Effect of Metal Ions and pH Values on the Conformational Transition of the Copolymer Chain in Aqueous Solutions

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ABSTRACT: A novel salts- and pH-responsive copolymer, poly(acrylic acid-co-ethyl 1-(4-chlorophenyl)-5-acrylamino-1,2,3-triazol-4-carboxylate), poly(AA-co-ECATC), was prepared by radical polymerization in methanol at room temperature with L-ascorbic acid (Vc) and peroxide hydrogen (H_2O_2) as initiators. The copolymer was characterized by Fourier Transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC). The copolymer possessed excellent fluorescence properties. The effects of metal ions with different charges, radius and outer-layer electron constructions and pH values on the conformation transition of copolymer chains were systematically investigated by the fluorescence intensity and the fluorescence anisotropy. It was found that fluorescence intensity and fluorescence anisotropy curves waved with the change of metal ions concentrations and pH values. Additionally, FTIR Subtraction spectroscopy was also carried out to confirm our results. The work provided a theory basis for the study of salts- and pH-sensitive smart hydrogels, which were always used in the slow- or controlled-release drugs. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 1714–1722, 2007

Key words: fluorescence; salts- and pH-sensitive; conformation transition of copolymer chain; solution properties

INTRODUCTION

Polymer hydrogels, smart hydrogels in particular, which have better response to external stimuli, such as pH and salts, have attracted much interest in both academic research and industrial applications since 1970s.[1–3] After several decades of the research and development, smart hydrogels have been widely used as controlled-release drugs carriers, molecular separation, material for improved biocompatibility, and as superabsorbents, because of peculiar physical and chemical properties.[4–6]

Among intelligent polymers, pH- and metal ion concentration-sensitive hydrogels are most widely investigated by fluorescence techniques, which can perform the examination of extremely-dilute solutions and purely intramolecular effects.[7–13] However, fluorescent techniques are quite silent with respect to the transformation of chelates with different coordination numbers in dilute solutions. One of the few methods that can be used to investigate the transformation process on such a level is FTIR subtraction spectroscopy. Indeed, several groups were able to characterize the transformation of complexes with different coordination numbers by analyzing changes in the FTIR subtraction spectroscopy of the copolymer with various concentrations of metal cations.[14–17]

Compounds with a 1,2,3-triazole nucleus have been reported as antibacterials,[18] antifungals,[19] antivirals, antiinflammatories, analgesics, inhibit tumor proliferation, invasion, metastasis, and anti-HIV properties.[20,21] However, we did not find any report on their fluorescence properties and applications as a fluorescence dye. Because poly(acrylic acid) (PAA) essentially adopts an open chain conformation at all values of pH,[22,23] it could not be studied by fluorescence techniques. After PAA was modified by ECATC through copolymerization, the resultant copolymer, poly(AA-co-ECATC), would be expected to possess salts- and pH-sensitive.

In this article we prepared the copolymer of poly(AA-co-ECATC), then the changes of fluorescence properties of this copolymer with the increase of salts concentrations and pH values were investigated. Additionally, FTIR subtraction spectroscopy experiment was carried out to confirm our results. The work provided a theoretical basis for the study

of salts- and pH-sensitive smart hydrogels, which were always used in slow- or controlled-release drugs.

EXPERIMENTAL

Materials

Acrylic acid (AA, Beijing Eastern Chemical Works, China) was distilled at reduced pressure (bp 20–21°C/ 0.5 mmHg) before use. Sodium azide (NaN$_3$), sodium nitrite (NaNO$_2$), ethyl cyanacetate (ECA), p-chlorophenylamine (PCPA), L-ascorbic acid (Vc), peroxide hydrogen (H$_2$O$_2$), sodium chloride (NaCl), magnesium chloride (MgCl$_2$), aluminum chloride (AlCl$_3$), calcium chloride (CaCl$_2$), zincum chloride (ZnCl$_2$), copper chloride (CuCl$_2$), barium chloride (BaCl$_2$) and other chemicals were all analytical grade, purchased from Xi'an Chemical Reagent Plant (China) and used directly without further purification. Water used in this work was deionized and then doubly distilled.
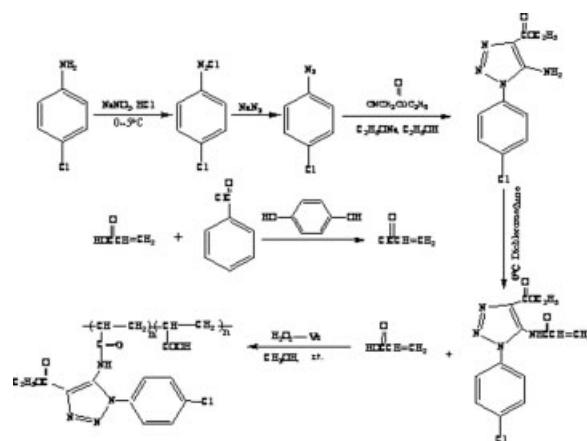
Synthesis

Synthesis of ethyl 1-(4-chlorophenyl)-5-amino-1,2,3-triazol-4-carboxylate.

The ethyl 1-(4-chlorophenyl)-5-amino-1,2,3-triazol-4-carboxylate compound was synthesized as in our previous work.[24]

Synthesis of ethyl 1-(4-chlorophenyl)-5-acrylamino-1,2,3-triazol-4-carboxylate (ECATC).

A solution of 20.0 mL acryloyl chloride dissolved in 10.0 mL dichloromethane was added dropwise to the solution of 32.0 g (0.1 mol) compound 1-(4-chlorophenyl)-5-amino-1,2,3-triazol-4-carboxylate and 21.0 mL (0.15 mol) triethylamine (to absorb HCl) previously dissolved in 150 mL dichloromethane at 0°C under N$_2$ atmosphere. The mixture was stirred for 6 h at 0°C and then stirred for 5 h at room temperature. The precipitated salt was removed by filtration and the filtered solution was washed with distilled water. After drying over anhydrous sodium sulfate, the dichloromethane was removed under reduced pressure. The crude product was purified by chromatography on a column of silica gel and eluted successively with ethyl acetate-petroleum ether (v/v, 1 : 6). The white solid was obtained and the yield (m.p.102–103°C) was 85.0 wt %. ^{1}HNMR(CDCl$_3$, δ ppm): 7.49(d, 2H, J = 6.6 Hz, Ar-3,5), 7.38(d, 2H, J = 6.6 Hz, Ar-2,6), 6.46(s, 1H, —NH—), 6.42(t, 1H, J = 9.3 Hz, —COCH=), 5.86(d, 2H, J = 9.3 Hz, =CH$_2$), 4.40(q, 2H, J = 6.9 Hz, —CO$_2$CH$_2$—), 1.36(t, 3H, J = 6.9 Hz, —CH$_3$). The results of ^{1}HNMR proved the resultant was ECATC.

Scheme 1 The synthetic scheme for poly(nAA-co-mECATC) (n = 448, m = 1).

Synthesis of poly(AA-co-ECATC)

Poly(AA-co-ECATC) was prepared by radical polymerization of AA and ECATC in methanol at room temperature. The initiator was Vc-H$_2$O$_2$. The polymerization process was similar to that of the literature.[25] The whole synthesis procedure of the poly(AA-co-ECATC) was shown in Scheme 1. The crude product was further purified by precipitation for five times with diethyl ether from a methanol solution and then dried under vacuum at room temperature to constant weight.

Preparation of the copolymer solutions

The concentration of copolymer solutions used in steady state fluorescence and fluorescence anisotropy measurements was 10^{-4} wt %. Poly(AA-co-ECATC) solutions were prepared from a stock solution (0.01 wt %), which was prepared freshly every day. The working solutions of poly(AA-co-ECATC) containing Na$^+$, Mg^{2+}, Al^{3+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, and Ba^{2+}, respectively, were prepared as follows. At first, a known amount of these cations salts were added to a 50-mL volumetric flask, then 40 mL of deionized water were added to the solution to dissolve them, after that 0.5 mL poly(AA-co-ECATC) stock solution was added with shaking, and finally the solutions were made up to 50 mL with deionized water. The pH of poly(AA-co-ECATC) solutions were adjusted by the addition of small amounts of 1M HCl or 1M NaOH, which was controlled with a pH meter (PHS-3C, China).

Fluorescence analytical methods

All fluorescence spectrums were recorded on a Perkin–Elmer LS 55 Luminescence Spectrometer. Fluorescence anisotropy measurements were performed on the same apparatus using the polarization accessory.

According to the previous literatures,[26,27] the fluorescence anisotropy, r, can be calculated by using eqs. (1) and (2), respectively.

$$G = I_{hv}/I_{hh} \qquad (1)$$

$$r = (I_{vv} - GI_{vh})/(I_{vv} + 2GI_{vh}) \qquad (2)$$

where I_{vv} and I_{vh} stand for the fluorescence intensities observed parallel and perpendicular, respectively. Similarly, I_{hv}, and I_{hh} were the fluorescence intensities measured perpendicular and parallel, respectively. G was the instrumental correction factor.

All measurements and calculations were carried out automatically by the system. Each measurement was performed using a quartz cuvette with a path length of 1 cm and was repeated at least 20 times. The fluorescence emission spectra (375 nm) were recorded at an excitation wavelength of 295 nm. The emission spectra were not corrected, except for quantum yield measurements. The excitation spectra were measured in the ratio mode. Solutions in study were not degassed, since the fluorescence intensities of poly(AA-co-ECATC) were not affected significantly by degassing for aqueous solutions. For measurements of the poly(AA-co-ECATC) emission spectra, the excitation slit widths were set at 10 nm (excitation) and 10 nm (emission). For measurements of spectra of poly(AA-co-ECATC), the excitation wavelength was set at 295 nm unless otherwise stated. The concentrations of poly(AA-co-ECATC) and 1% attenuation were adjusted to make peak areas and peak heights in proportion. All samples were allowed to equilibrate for 10 min at 25°C to measure at the same temperature. Each value was the average of at least three measurements.

Differential scanning calorimetry (DSC) analytical methods

The DSC analysis for all samples was done on a Sapphire DSC (Perkin–Elmer Corp., Wilton, CT) at a scan rate of 10°C/min under a dry nitrogen atmosphere. The weight of the samples ranged within 8–12 mg. The second scan was taken into consideration.

FTIR measurements

The FTIR spectroscopy of the poly(AA-co-ECATC) and the FTIR Subtraction spectroscopy of poly(AA-co-ECATC) in aqueous solutions were obtained on a Nicolet NEXUS 670 spectrometer (American Nicolet, Madison, WI). The FTIR Subtraction spectroscopy of poly(AA-co-ECATC) in aqueous solutions was obtained by subtraction of the solvent spectrum recorded under the same condition. The resolution

was 4.0 cm^{-1}, and the scanned wave number ranges from 4000 to 400 cm^{-1}.

Determination of the weight-average molecular weight of the copolymer

The weight-average molecular weight of the copolymer was determined by Laser Light Scattering (LLS) (BI-200 SM, Brookhaven Instruments, Long Island, NY).

RESULTS AND DISCUSSION

Characterization of poly(AA-co-ECATC)

Figure 1 showed the FTIR spectroscopy of poly(AA-co-ECATC). From which it could be found that IR absorption peaks at 1739 cm^{-1}, 1708 cm^{-1}, and 1656 cm^{-1} were assigned to C=O in ester carbonyl-group, carboxylic-group, and carboxamido-group, respectively. The typical vibration bands of N—N=N in 1H-1,2,3-triazole ring was at 941 cm^{-1}, this was the same as that of our previous work.[28] All of these indicated that the resultant was poly(AA-co-ECATC).

The results of DSC were shown in Figure 2. It could be found that PAA showed a T_g (glass transition temperature) of 129.6°C, and ECATC exhibited a melt temperature at 156.6°C, while poly(AA-co-ECATC) showed a T_g of 136.5°C. There was only one T_g in the DSC curve of the poly(AA-co-ECATC), which was just in the middle of that of PAA and ECATC. The results showed that the copolymer was poly(AA-co-ECATC).

The result of the elemental analysis showed that the content of nitrogen was 0.14%. Then, the value of m and n could be calculated from it, and they were 1 and 448, respectively. The weight-average molecular weight of the copolymer determined by Laser Light Scattering technique was 3.39×10^4 g/mol. In the study of the intermolecular and the intramolecular interactions among polymer chains with
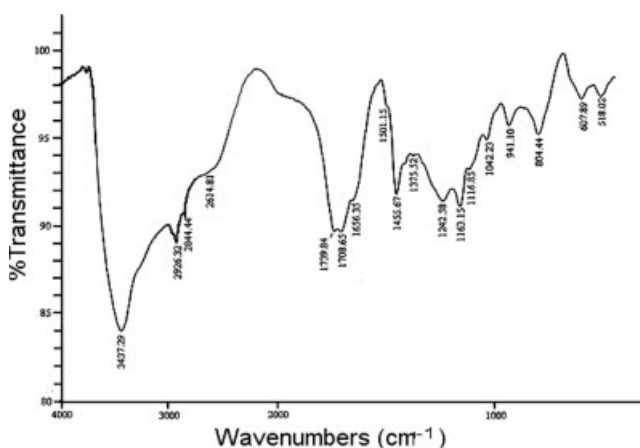

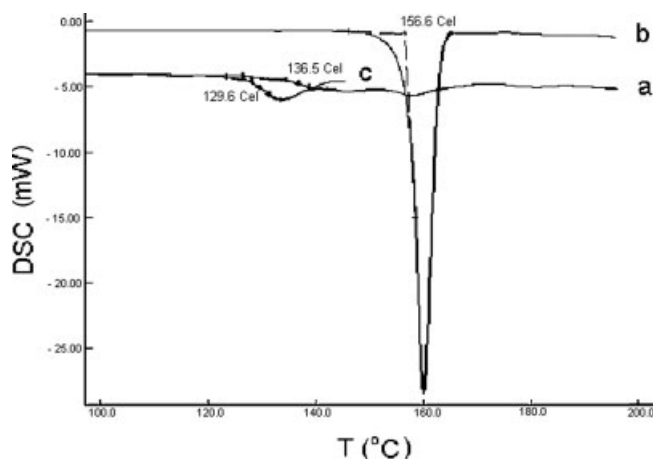**Figure 1**   FTIR Spectrum of poly(AA-co-ECATC).

Figure 2 DSC curves of poly(AA-co-ECATC) (a), ECATC (b), and PAA(c).

fluorescence techniques, the molecular weight of the polymer plays an important role. The most suitable molecular weight in the study of the conformation transition of polymer chains reported in literature[29,30] was about 10^4, so the copolymer was suitable for the investigation of fluorescence properties in dilute aqueous solutions.

The fluorescence property of poly(AA-co-ECATC)

The fluorescence emission spectra (λ_{ex} = 295 nm) of poly(AA-co-ECATC) solutions (10^{-3} wt %) with various solvents was shown in Figure 3. From Figure 3, it could be seen that when the wave length of the excitation light kept a constant, the maximum emission wave length of the emission light changed little, but the fluorescence intensity changed greatly. So the ECATC could be used as a fluorescence dye, which
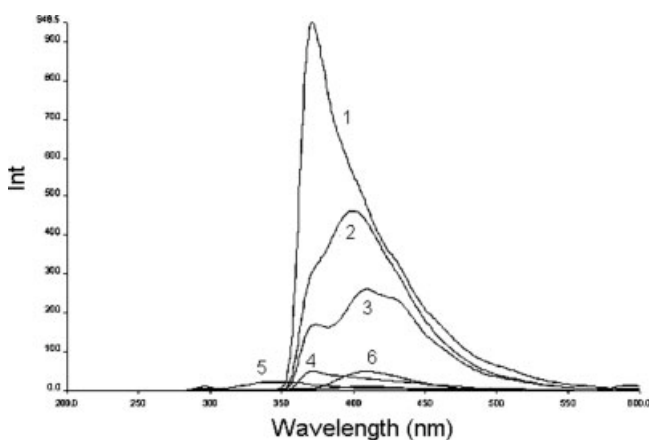
means we could investigate the conformation transition of this copolymer chains by fluorescence techniques directly. Therefore, the negative influences of the fluorescence label dye, which was always introduced into the polymer chains to probe the conformation transition of them, were avoided and the experimental results became more objective.

The effect of metal ions charge on the conformation transition of copolymer chains

Figure 4 showed the effect of the metal ions' charge on fluorescence intensity of solutions. From Figure 4, it could be found that as increasing concentration of metal ions in copolymer solutions, the fluorescence intensity experienced several increasing-decreasing-increasing stages, and, there were more than one maximum and minimum. Above phenomenon was also shown in the curves of fluorescence anisotropy of Mg^{2+} and Al^{3+} in the same conditions (Fig. 5). From Figure 5, it could be found that the curves of fluorescence anisotropy waved with the increasing of metal ions concentrations, whereas the trends were contrary to that of the fluorescence intensity. The possible reason was the change of coordination numbers during the chelation between the copolymer and metal ions.

It was well known that PAA was a favorable ligand, it could coordinate with metal ions.[31] Because AA segments were the main component in the poly (AA-co-ECATC) chains, the copolymer properties would be similar to that of PAA. The coordination of AA segments in copolymer chains were influenced by the cations type in copolymer solutions. The more stable the complexes of metal ions with the copolymer were, the larger the rigid of copolymer chains were, and the more difficult the confor-
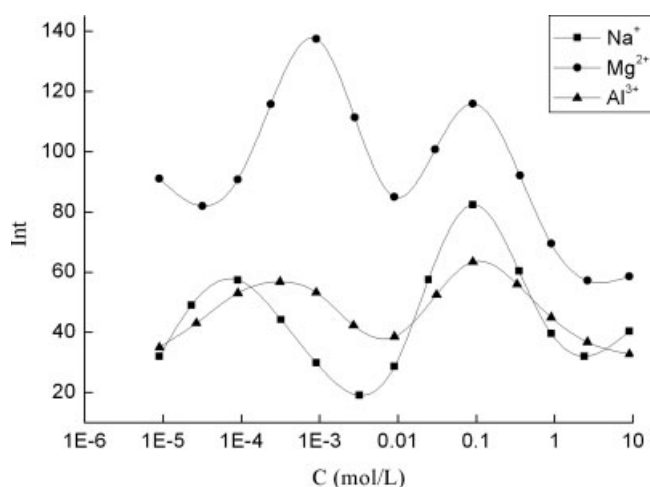

Figure 3 The florescence emission spectra (λ_{ex} = 295 nm) of poly(AA-co-ECATC) solutions(10^{-4} wt %) with different solvents. (1: ethanol; 2: tetrahydrofuran; 3: N,N-dimethylformamide; 4: water; 5: dimethylsulfoxide (1% attenuation); 6: methanol (1% attenuation)).

Figure 4 The fluorescence intensity for a series of poly (AA-co-ECATC) solutions (10^{-4} wt %) with different concentrations of Na^+, Mg^{2+} and Al^{3+}. ($\lambda_{ex}/\lambda_{em}$ = 295/375 nm).
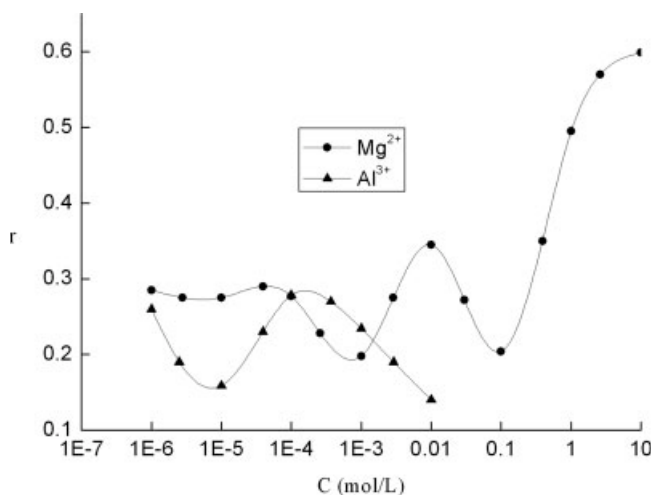
Figure 5 The fluorescence anisotropy for a series of poly (AA-co-ECATC) solutions (10^{-4} wt %) with different concentrations of Mg^{2+} and Al^{3+}. ($\lambda_{ex}/\lambda_{em}$ = 295/375 nm).

mation transition of copolymer chains was. So the addition of metal cations could affect the status and the conformation transition of copolymer chains, which could be probed by fluorescence techniques. Besides, it was well known that the most stable complexes would be made up of oppositely charged ions. Moreover, the greater the charge of ions, the greater the stability should be. On the other hand, when the concentrations of cations were large, there was a competition of metal ions for ligands; otherwise, there was a competition of ligands for metal ions. That was to say, the coordination number would redistribute and tend to average anyway.[32]

When the concentration of metal ions was low, the quantity of the copolymer in solutions was relatively high. Because of the surplus of ligands in copolymer, there existed the phenomenon of ligands contending for metal ions. As a result, the copolymer chains extended and the fluorescence intensity increased and the fluorescence anisotropy decreased. Along with the increasing of metal ions concentration, the ligands on copolymer chains would decrease, and copolymer chains coiled gradually. When metal ions concentration reached a certain degree, almost all the ligands on copolymer chains were chelated, thus, copolymer chains coiled further, and the fluorescence intensity of solutions reached to a minimum, and the fluorescence anisotropy increased to a maximum. When the concentration of metal ions went on to increase, metal ions would fight for ligands, and the ligands would distribute again. The intermolecular and intramolecular interaction were disturbed, thus the relative stability was destroyed. As a result, copolymer chains extended gradually, the fluorescence intensity and the fluorescence anisotropy of the solution increased and decreased relatively.

When the concentration of metal ions increased to a certain degree, another chelate with lower coordination number was formed, then, copolymer chains coiled and the conformation transition of it became difficult again, thus, the fluorescence intensity and the fluorescence anisotropy of the solution decreased and increased, respectively. In a word, along with the change of metal ions concentration, the chelates between metal ions and the copolymer would transform from high to low coordination numbers gradually, which was the result of the competing chelation between metal ions and ligands. When the concentration of metal ions increased, the competing for the chelation strengthened, and the extending degree of copolymer chains enhanced again. Therefore, the conformation transition of copolymer chains changed from extending to coiling to extending. Correspondingly, the curves of the fluorescence intensity and the fluorescence anisotropy waved.

From Figure 4, it could be seen that Al^{3+} concentration corresponding to the first minimum in the fluorescence intensity curve was larger than those of Na^+ and Mg^{2+}. Because there was a priority-choosing rule when more than one ligand existed, the hydration of Al^{3+} could not be negligible when the concentration of the copolymer was very dilute. Because of its strong ability of hydration, the ability of Al^{3+} chelation was weakened, therefore, the number of Al^{3+} chelating with the copolymer decreased. As a result, the concentration of the first minimum was high. At the same time, because the stability of Al^{3+} chelate was high and the chelate was difficult to break down, copolymer chains coiled strongly. Thus the fluorescence intensity curve of Al^{3+} was low.

For Mg^{2+}, its hydration ability was weaker than that of Al^{3+}. And because the copolymer was a polydentate ligand, it could form more stable complexes than other monodentate analogs generally, as a result, the chelation ability of Mg^{2+} with copolymer was stronger than its hydration ability. So Mg^{2+} concentration corresponding to the first minimum in the fluorescence intensity curve was lower than that of Al^{3+}. Moreover, the chelate stability of Mg^{2+} was weaker than that of Al^{3+}, so Mg^{2+} chelate were easy to produce and destroy. This made the competition for chelation between Mg^{2+} and the copolymer strong relatively, consequently copolymer chains extended. Therefore, the fluorescence intensity curve of Mg^{2+} was high.

For Na^+, its abilities of hydration and chelation were all weak. However, because the copolymer was a polydentate ligand, it could form more stable complexes than other monodentate analogs, thus Na^+ was inclined to chelation. Because of the weak stability of Na^+ chelate, the effect of Na^+ on the coiling and extending of copolymer chains was low. As a

result, ion strength became the main factor that affected the conformation transition of copolymer chains, which would be discussed later in detail.

In brief, the exceptional phenomenon of the fluorescence intensity curves of Mg^{2+} and Al^{3+} chelate were caused by the hydration effects of them in dilute aqueous solutions. The waved curves of the fluorescence intensity were the results of the change of coordination numbers between metal ions and the copolymer.

In a word, the stability of the chelate increased as the increasing of ions charge, and the exception may be attributed to the competition between the chelation and the hydration.

To further prove above illuminations, FTIR subtraction spectroscopy of different concentrations Mg^{2+} was carried out. The results were shown in Figure 6. From Figure 6, it could be seen that the absorbing peaks of poly(AA-co-ECATC) solution (10^{-4} wt %) without Mg^{2+} [Fig. 6(a)] observed at 1749 cm^{-1} and 1662 cm^{-1} corresponded to 1 and 2 C=O in the structure of Figure 7 (I). After the addition of Mg^{2+}, there were many new peaks in FTIR subtraction spectroscopy, some of them were upward shift or downward shift of the old peaks, and some of them were new peaks due to the addition of Mg^{2+}.

The absorption bands at 1643 cm^{-1} [Fig. 6(b)], 1645 cm^{-1} [Fig. 6(c)] and 1653 cm^{-1} [Fig. 6(d)] ascribed to 1 C=O and 1691 cm^{-1} [Fig. 6(b)], 1691 cm^{-1} [Fig. 6(c)] and 1695 cm^{-1} [Fig. 6(d)] ascribed to 2 C=O in the structure of Figure 7 (II). The 1 C=O in the structure of Figure 7 (III) can be designated to 1580 cm^{-1} [Fig. 6(b)], 1577 cm^{-1}
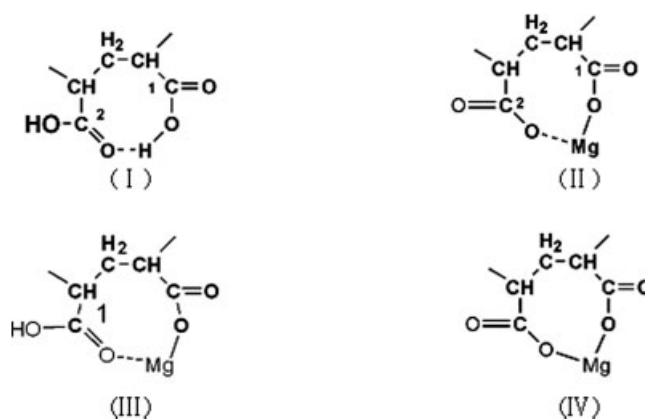

Figure 7 The sketch of intramolecular interaction ((I)), interaction between copolymer and Mg^{2+} ((II), (III), (IV)).

[Fig. 6(c)], and 1575 cm^{-1} [Fig. 6(d)]. The absorption bands at 1541 cm^{-1} [Fig. 6(b)], 1542 cm^{-1} [Fig. 6(c)] and 1543 cm^{-1} [Fig. 6(d)] are attributed to the C=O in the structure of Figure 7 (IV). When the Mg^{2+} concentration was 0.01 mol/L, the absorbing peak at 1749 cm^{-1} [Fig. 6(c)] was the lowest in FTIR subtraction spectroscopy. The concentration corresponded to the minimum in the fluorescence intensity curve where chelate was formed. From the above analysis, it could be concluded that there was new force between Mg^{2+} and the copolymer, i.e., Mg^{2+} could interact with copolymer. This could further prove the chelation between metal ions and the copolymer.

The effect of metal ions radius on the conformation transition of copolymer chains

Figures 8 and 9 showed the effects of concentration of metal ions with same charge and different radius on the fluorescence intensity and fluorescence anisotropy. From above two Figures, it could be found that when the concentrations of metal ions changed from low to high, the curves of the fluorescence intensity and the fluorescence anisotropy were waved also. The reason for the phenomenon was the same as the above illustrations, i.e., the conversion of chelates with different coordination numbers. It was well known that the smaller the ions radius, the greater the stability should be.[32] For Ba^{2+}, due to its big radius, the ability of chelation with the copolymer was weak, so ion strength became the main factor that affects the conformation transition of the copolymer chain. The addition of Ba^{2+} made the static electricity exclusion interaction of the copolymer chain decrease, thus the copolymer chain coiled and the fluorescence intensity of the solution was low. The hydration ability of Mg^{2+} was stronger than that of Ca^{2+}, so the chelation ability of Mg^{2+} with the copolymer was weakened more than that of Ca^{2+}, besides, Mg^{2+} chelates with the copolymer were
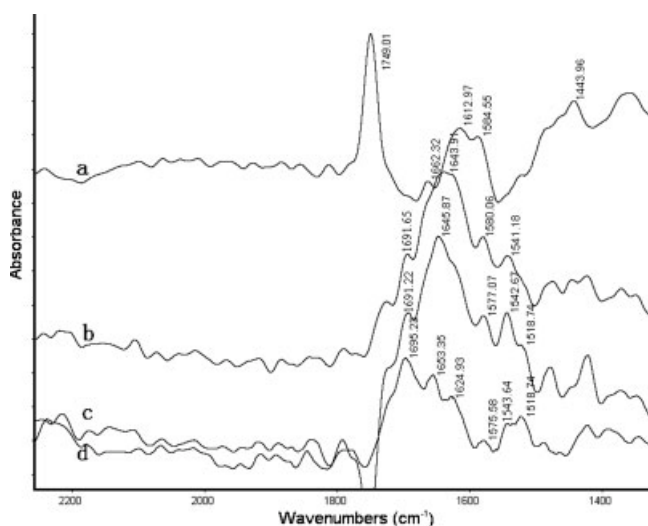

Figure 6 The FTIR subtraction for a series of poly(AA-co-ECATC) solutions (10^{-4} wt %) with different concentrations of Mg^{2+}. (a: No Mg^{2+}; b: 0.001 mol/L Mg^{2+}; c: 0.01 mol/L Mg^{2+}; d: 0.09 mol/L Mg^{2+}).
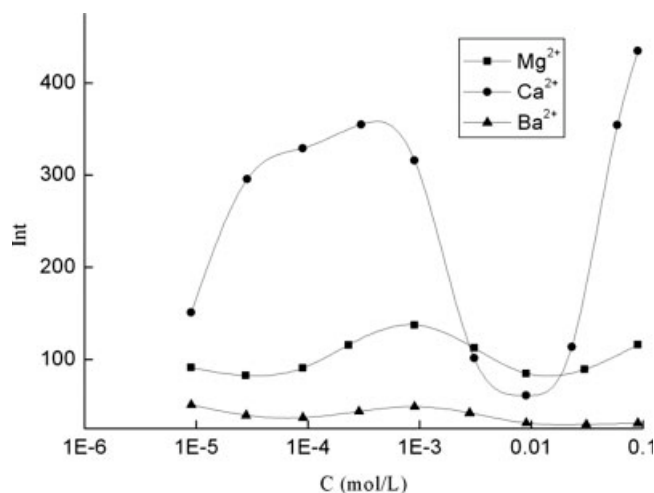
Figure 8 The fluorescence intensity for a series of poly(AA-*co*-ECATC) solutions (10^{-4} wt %) with different concentrations of Mg^{2+}, Ca^{2+}, and Ba^{2+}. ($\lambda_{ex}/\lambda_{em}$ = 295/375 nm).

Figure 10 The FTIR subtraction for a series of poly(AA-*co*-ECATC) solutions (10^{-4} wt %) with different concentrations of Ca^{2+}. (a: No Ca^{2+}; b: 9×10^{-6} mol/L Ca^{2+}; c: 10^{-3} mol/L Ca^{2+}; d: 0.01 mol/L Ca^{2+}).

more stable than those of Ca^{2+} and harder to destroyed. As a result, the copolymer chains coiled and the fluorescence intensity of Mg^{2+} solution was lower than that of Ca^{2+}.

To further prove the validity of the above illustrations, the FTIR subtraction spectroscopy of different concentrations of Ca^{2+} was carried out. The results were shown in Figure 10. From Figure 10, it could be seen that there were many new peaks in FTIR subtraction spectroscopy, some of which were upward shift or downward shift of the old peaks, and some of which were new peaks due to the addition of Ca^{2+}. The appearance of absorption bands at 1714 cm^{-1} [Fig. 10(b)], 1701 cm^{-1} [Fig. 10(c)], and 1701 cm^{-1} [Fig. 10(d)] attributed to 1 C=O in the structure of Figure 11 (V). The absorption bands at
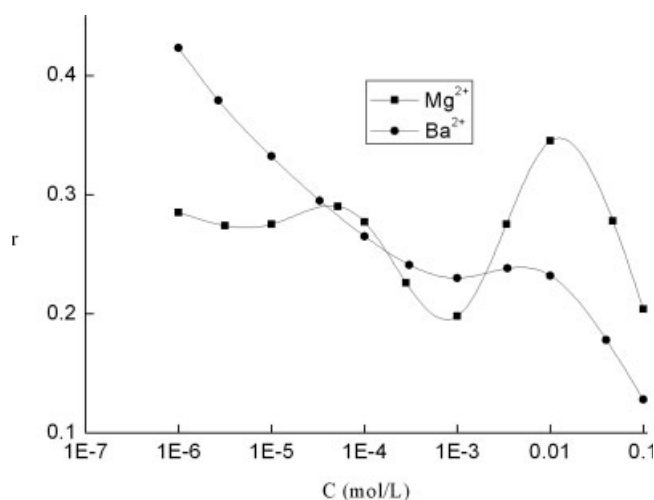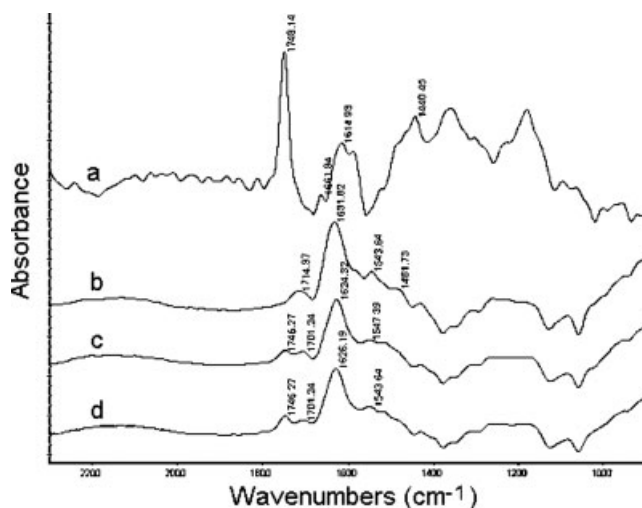
1631 cm^{-1} [Fig. 10(b)], 1624 cm^{-1} [Fig. 10(c)], and 1626 cm^{-1} [Fig. 10(d)] ascribed to C=O in the structure of Figure 11 (VI). The C=O in the structure of Figure 11 (VII) can be designated to 1543 cm^{-1} [Fig. 10(b)], 1547 cm^{-1} [Fig. 10(c)], and 1543 cm^{-1} [Fig. 10(d)].

From the above analysis, we found the direct evidence for the interaction between Ca^{2+} and copolymer and it could be concluded that there was new force between Ca^{2+} and copolymer. This could further prove the chelation between metal ions and copolymer.

In all, the stability of the chelates decreased with the increase of ions radius, and the exception could be the result of the synergetic effect of the chelation and the hydration. For Ba^{2+}, whose ability of chelation was weak, the ion strength was the main factor that affected the stability of chelates.

The effect of outer-layer electron construction of metal ions on the conformation transition of the copolymer chain

Figure 12 showed the effects of the concentration of metal ions with different outer-layer electron constructions on the fluorescence intensity. From Figure 12, it could be found that the fluorescence inten-
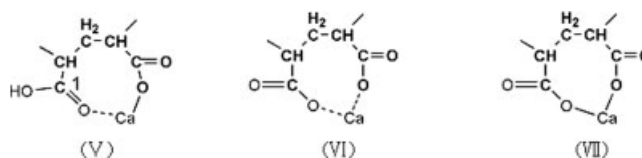

Figure 9 The fluorescence anisotropy for a series of poly (AA-*co*-ECATC) solutions (10^{-4} wt %) with different concentrations of Mg^{2+} and Ba^{2+}. ($\lambda_{ex}/\lambda_{em}$ = 295/375 nm).

Figure 11 The sketch of interaction between copolymer and Ca^{2+} ((V), (VI), (VII)).
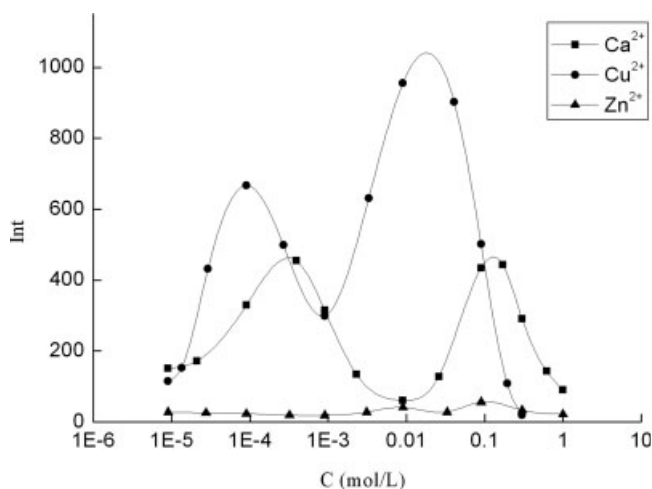
Figure 12 The fluorescence intensity for a series of poly (AA-*co*-ECATC) solutions (10^{-4} wt %) with different concentrations of Ca^{2+}, Cu^{2+}, and Zn^{2+}. ($\lambda_{ex}/\lambda_{em} = 295/375$ nm).

sities of Cu^{2+}, Ca^{2+} and Zn^{2+} in dilute aqueous solutions decreased, successively, and the fluorescence intensity of Cu^{2+} changed sharply. It was reported in the literature[32] the stronger the ions' polarization and deformability, the greater the stability should be. For Cu^{2+} and Zn^{2+}, the outer-layer electron constructions were 9–17e and 18e, respectively. The abilities of their polarization and deformability were stronger than those of Ca^{2+}, which outer-layer electron construction was 8e. Therefore, the chelation abilities of Cu^{2+} and Zn^{2+} with the copolymer were stronger than that of Ca^{2+}, and the stabilities of chelates were higher also. As a result, copolymer chains coiled strongly and the fluorescence intensity of the solution decreased. The reason for the special change of the fluorescence intensity of Cu^{2+} solution was certainly related to the fact that there was a single electron in the outermost layer of Cu^{2+}. When excited, the single electron could

leap and transmit fluorescence.[33] The waved curves of the fluorescence intensity were also the results of the change of coordination numbers between metal ions and the copolymer.

Obviously, for Ca^{2+}, Cu^{2+}, and Zn^{2+}, the stability of chelates increased successively. The reasons for the behavior may be attributed to the effect of the ion polarization and the deformability. The exception of Cu^{2+} might be due to the leap of the excited single electron in the outermost layer.

The effect of pH of solution on the conformation transition of the copolymer chain

Figure 13 showed the effects of pH on the conformation transition of the copolymer chain. From Figure 13, it could be seen that when the pH changed from 1 to 8, the fluorescence intensity increased on the whole despite that the curve was waved, and when the pH was larger than 9, the fluorescence intensity of the solution decreased rapidly. The possible cause for the phenomena could be attributed to two reasons. On the one hand, when pH was low, copolymer chains in the solution coiled owing to the intra- and intermolecular hydrogen bonds. Therefore, the fluorescence intensity of the solution was weak. Along with the increasing of pH, the AA segments in the poly(AA-*co*-ECATC) began to ionize and produce —COO^-. Under the influence of static electricity exclusion, the polymer chain extended gradually. The higher the pH was, the stronger the static electricity exclusion interaction was, the more stretched the copolymer chain was, the higher the fluorescence intensity was. The waved change of the fluorescence intensity was caused by the chelation between Na^+ and the copolymer, just as illustrated in Figure 4. On the other hand, when pH of the solution was larger than 9, the ion strength increased, the exclusion force among the negative charge group
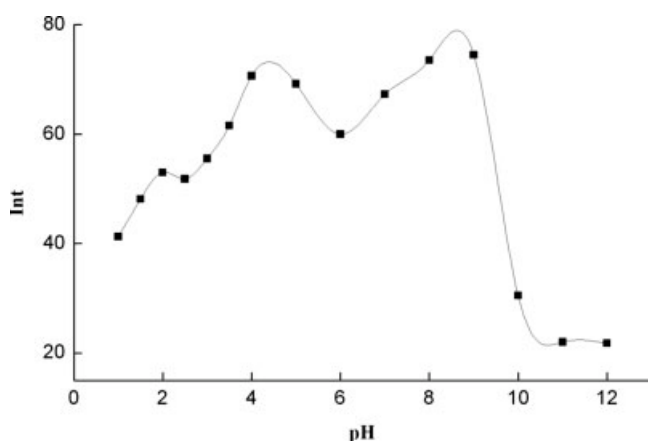

Figure 13 The fluorescence intensity of poly(AA-*co*-ECATC) solutions (10^{-4} wt %) with different pH. ($\lambda_{ex}/\lambda_{em} = 295/375$ nm).
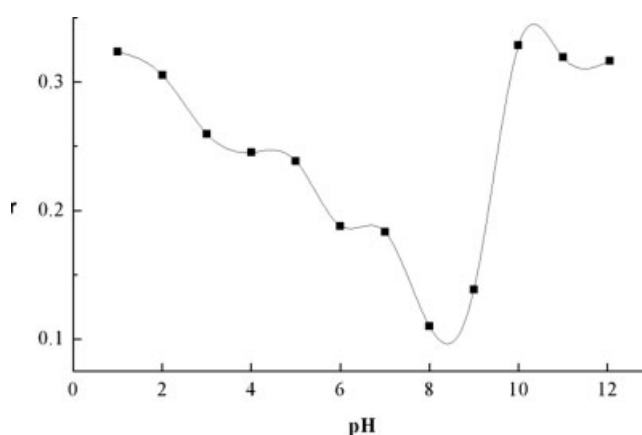

Figure 14 The fluorescence anisotropy of poly(AA-*co*-ECATC) solutions (10^{-4} wt %) with different pH. ($\lambda_{ex}/\lambda_{em} = 295/375$ nm).

decreased, as a result, the copolymer chains coiled and the fluorescence intensity decreased. When pH went to increase, the static electricity exclusion vanished completely due to the high ion strength, thus the copolymer chain became compact globule structure, and the fluorescence intensity reached the minimum. The results were in agreement with the previous literature.[34]

The above phenomena could also be proved by the curve of fluorescence anisotropy (Fig. 14) on the same conditions. The result was in accordance with the curve of the fluorescence intensity.

CONCLUSIONS

A novel salts- and pH-responsive copolymer, poly (AA-*co*-ECATC), was prepared by radical polymerization in methanol at room temperature with Vc-H_2O_2 as an initiator system. The prepared copolymer possessed the fluorescence properties due to the introduction of ECATC, and it was employed to study the conformation transition of copolymer chains in solutions. The results indicated that metal ions with different charges, radius and outer-layer electron constructions and pH values could affect the conformation transition of copolymer chains in dilute aqueous solutions. When metal ions concentrations and pH values changed, the conformation transition of the copolymer chains changed from extending to coiling to extending because of the change of coordination numbers between metal ions and the copolymer, correspondingly, the fluorescence intensity and the fluorescence anisotropy curves of the copolymer solution waved. The above conclusions were also confirmed by FTIR Subtraction spectroscopy.

The more stable of the chelates, the slower the conformation transition of copolymer chains was, and then the lower the fluorescence intensity was. For the fluorescence intensity curves, it could be concluded that stabilities of the chelates between metal ions and the copolymer were as followings: for Na^+, Mg^{2+} and Al^{3+}, the stability of the chelates increased as the increasing of ions charge, and the exception could be attributed to the competition between the chelation and the hydration. For Mg^{2+}, Ca^{2+}, and Ba^{2+}, the stability of the chelates decreased with the increase of ions radius, and the exception could be result of the synergetic effect of the chelation and the hydration. For Ba^{2+}, whose ability of chelation was weak, the ion strength was the main factor that affected the stability of chelate. For Ca^{2+}, Cu^{2+}, and Zn^{2+}, the stability of chelates increased successively. The reasons for the behavior may be attributed to the effect of the ion polarization and the deformability. The exception of Cu^{2+} might be due to the leap of the excitated single electron in the outermost layer.

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