{g} \cdot \text{mol}^{-1})$	$\overline{M}_z (\times 10^4) (\text{g} \cdot \text{mol}^{-1})$	$\overline{M}_v (\times 10^4) (\text{g} \cdot \text{mol}^{-1})$	D ($\overline{M}_w/\overline{M}_n$)
CPA	1.72	3.57	5.95	3.57	2.08
F-CPA	2.14	4.54	6.34	4.04	2.12

tions with good product yield. F-CPA can be dissolved in many commonly used organic solvents, so it is very important to select the appropriate solvent for purification with the method of dissolution/deposition. In our case, acetone/petroleum ether system was chosen to purify the primary F-CPA. The results of control experiments showed that no fluorescence was detectable on the simple mixture of fluorescein and CPA after five cycles of dissolving and depositing with acetone/petroleum ether system. However, the fluorescence of F-CPA was very strong even after ten such cycles used for rough F-CPA products. It is proved that the fluoresceins have already been grafted on the main chain of CPA.

Fig. 1 shows the FT-IR spectra of fluorescein (curve a), CPA (curve b) and F-CPA (curve c). Fluorescein (curve a) IR (KBr): 3,533.44 (vs, OH), 3,069.92 (vs, =CH), 1,689.56 (vs, C=O), 1,595.47, 1,467.47, 795.54, 706.55 cm^{-1} (vs, Ar); CPA (curve b) IR (KBr): 3,548.53 (vs, OH), 2,955.67 (vs, CH₃), 1,733.23 (vs, C=O). After the esterification reaction of fluorescein and CPA, a strong characteristic peak of benzene ring appears at around 1,595.69, 1,466.69, 795.36 and 705.46 cm^{-1} . It is well known that there is a big benzene ring in flu-

orescein. All these facts reflect that the fluoresceins have already been grafted on the CPA through esterification reaction.

Table 1 shows molecular weight and molecular weight distribution of CPA and F-CPA. The results show that number-average molecular weight (\overline{M}_n) and weight-average molecular weight (\overline{M}_w) of F-CPA are 2.14×10^4 and 4.54×10^4 , while \overline{M}_n and \overline{M}_w of CPA are 1.72×10^4 and 3.57×10^4 , respectively. Both \overline{M}_n and \overline{M}_w of F-CPA are larger than that of CPA, indicating the successful fluorescein incorporation. The molecular distribution index D remains at 2.08 around, which implies the same molecular weight distribution before and after grafting. The average number of fluorescein grafting on a CPA molecule in this work should be $(2.14 \times 10^4 - 1.72 \times 10^4) / 332.31 = 12.64$, and the relevant theoretical grafting data is $(1.72 \times 10^4 \times 10\%) / 116.12 = 14.81$, where 10% is the content of HEA in CPA, and 116.12 is the molecular weight of HEA. It is proved that the fluoresceins have already been grafted on the main chain of CPA and the grafting ratio reaches 85.34%.

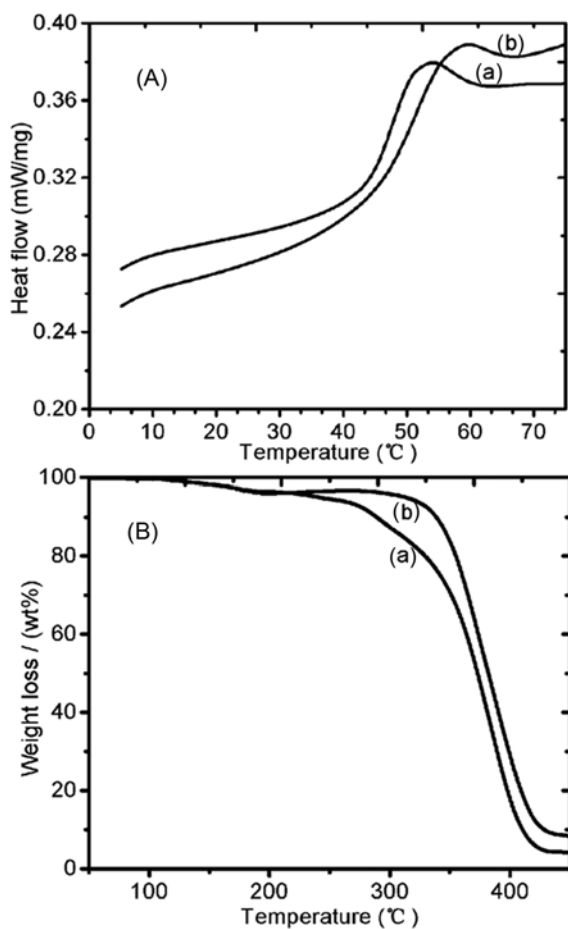
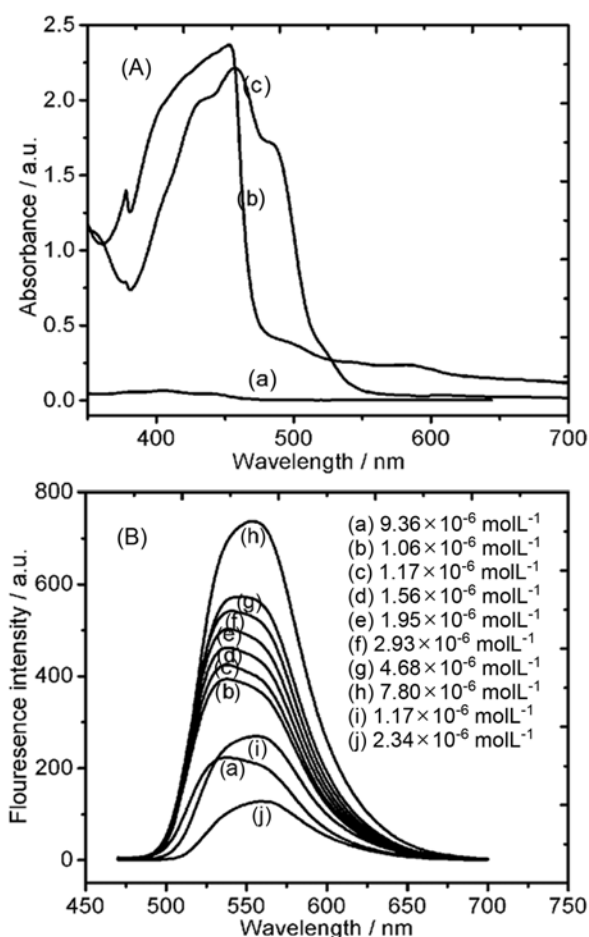
**Fig. 2. The DSC (A) and TGA (B) of CPA (a) and F-CPA (b).****Fig. 3. The absorption spectra of CPA (a), fluorescein (b) and F-CPA (c) in acetone (A). The emission spectra of F-CPA in CHCl₃ at the 10 concentrations indicated (B).**

Fig. 2(A) shows the DSC curves of CPA (a) and F-CPA (b), while Fig. 2(B) shows the TGA curves of CPA (a) and F-CPA (b). It is obvious that the glass transition temperature (T_g) of F-CPA (60°C) is higher than that of CPA (52°C). Moreover, F-CPA exhibit higher thermal stability than CPA, since the onset degradation temperature of F-CPA is 300°C , while that of CPA is 240°C . These results confirmed the fluorescein molecules were indeed introduced to the chain of the CPA, enhancing the rigidity of the resulting F-CPA and thus improving their thermal stability.

Fig. 3(A) shows the UV-Vis absorption spectra of CPA (a), fluorescein (b) and F-CPA (c) in solvent acetone. According to the absorption data for the CPA, fluorescein and F-CPA, CPA is no optical absorption and the absorption peak at 450 nm of F-CPA is essentially identical with that observed for the fluorescein in the visible region. The maximum absorption peak of F-CPA is very close to that of fluorescein. Due to the presence of the rigid conjugated system of fluorescein, the long wavelength band in the absorption spectra has a charge transfer character. It is a $\pi \rightarrow \pi^*$ electronic transition, from ground to the first excited singlet state ($S_0 \rightarrow S_1$). The absorption spectra provide the evidence for the graft of fluorescein on the main chain of CPA.

2. Fluorescence Behavior of F-CPA

Fig. 3(B) shows the fluorescence spectra of F-CPA in solvent chloroform under different concentrations. The fluorescence of F-CPA in solution was very stable. No significant difference of the fluorescence spectra was observed when the spectrum was meas-

ured as soon as F-CPA was dissolved in chloroform and kept after 48 h. From Fig. 3(B) we can see that the fluorescence intensity of F-CPA strengthens with the increase of the concentration within a certain range. At higher concentration, the fluorescence intensity of F-CPA begins to decrease. This is attributed to the concentration self-quenching. The mechanism of concentration self-quenching has been attributed either to resonance energy transfer or to molecular interactions. The maximum fluorescence intensity of F-CPA appears when the concentration of F-CPA reaches around $7.80 \times 10^{-5}\text{ molL}^{-1}$.

Fig. 4(A) shows the fluorescence spectra of F-CPA in different polar solvents under the same concentrations. We can see that the fluorescence intensity and red shift of fluorescence spectra are gradually enhanced on increasing the solvent polarity. For example, when F-CPA dissolve in THF and acetone, the fluorescence emission max peaks appear at 550 and 552.8 nm , respectively, but in DMF and DMSO the fluorescence emission max peaks appear at 565.6 and 568.8 nm , respectively. As the dipole moment of the molecule is enhanced upon excitation due to electron density redistribution, the excited molecule is better stabilized in polar solvents because of the stronger interactions with the solvent dipoles [31]. This effect causes a red shift in the fluorescence maxima.

Fig. 4(B) shows the fluorescence intensity of F-CPA in solution under different pH conditions. The fluorescence intensity was measured in acetonitrile/water (1 : 4 v/v) and the pH adjusted by adding HCl or NaOH. The polymer concentration in the solution was fixed at $3.0 \times 10^{-6}\text{ molL}^{-1}$. The experiments were performed in the pH range

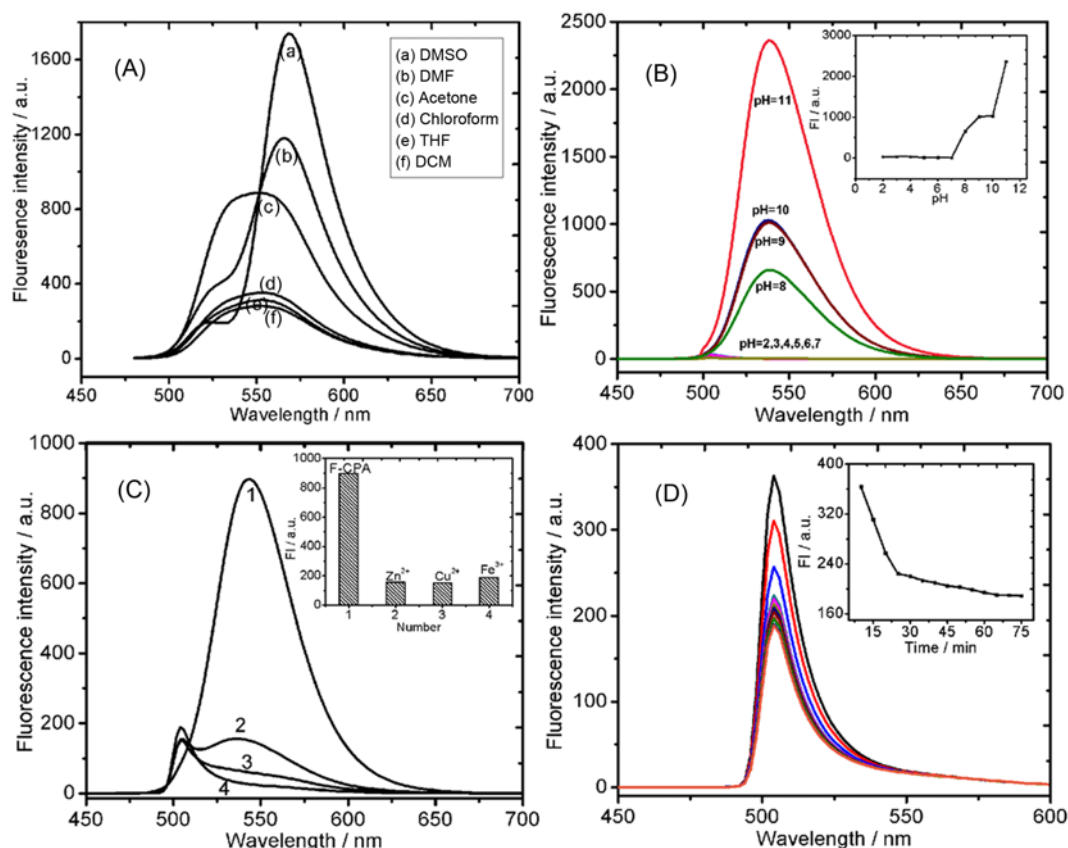


Fig. 4. The emission spectra of F-CPA in the different solvents (A), the emission spectra of F-CPA in the mixtures of acetonitrile and water at different pH (B), the emission spectra of F-CPA in the mixtures of acetonitrile and water in the presence of the different metal cations (C), and the emission stability (D).

from 2 to 11. From Fig. 4(B), we can see that the fluorescence intensity of F-CPA completely losses at very acidic pH condition. On the contrary, the fluorescence intensity rapidly increases when the pH is higher than 7. The pH-dependent fluorescence character could be explained by the electron density change of different F-CPA form under different pH values. The phenolic hydroxyl groups of fluorescein molecules have different forms changing with pH values. Under basic condition, these phenolic hydroxyl groups were deprotonated and became the monoanion form of F-CPA, whose electron density of conjugated system for fluorescein molecules was much enhanced, causing the fluorescence quantum yield to be much higher than that of its acidic and neutral molecule.

Fig. 4(C) shows the influences of metal cations (Zn^{2+} , Cu^{2+} and Fe^{3+}) on the fluorescence spectra of F-CPA. The concentration of these three different metal cations was $10^{-4} \text{ mol L}^{-1}$. The fluorescence intensity was measured in acetonitrile/water (1 : 4 v/v) medium and the spectrum was collected after the metal ions were mixed with F-CPA for 30 min. From the picture, we can see that the presence of metal ions significantly influences the fluorescence property since above 70% of the fluorescence intensity was quenched for each metal ion. As we know, the metal cations could probably coordinate with the carbonyl groups from the fluorescein; thus the electron-withdrawing ability of the formed complex is increased, and as a result, a decrease of the fluorescence intensity has been observed [32].

Fig. 4(D) plots the time dependent fluorescence intensity of F-CPA. The inset shows the decrease in the fluorescence intensity vs. time in the presence of Fe^{3+} cations. In the presence of Fe^{3+} cations the fluorescence intensity decreases without change in the position of the wavelength maxima. The rigid polymer chains trouble the diffusion of metal cations and their contact with the fluorophore receptors. Therefore, F-CPA needs a longer time to exhibit its probe abilities.

CONCLUSIONS

We succeeded in synthesizing the novel fluorescent polymer (F-CPA) through the method of esterification reaction, which has excellent photoluminescence property, solubility and film-forming ability. In addition we studied the influences of solvents, pH and metal cations (Zn^{2+} , Cu^{2+} and Fe^{3+}) on the fluorescent behaviors of F-CPA. In the solution state, the designed fluorescent polymer exhibits fluorescence quenching phenomenon in the presence of Zn^{2+} , Cu^{2+} and Fe^{3+} cations. This fact suggests its good prospects as a polymer probe for Zn^{2+} , Cu^{2+} and Fe^{3+} cations. It is expected that it may be used in fluorescent colorants and photoluminescence devices in the future because its raw materials are commercially available and it can be produced conveniently in large scale.

ACKNOWLEDGEMENTS

The current investigations were financially supported by the Science and Technological Department of Sichuan Province (NO. 2011FZ0107).

REFERENCES

1. R. W. Tsien and Randall, *Chem. Eng. News*, **72**, 34 (1994).
2. X. L. Hou, X. F. Tong, W. J. Dong, C. Dong and S. M. Shuang, *Spectrochim. Acta Part A: Mol. Biomole Spectro.*, **66**, 552 (2007).
3. Y. Guan, W. Zhou, X. H. Yao, M. P. Zhao and Y. Z. Li, *Anal. Chim. Acta*, **570**, 21 (2006).
4. Y. Nakamura, R. Miyatake, Matsubara, H. Kiyota and M. Ueda, *Tetrahedron*, **62**, 8805 (2006).
5. Y. Tang, F. He, M. H. Yu, F. D. Feng, L. L. An, H. Sun, S. Wang, Y. L. Li and D. B. Zhu, *Macromol. Rapid Commun.*, **27**, 389 (2006).
6. R. Métivier, L. Leelay and B. Valeur, *Chem. Commun.*, **8**, 996 (2003).
7. J. F. Fernández-Sánchez, A. Segura-Carretero and C. Cruces-Blanco, *Talanta*, **60**, 287 (2003).
8. C. D. Geddes, P. Douglas, C. P. Moore, T. J. Wearc and P. L. Eger-ton, *Dyes and Pigments*, **43**, 59 (1999).
9. C. G. Niu, A. L. Guan, G. M. Zeng, Y. G. Liu and Z. W. Li, *Anal. Chim. Acta*, **577**, 264 (2006).
10. Z. Q. Wu and L. Z. Meng, *Progress in Fluorescent Polymers*, **19**, 1381 (2007).
11. M. D. Disney, J. Zheng, T. M. Swager and P. H. Seeberger, *J. Am. Chem. Soc.*, **126**, 13343 (2004).
12. C. G. Niu and Z. Z. Li, *Anal. Bioanal. Chem.*, **372**, 519 (2002).
13. S. Santra, P. Zhang, K. Wang, R. Tapeç and W. Tan, *Anal. Chem.*, **73**, 4988 (2001).
14. D. S. Prasanna, G. H. Q. Nimal and T. Gunnlaugsson, *Chem. Rev.*, **97**, 1515 (1997).
15. H. M. Hung, K. M. Wang, W. H. Tan, D. L. An and X. H. Yang, *Angew. Chem. Int. Ed.*, **43**, 5635 (2004).
16. X. Q. Liu, F. S. Du, Z. C. Li and F. M. Li, *J. Appl. Polym. Sci.*, **70**, 1191 (1998).
17. Y. Tao, B. A. Doat and J. Lam, *Thin Solid Films*, **363**, 298 (2003).
18. L. F. Campo, F. S. Rodembusch and V. Stefani, *J. Appl. Polym. Sci.*, **99**, 2109 (2006).
19. R. B. Qaish and M. M. Amiji, *Carbohydr. Polym.*, **38**, 99 (1999).
20. K. Tommerass, S. P. Strand, W. Tian, L. Kenne and K. M. Varum, *Carbohydr. Res.*, **336**, 291 (2001).
21. X. Guo, J. Lu, H. Li, S. Yao and L. Wang, *J. Appl. Polym. Sci.*, **97**, 2067 (2005).
22. H. A. Klok, S. Becker, F. Schuch, T. Pakula and K. Mullen, *Macromol. Chem. Phys.*, **203**, 1106 (2002).
23. K. Teranishi and T. Nishiguchi, *Anal. Biochem.*, **325**, 185 (2004).
24. W. C. Yang, M. J. Schmeer and E. S. Yeung, *Anal. Chem.*, **77**, 4489 (2005).
25. X. L. Guan, X. Y. Liu and Z. X. Su, *J. Appl. Polym. Sci.*, **104**, 3960 (2007).
26. H. F. Gao, C. C. Wang and W. L. Yang, *J. Macrom. Sci. Part A*, **41**, 357 (2004).
27. K. C. Heui, L. Saifullah, D. K. Dae and H. C. Joon, *Polymer*, **50**, 2357 (2009).
28. N. Nakayama-Ratchford, S. Bangsaruntip and X. M. Sun, *J. Am. Chem. Soc.*, **129**, 2448 (2007).
29. A. Nese, N. V. Lebedeva, G. Sherwood and S. Averick, *Macromolecules*, **44**, 5905 (2011).
30. B. Yang and B. B. Sun, *Hebei Chemical Industry*, **31**, 7 (2008).
31. K. Rurack, *Spectrochim. Acta Part A*, **57**, 2161 (2001).
32. I. Grabchev, X. Qian, Y. Xiao and R. Zhang, *New J. Chem.*, **26**, 920 (2002).