Unexpected Fluorescence Emission of Poly(α,β-L-malic Acid) in Aqueous Medium

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ABSTRACT: Unexpected fluorescence of poly(α,β-L-malic acid) (α,β-PMA) without traditional fluorophore was observed firstly. This fluorescent polymer was synthesized via melt polycondensation of L-malic acid. The polymer was characterized by gel permeation chromatography (GPC), nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), thermogravimetry (TG), Fourier transform infrared spectroscopy (IR), Fourier transform Raman spectroscopy (Raman), and X-ray powder diffractometry (XRD). The high molecular weight α,β-PMA was synthesized by the optimum polycondensation at 130°C for 15 h, followed by fractional precipitation with diethyl ether and petroleum ether. The degree of branching of α,β-PMA was from 10% to 20% according to the reaction condition. Terminal group of α,β-PMA was mainly hydroxycarboxylic group companied with a few —CH=CHCOOH groups owing to dehydration of a normal terminal during the melt polycondensation. A fluorescence emission maximum of α,β-PMA in water appeared at 420 nm when it was excited at 340 nm. Further study indicated that the fluorescence intensity was concentration-dependent, pH-dependent, and molecular-weight-dependent. The fluorescence formation may result from multichain aggregations, which was formed readily in aqueous solution due to intermolecular hydrogen bonds between branched α,β-PMA. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 1640–1647, 2007

Key words: association; fluorescence; biopolymers; biocompatible luminescent material; polycondensation

INTRODUCTION

Poly(L-malic acid) (PMA) has attracted a significant interest because it can be used as a pro-drug or in drug-delivery system.[1] PMA is a water-soluble, biodegradable, and bioabsorbable polymer without toxicity. Its degradation product, L-malic acid, can be metabolized in the mammalian tricarboxylic acid cycle.[2] Carboxyl group in the side chain can readily react with other functional groups. Thus, small molecule drugs can be introduced into the polymer chain.[3] In addition, the polymer can gelate other biopolymers such as collagen.[4]

A small quantity of poly(β-L-malic acid) (β-PMA) was obtained directly from natural resource and extracted from fermentative production.[5–10] Poly(α-L-malic acid) (α-PMA) and β-PMA with a high molecular weight were mainly synthesized by ring-opening polymerization.[11–23] However, many steps including repeated purification were involved in the reaction cycle and the total yield was very low (<20%).[23] To simplify the reaction steps and increase the yield, a direct polycondensation as a one-step reaction was applied to obtain poly(α,β-L-malic acid) (α,β-PMA). However, the product was in a wide molecular weight distribution, with a number average molar mass ($\overline{M}_n$) of 2800 g/mol and a weight average molar mass ($\overline{M}_w$) of 5300 g/mol.[24–28] In our laboratory, α,β-PMA having a narrow molecular weight distribution was prepared by direct polycondensation under the optimum condition. The properties and its branched structure were characterized in detail. Interestingly, a fluorescence phenomenon of α,β-PMA in water was firstly observed during the investigation.

Recently, strong fluorescence of poly(amido amine) dendrimer (PAMAM) and poly(propyleneimine) dendrimer (PPI) without any traditional fluorophore was observed.[29,30] Moreover, the fluorescence could be displayed from inorganic/PAMAM nanocomposite with a suitable morphology.[31] According to previous reports, a part of β-PMA existed as multichain aggregates with a dimension of about 100 nm in aqueous solution,[32] which was similar to morphology of PAMAM dendrimers in water.[33] A mechanism for the fluorescence of α,β-PMA would be similar with that of PAMAM and PPI. Actually, a branched structure of α,β-PMA

might be formed during the direct polycondensation since L-malic acid has two carboxyl groups per molecule. The branched α,β-PMA contains a great deal of the carboxylic, hydroxyl and carbonyl groups, which can interact each other to form many hydrogen bonds. Therefore, the functional groups may become crowded and cause α,β-PMA to take a densely packed structure like PAMAM dendrimers. The fluorescence emitted from α,β-PMA was elucidated and the mechanism of fluorescence formation was investigated in the present work.

EXPERIMENTAL

Materials

L-Malic acid (purity > 99.5%, batch number 20050523) was obtained from Shanghai Yuanji Chemical (China). Anhydrous tin (II) chloride (purity > 98%) was purchased from Acros Organics. Deuterated acetone (purity > 99.9%) was purchased from Cambridge Isotope Laboratories. All of the other chemicals were of analytical purity and were used without further purification.

Synthesis of α,β-PMA

α,β-PMA was synthesized via melt polycondensation of L-malic acid catalyzed by anhydrous tin (II) chloride according to the modification of Kajiyama methods.[24] The reaction product was dissolved in acetone. The solution was first precipitated in diethyl ether to obtain the fraction I. The obtained supernatant was then precipitated in petroleum ether to afford the fraction II.

Characterization of α,β-PMA

^{1}H-NMR spectra and ^{13}C-NMR spectra were performed on a VARIAN model Mercury Vx300 at room temperature. Deuterated acetone was used as the solvent. Tetramethylsilane (TMS) was used as the internal standard. The molecular weight of α,β-PMA was estimated in tetrahydrofuran by gel permeation chromatography (GPC； Waters 1525 binary HPLC pump, Waters 2414 RI detector, column: Styragel HT2 + Styragel HT3 + Styragel HT4, standard: polystyrenes). The infrared (IR) spectra were recorded as KBr pellets on a Fourier transform infrared spectrometer (Bio-Rad FTS 6000). The Raman spectra were obtained by using a Fourier transform Raman spectrometer with a liquid nitrogen-cooled Ge-diode detector (Bruker RFS100/S). They were excited by 1064 nm line radiation from an Nd:YAG laser. Thermograms from −50°C to 300°C were measured by a differential scanning calorimeter (DSC; NETZSCH DSC 204) under a heating/cooling rate of 10°C/min or 2°C/min. Thermal properties were also determined with a thermogravimetric analyzer (TG; NETZSCH TG 209) under a heating rate of 10°C/min. The X-ray powder diffraction (XRD) was performed on an X-ray powder diffractometer (Rigakudmax) using monochromatized CuKα$_1$ radiation (λ = 1.5406 Å) in the 2θ range of 3–80°.

Fluorescence measurement of α,β-PMA

In the fluorescence experiment, Millipore water was used to prepare the α,β-PMA solution. α,β-PMA ($\overline{M}_n$ = 4500 g/mol) was used unless the other molecular weight samples were indicated. α,β-PMA was dissolved in water with concentration ranging from 0.1 to 25 mg/mL. 1M NaOH or 1M HCl was used to adjust the pH of the solutions (15 mg/mL) from 2 to 12, so that the concentrations were kept constant. The fluorescence spectra were measured at room temperature on a Fluoro Max-P fluorometer (JY). The fluorescence quantum yield was measured using quinine sulfate as a standard.

Particle size measurement of α,β-PMA aggregates in water

α,β-PMA with different molecular weights ($\overline{M}_n$ = 2300, 3500, 3600, 4500 g/mol) were dissolved in Millipore water to form the solutions with a certain concentration from 0.1 to 25 mg/mL. They were filtered through a cellulose film (pore size 0.45 μm). The solutions were measured on a laser particle-size analyzer (BI-90Plus, Brookhaven Instruments) to observe the formation of α,β-PMA aggregates. Effect of pH on particle size was also investigated.

RESULTS AND DISCUSSION

Synthesis of α,β-PMA

PMA can be synthesized through intermolecular dehydration of L-malic acid under a suitable condition. Due to similar reactivity of two carboxyl groups in each L-malic acid molecule, PMA via direct polycondensation was a random copolymer of α- and β-type L-malic acid residues, called α,β-PMA.[24–28] α,β-PMA from solution polycondensation had only a low molecular weight ($\overline{M}_n$ = 1300 g/mol).[26] Melt polycondensation may result in α,β-PMA with a relatively higher molecular weight. Reaction temperature for the melt polycondensation should be controlled lower than 150°C. When L-malic acid was heated up to 150°C, its intramolecular dehydration would take place to produce fumaric acid and maleic acid.[26] Anhydrous tin (II) chloride was used as a catalyst. Although four catalysts including tin powder, anhy-

Sample no.	Fraction	Time (h)	$\overline{M}_w$ (g/mol)	$\overline{M}_n$ (g/mol)	$\overline{M}_w/\overline{M}_n$	Yield (%)
Stirring type: Magnetic						
A	I	7	2400	2000	1.2	2
	II		2100	1900	1.1	73
Stirring type: Mechanical						
B	I	7	2800	2300	1.2	9
	II		2200	2000	1.1	73
C	I	9	4100	3500	1.2	9
	II		2500	2300	1.1	83
D	I	15	5400	4500	1.2	35
	II		2300	2100	1.1	57
E	I	20	4400	3600	1.2	25
	II		2500	2300	1.1	62

drous tin (II) chloride, tin (II) oxide, and tin (II) acetate, could be used to catalyze the melt polycondensation, anhydrous tin (II) chloride had a better catalytic efficiency.[24–28]

The molecular weight and yield of α,β-PMA are listed in Table I. It was found that α,β-PMA with the molecular weight of several thousands was synthesized by melt polycondensation of L-malic acid catalyzed with anhydrous tin (II) chloride at 130°C. The molecular weight was dependent on the reaction time. The $\overline{M}_w/\overline{M}_n$ of both fraction I and II was 1.1–1.2. It indicated that α,β-PMA with a narrow molecular weight distribution could be obtained by fractional precipitation. As seen in Table I, fraction II had a lower molecular weight than fraction I, but had higher yield. The replacement of magnetic stirring by mechanical stirring would result in an increase in the molecular weight and the yield. The reason was the effect of high viscosity in the reaction system. When the reaction time exceeded 5 h, a magnetic stirrer would not work well due to the high viscosity. The total yield of two fractions was 75–92%. In the synthesis of α,β-PMA, the reaction time had better be limited to about 15 h so as to obtain α,β-PMA with a high molecular weight and a high yield.

Structure of α,β-PMA

In ¹H-NMR spectrum, there were two peaks with approximately equivalent intensity at 5.5–5.6 ppm. The peak at 5.6 ppm was attributed to methine in α-type units, and the other peak at 5.5 ppm belonged to methine in β-type units.[13] Similarly, two methine peaks were also seen at 69.8–70.3 ppm in ¹³C-NMR spectrum. In ¹³C-NMR spectrum, carbonyl carbon of α-type ester (168.2 ppm) or β-type ester (169.0 ppm), carboxylic carbon of α-type side chain (169.5 ppm) or β-type side chain (170.8 ppm) could be observed. It was concluded that the polymer consisted of both

α- and β-type units in almost equivalent ratio, similar to previous results.[24,26]

The characteristic peaks of terminal groups could distinctly observed in the NMR spectra. In ¹H-NMR spectrum, two weak peaks near 4.6 ppm were ascribed to terminal methine.[34,35] The peak at 4.7 ppm was assigned to methine protons of —OCH*(COOH)-CH₂COOH, and the other at 4.5 ppm belonged to methine protons of —COCH*(OH)CH₂COOH and —COCH₂CH*(OH)COOH. It should be pointed out that a much weak peak near 6.8 ppm was observed in the ¹H-NMR spectrum. Referring to the ¹H-NMR standard spectra of fumaric acid,[36] the peak was assigned to olefinic hydrogen formed by intramolecular dehydration of L-malic acid residues in the terminal of the α,β-PMA molecular chain. This reaction would compete with the chain propagation reaction (i.e., intermolecular dehydration). The ratio of both terminals, terminal olefinic groups and terminal hydroxyl groups, could be calculated by the peak area of olefinic protons and that of methine protons of the terminal hydroxyl group in the ¹H-NMR spectrum. For the fraction I, the ratio of terminal hydroxyl groups was 80–95%, as listed in Table II, much higher than the result (40–70%) in the previous report.[37]

The pKa of α,β-PMA determined by acid-base titration was 4.0, a little lower than that of β-PMA (4.2).[38] This phenomenon may result from the branched structure. An increase of a branched unit means an increase of a carboxylic group accordingly to enhance the acidity of this polyester, while the number of structural units is kept constant. The degree of branching (DB) of α,β-PMA can be calculated by the peak areas of methine protons (or methylene carbons) in the inner part and in the terminal group in the ¹H-NMR (or ¹³C-NMR) spectra.[39,40] As shown in Table II, the DB of α,β-PMA was 9–22%, and increased with the reaction time. For the polymer from a AB₂-type monomer with two B groups of equal reactivity, its maximum DB is 50% accord-

Sample no.	$\overline{M}_n$ (g/mol)		Degree of branching[b] (DB) (%)		Terminal group ratio[c] (%)	
	GPC	^{1}H NMR[a]	^{1}H NMR	^{13}C NMR	Hydroxyl	Olefinic
A(I)	2000	1800	19	22	95	5
B(I)	2300	1900	18	20	95	5
C(I)	3500	2900	16	9	89	11
D(I)	4500	4300	13	10	80	20
E(I)	3600	3300	14	11	88	12

[a] $\overline{M}_n$ was estimated by the peak areas of the methine proton in the inner part and in the terminal group in the ^{1}H NMR spectra.

[b] Degree of branching was calculated respectively by using the characteristic peak areas of methine protons in the ^{1}H NMR spectra or those of methylene carbons in the ^{13}C NMR spectra.

[c] The ratio was calculated by the peak area of olefinic protons and that of methine protons linked with terminal hydroxyl groups in the ^{1}H NMR spectra.

ing to the statistical theory.[41–44] The DB of α,β-PMA is much less than 50%, mainly because the reactivity of a carboxyl group in L-malic acid will be reduced due to steric hindrance after one carboxyl group is esterified. In addition, the molecular weight of α,β-PMA could be determined not only by GPC, but also by the peak areas of methine protons in the inner part and in the terminal group in the ^{1}H-NMR spectra. As shown in Table II, the $\overline{M}_n$ calculated by ^{1}H-NMR was close to that from GPC.

The peak near 3270 cm^{-1} in IR spectrum indicated that hydrogen bond was formed between carbonyl and hydroxyl in α,β-PMA. Vibration absorption of ester carbonyl C=O occurred at 1749 cm^{-1} and several signals of ester C—O—C appeared at 1175, 1088, and 1055 cm^{-1}. As seen in Figure 1, Raman peaks of α,β-PMA were broadened obviously, compared with those of L-malic acid. It was connected with the formation of α,β-PMA polycondensate and the decrease of its structural symmetry. A strong peak attributed to methylene appeared at 2943 cm^{-1},

and a weak peak ascribed to methine of CHOH appeared at 2883 cm^{-1}. A medium peak of carbonyl was shown at 1749 cm^{-1}, but the characteristic peak of olefinic groups was not observed in the range of 1700–1600 cm^{-1}. The reason is that the terminal olefinic groups are rare in α,β-PMA. Another reason is the overlap between carbon–carbon and carbon–oxygen double bond signals.

Thermal properties and X-ray data of α,β-PMA

Thermal properties of α,β-PMA were measured by TG and DSC, and the results are shown in Figures 2 and 3. The thermal decomposition of α,β-PMA was initiated at about 200°C and ended near 270°C. Its thermal decomposition temperature determined by TG was 250°C and the corresponding enthalpy measured by DSC was 870 J/g. Marked endothermic peaks around 200–270°C in the DSC curve might be largely attributed to decomposition of chain structure of the polymer releasing one mole of water from each connecting site of the repeating units of
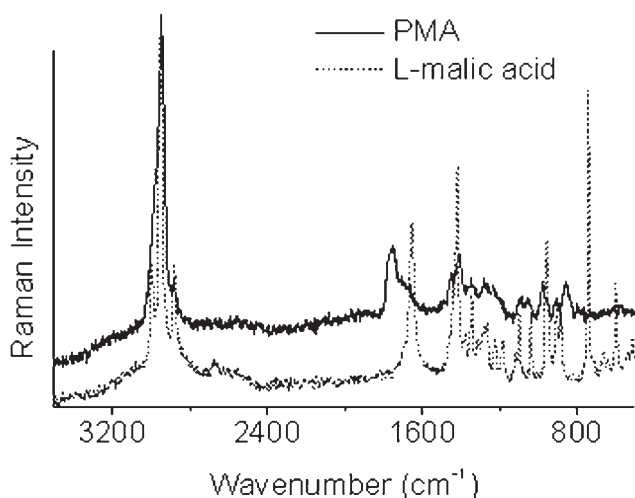

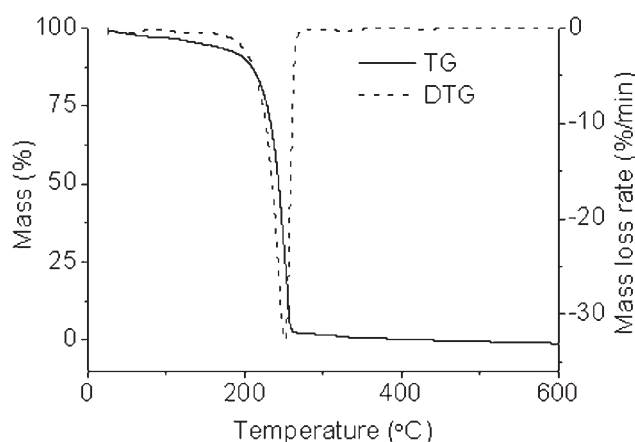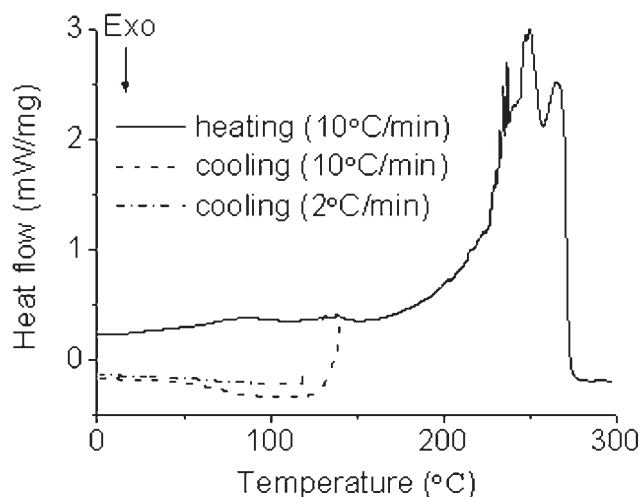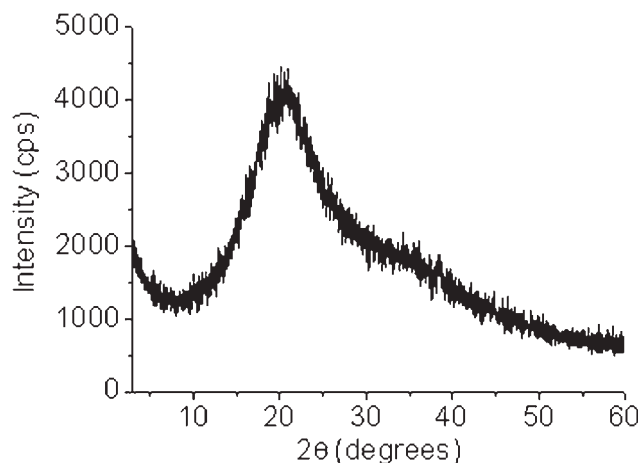**Figure 1** Raman spectra of L-malic acid and the fraction I of α,β-PMA from melt polycondensation at 130°C for 15 h.

Figure 2 The TG and DTG curves of the fraction I of α,β-PMA from melt polycondensation of L-malic acid at 130°C for 15 h.

Figure 3 The DSC curves of the fraction I of α,β-PMA from melt polycondensation of L-malic acid at 130°C for 15 h.

α,β-PMA.[45] The polymer existed as an amorphous state since no melting peak and crystallization peak was observed in the DSC curve. A probable reason for this phenomenon should be that a great deal of hydrogen bonds in α,β-PMA would prevent the chain segment from moving to arrange regularly. Furthermore, it was difficult for molecular chains with the branched structure to pack orderly to form crystals. It was also noted that the baseline drifted at 72°C in the second DSC heating curve. It might result from some order-disorder transformation caused by breakage of hydrogen bonds, since the glass transition temperature (T_g) in air was about 1°C according to the previous report.[27]

XRD was performed to further investigate crystallization information of α,β-PMA. Except a dispersion peak at around 20°, no diffraction peak was observed in the XRD spectrum of α,β-PMA (Fig. 4). It further confirms that α,β-PMA has no crystal at room temperature.

Fluorescence emission from α,β-PMA

Strong fluorescence emission from α,β-PMA in aqueous medium was observed although this polymer had no traditional fluorophore. The excitation and emission fluorescence spectra of α,β-PMA solution (25 mg/mL) are shown in Figure 5. α,β-PMA presented an excitation maximum at 340 nm and a fluorescence emission maximum at 420 nm. It was different from the NH$_2$-terminated PAMAM dendrimer, which has two excitation maxima and one fluorescence emission maximum.[30] It indicated that the polymer species and molecular structure significantly affected the fluorescence properties. For α,β-PMA with $\overline{M}_n$ of 4500 g/mol, the fluorescence quantum yield (ϕ) was about 0.09 (using quinine sulfate as a standard), lower than that of quinine sulfate (ϕ = 0.58, which was measured in 0.1M H$_2$SO$_4$ at an excitation wavelength of 350 nm at 22°C) and that of rodamine B (ϕ = 0.31, which was determined in water at excitation wavelength of 514 nm).[46,47]

Compared to the samples, the pure solvent has a much weak peak near 385 nm when excited at 340 nm. It confirms that the samples can show a fluorescence emission band at 420 nm. Furthermore, the fluorescence phenomenon of α,β-PMA was highly reproducible.

In the fluorescence spectra of α,β-PMA with different molecular weight, the fluorescence emission maximum appeared almost at the same position and the intensity increased gradually with increasing the $\overline{M}_n$ from 1900 g/mol to 3600 g/mol, as shown in Figure 6. It is noteworthy that α,β-PMA with $\overline{M}_n$ of 1900 g/mol presented a much weak emission band. The observation suggested that low molecular

Figure 4 X-ray powder diffraction spectrum of the fraction I of α,β-PMA from melt polycondensation of L-malic acid at 130°C for 15 h.
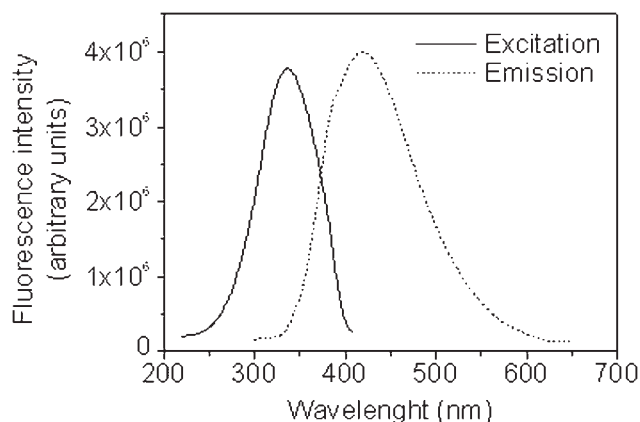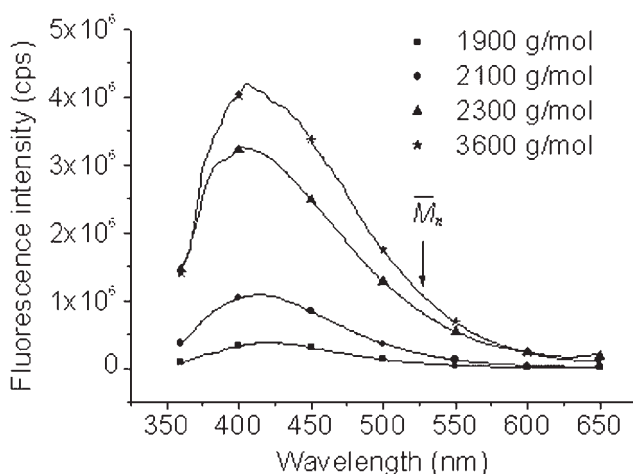

Figure 5 Fluorescence spectra of α,β-PMA (25 mg/mL) in Millipore water. Excitation spectrum was emitted at 420 nm and emission spectrum was excited at 340 nm.

Figure 6 Fluorescence spectra of α,β-PMA with different number average molar masses $\overline{M}_n$= 3600, 2300, 2100, 1900 g/mol from top to bottom) in the aqueous solution (15 mg/mL). Excitation: 340 nm.

weight was disadvantageous to fluorescence formation of α,β-PMA.

α,β-PMA solutions with different concentrations from 1 to 25 mg/mL were investigated. Results showed that the fluorescence intensity of α,β-PMA was concentration-dependent. The fluorescence of α,β-PMA was also pH-sensitive, as demonstrated in Figure 7. The fluorescence intensity decreased gradually with increasing the pH from 2.2 to 12, and the maximum was achieved at pH 2.2. It was noted that a fairly strong fluorescence was still emitted from α,β-PMA solution (15 mg/mL) even though the pH was increased to 12. It differed from the NH_2-terminated PAMAM dendrimer that emitted a strong fluorescence below pH 6 but did a much weak fluorescence over pH 6.[30] It might result from their difference in molecular structure. The reason for concentration-, molecular-weight-, and pH-dependence of this fluorescence intensity will be discussed later.

Three assumptions may be brought forward as the reason for fluorescence emission of α,β-PMA in water. The first assumption is that the chemical reaction took place in α,β-PMA solution has produced new fluorescence chemical species. Based on the previous reports, the possible by-products in the polycondensation process of α,β-PMA were fumaric acid, L-malic acid and its oligomers,[37] and the hydrolysis products of α,β-PMA were L-malic acid and its oligomers.[48] In our experiments, however, L-malic acid in water or fumaric acid (poor water-solubility) in ethanol did not show any detectable fluorescence emission maximum at 420 nm at the excitation wavelength of 340 nm. All results suggested that there was no new fluorescence chemical specie formed in α,β-PMA solution. The second assumption

is that a little olefinic group in the terminal group of α,β-PMA might induce the fluorescence emission due to the π-π conjugation between carbon–carbon double bond and adjacent carbon–oxygen double bond. But a series of monomers (methacrylic acid, methyl methacrylate, maleic acid and fumaric acid) were not fluorescent at 420 nm when excited at 340 nm. It indicated that a little olefinic groups should not be the reason for fluorescence emission of α,β-PMA. The third assumption is that a novel fluorescent center may be formed in α,β-PMA solution. It was known that multichain aggregates existed in aqueous β-PMA solution.[32] Similar to β-PMA, α,β-PMA was possible to form multichain aggregates in aqueous solution. The measurement with a laser particle-size analyzer showed that a part of α,β-PMA existed as aggregates with an average diameter of about 90 nm. Meanwhile, within the experimental error, there was little change in the aggregate dimension with increasing the $\overline{M}_n$ from 2300 to 4500 g/mol and the α,β-PMA concentration from 1 to 25 mg/mL. From further experiments, these aggregates always appeared in the aqueous α,β-PMA solution (15 mg/mL) even though the pH was adjusted from 2 to 12. Intermolecular hydrogen bond between carbonyl and hydroxyl from the nonionized carboxyl groups of different monomer units should be responsible to aggregate formation.[49] The fact implied that the hydrogen bond associations were very strong and the structure was probably organized in microdomains. Based on above discussion, it was believed that multichain aggregates readily formed in aqueous α,β-PMA solution should be the novel fluorescence center. According to the previous report, polystyrene and poly(vinyl naphthalene) exhibit the exceptional fluorescence arising from an eximer which is formed by strong interactions
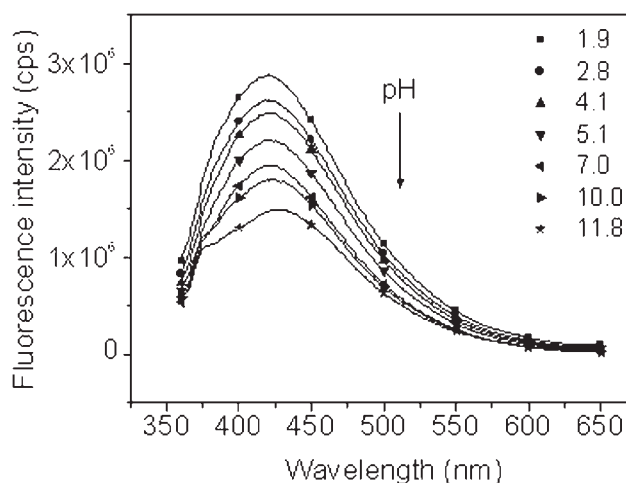
Figure 7 pH-dependent fluorescence of α,β-PMA (15 mg/mL). The wavelengths of excitation and emission were 340 nm and 420 nm, respectively.

between two neighboring plane structures such as phenyl and naphthyl.[50] Correspondingly, carbonyl groups will be packed in the multichain aggregates of α,β-PMA due to strong hydrogen bonds. The intermolecular carbonyl conjugation occurs between the neighboring molecules. The fluorescence emission probably arises from the π-π* electronic transition in this conjugated structure. It should be noted that the fluorescence mechanism of α,β-PMA is not entirely the same as that of polystyrene and poly (vinyl naphthalene) because the former has not traditional fluorophores but the latter has.

With increase of the molecular weight, the population of hydrogen bonds per polymer chain increased so that the quantity of multichain aggregates grew up. It results in the enhanced fluorescence intensity. The monomer and malate oligomers cannot produce fluorescence owing to no aggregate formation in the aqueous medium.

The fluorescence intensity of α,β-PMA was concentration-dependent, because the aggregates of α,β-PMA increase linearly with concentration. The ionization of α,β-PMA at high pH would lead to the enhanced repulsive interactions between the negatively-charged carboxyl groups,[32] so that the aggregates might be destroyed, especially in the diluted solution. For examples, the fluorescence could not be observed in 0.1 mg/mL α,β-PMA at pH 3.8 (or 1.0 mg/mL α,β-PMA at pH 12) while the aggregates were hardly detected by the laser particle-size analyzer. In the higher concentration solution, however, a part of α,β-PMA multichain aggregates can still survive at basic pH. Because α,β-PMA could form the aggregates at a higher concentration, only outer shell of the aggregates were ionized when the pH was adjusted. As a result, although the fluorescence of α,β-PMA with a high concentration was pH-dependent, it was still strong even when the pH increased up to 12.

It is noteworthy that α,β-PMA had a large Stokes shift (the difference in wavelength between the emission and excitation maximum), ~ 80 nm. Since α,β-PMA is surrounded by lots of water molecules, its excited state will reduce the energy through rearrangement of the solvent "cage" around the polymer prior to emission. More importantly, the fluorescence emission is attributed to the π-π* electronic transition in the conjugated carbonyl groups of multichain aggregates that formed in aqueous α,β-PMA solution, and hence the excited state will undergo charge transfer between the conjugated structures.

CONCLUSION

Branched α,β-PMA with random sequences of both α- and β-type units in almost equivalent ratio was synthesized by melt polycondensation of L-malic acid. α,β-PMA existed as an amorphous solid powder at room temperature. In the α,β-PMA molecular chain, strong hydrogen bonds were formed between carbonyl and hydroxyl groups. The molecular weight and the chemical structure of α,β-PMA were dependent on the reaction time. With increasing the reaction time, the primary structure of α,β-PMA was unchanged; however, the DB was changed from 9% to 22% and the content of the terminal hydroxyl group was changed from 80% to 95%. α,β-PMA with a high molecular weight and a narrow distribution ($\overline{M}_n$ = 4500 g/mol, $\overline{M}_w$ = 5400 g/mol) could be obtained by the optimum polycondensation at 130°C for 15 h.

α,β-PMA emitted fluorescence in water. It presented an excitation maximum at 340 nm and a fluorescence emission maximum at 420 nm. A fluorescence emission maximum appeared at the same position for different molecular weights of α,β-PMA. However, the intensity increased with the molecular weight. The fluorescence intensity increase linearly as the α,β-PMA concentration increase and the pH decrease. Multichain aggregates, which resulted from intermolecular hydrogen bond between carbonyl and hydroxyl from the nonionized carboxyl groups of different monomer units, should be mainly responsible to the fluorescence formation in α,β-PMA solution.

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