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Synthesis and Striking Fluorescence Properties of Hyperbranched Poly(amido amine)

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Hyperbranched poly(amido amine) (h-PAMAM) was synthesized from different feed ratios of diethylene triamine and methyl acrylate by the simple one-pot and commercial synthesis method. Reaction procedures and products were intensively studied by FTIR, inherent viscosity and fluorescence techniques. The ill-structured h-PAMAM shared similar chemical and physical properties with well defined poly(amido amine) (PAMAM) dendrimers in generation 2 or 3. Its strong fluorescence properties were influenced by pH values, solvents, concentrations, terminal groups and other factors.

Keywords: hyperbranched; PAMAM; fluorescence

1 Introduction

As the two most important subsets of the fourth major class polymers (1, 2), both dendrimers (3–6) and hyperbranched polymers (7–11) have exciting architectures that have been developed in the recent past. They are unique polymers with high branching density, repeated branched building blocks and a large number of terminal units. If made from similar monomers, hyperbranched polymers and dendrimers have many similarities in composition, structure and property: a large number of functional groups at their periphery, lower viscosity and better solubility compared with linear polymers. These remarkable features have paved the way for a new class of materials with promising applications in different fields (12–14), ranging from chemistry to physics, biotechnology, and life science. On the other hand, there are some distinct differences (1) between the two polymers. For example, dendrimers have controllable structures without defects by chemical reactions, while hyperbranched polymers exhibit random branched structure and their functional groups are not located at ordered positions. As a result, perfect dendrimers always require stepwise synthesis (1) via divergent or convergent approaches and repeated purification, which extremely consumes time and greatly enhances commercial cost. In contrast, hyperbranched polymers are usually prepared by one-pot synthesis from specific monomers with branching potential. In this case, hyperbranched polymers are sometimes preferred to be used instead of dendrimers.

Among the dendrimer families, poly (amidoamine) dendrimers (PAMAM) (12, 15) are the first to be commercialized, and represent the most extensively characterized and best understood series at this time. PAMAM composes of a central core (e.g. NH_3, ethylene diamine), repeated amides and tertiary amines branching unites throughout the interior, and a high density of functional groups (e.g. NH_2, $COOCH_3$ and OH) at the outmost shell. Owing to their commercial availability, well-defined architectures and biocompatible abilities, PAMAM materials have attracted much attention in a variety of fields (12, 16, 17). However, just as others, these dendrimers are synthesized by multiple steps. As a result, based on the knowledge of properties of hyperbranched polymers, some research groups have developed modified methods (18–20) to prepare aliphatic hyperbranched poly (amido amine) used as a substitute for PAMAM in some fields. As expected, these routes are more simple and efficient in comparison with that of PAMAM, but they still require designing specific monomers for building up hyperbranched structures.

Another exciting feature of PAMAM is its intrinsic fluorescence property (21–26) under certain conditions. From the classic viewpoint (27, 28), the fluorescence signals of organic molecules (for example, fluorescein, anthrance, and other aromatic hydrocarbons) are usually from some units

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possessing extensive delocalized system of conjugated double bonds, leading to a relatively rigid structure. As a result, the fluorescence property of PAMAM is rather interesting in the absence of these special structures. Recently, several researchers (24–26) have reported the fluorescence property of different types of PAMAM. Larson et al. (24) studied fluorescence emission from carboxyl-terminated PAMAM via two fluorescent techniques, i.e., excitation-emission matrices and lifetime. They suggested that the weak but detectable fluorescence is due to an n-π^* transition from amido groups throughout the dendritic structure. Lee et al. (25) reported the strong photoluminescence from OH-terminated PAMAM when oxidated by $(NH_4)_2S_2O_8$, while the amine-terminated PAMAM showed weak luminescence under the same conditions. Wang et al. (26) found a strong fluorescence emission from amine-terminated PAMAM under acidic conditions. They assumed that backbones of PAMAM, not terminal groups as Lee addressed (25), played the key role to form the fluorescence center. However, to date, the satisfying conclusions have not been provided for the luminescence property of PAMAM.

In this paper, we developed an easy and one-pot method to prepare hyperbranched poly (amido amine) (h-PAMAM) (Figure 1) with striking fluorescence properties. This method was applied to widely commercialized monomers diethylene triamine and methyl acrylate via repeated Michael addition and amidation. The h-PAMAM showed analogous physicochemical properties as well as the backbone and molecular weight in comparison with PAMAM in generation 2 (G2) or generation 3 (G3). It is more important that the h-PAMAM also has strong intrinsic fluorescence properties in acidic solutions which can be directly observed under the radiation of the UV-lamp. Herein, with the help of some experiments, a further understanding was formed for the fluorescence property of h-PAMAM.

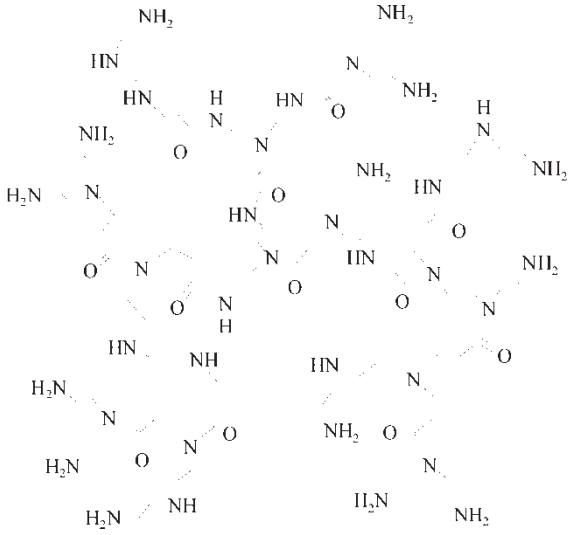


Fig. 1. Architecture of hyperbranched PAMAM.

2 Experimental

2.1 Materials

Diethylene triamine (DETA), methyl acrylate (MA), acrylic acid, formic acid (88%) and formaldehyde (37%) were purchased from Shanghai Chemical Reagent Company and used without further purification. All the other chemicals were reagent grade unless otherwise described.

2.2 Synthesis of h-PAMAM

To a one-neck flask was added a solution of 20.6 g diethylene triamine in 25 mL of methanol. Then, 20.6 g methyl acrylate was added dropwise into the reaction system. The mixture was stirred at room temperature for 48 h. Then the flask was equipped onto a rotary evaporator to remove the methanol under the vacuum at room temperature. The mixture was then reacted for 1 h at 60°C, 1 h at 80°C, 1.5 h at 100°C, 1.5 h at 120°C, and 3 h at 140°C under the rotary evaporator in vacuum. The yellow product was precipitated in diethyl ether three times and kept in the sealed container.

2.3 Synthesis of Tertiary Amine Terminated h-PAMAM

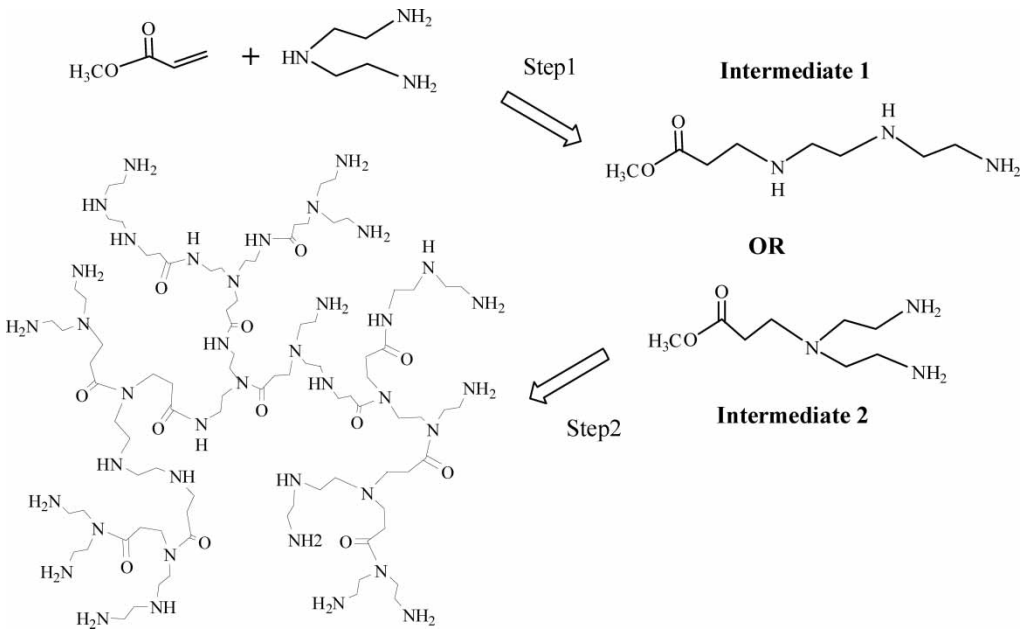
To 10 g formic acid (88%), which was cooled by cold water, was gradually added 0.5 g h-PAMAM and then 10 g formaldehyde (37%). The mixture was refluxed at 90–100°C for about 9 h. The solvent was removed under vacuum and the resulting slurry was dried at 40°C under vacuum.

2.4 Synthesis of Carboxyl Terminated h-PAMAM

Absolute excess acrylic acid was added dropwise into the methanol solution of h-PAMAM. The reaction was stirred at room temperature for two days. The product was precipitated in diethyl ether three times and kept in the sealed container.

2.5 Instrument Analyses

Fourier Transform Infrared (FTIR) spectroscopy measurements were preformed on a Nicolet Magna-550 spectrometer and the samples were prepared by dropping and drying the methanol solution onto a NaCl plate. ^{1}H-NMR and ^{13}C-NMR measurements were conducted on a Philips DMX500 Spectrometer using $CDCl_3$ as solvent. Viscosity experiments were performed in the Ubbelohde Capillary Viscometer. The samples in 5 mg/mL methanol solution were kept for 20 min in a 25 $\pm$ 0.1°C water bath before the test. Gel Permeation Chromatography (GPC) measurements were carried out on a HP series 1100 Chromatograph equipped with Zorbax HV1618 columns and RI/UV dual-mode detectors. The elution rate of the sample (1 mg/mL in H_2O) was 1 mL/min and the calibration standard was PEG.



Sch. 1. Reaction scheme of h-PAMAM.

Fluorescence spectra were determined by a FL920 spectrofluorimeter of Edinburgh Instruments. All excitation and emission slit widths were 5 nm and the scan intervals were 10 nm. The emission wavelength varied from 340 nm to 600 nm and the excitation wavelength from 200 nm to 360 nm. Prior to fluorescence measurements, all the sample solutions need to keep for 24 h at room temperature.

3 Results and Discussion

3.1 Synthesis and Characteristics of h-PAMAM

The synthesis of h-PAMAM was based on a procedure described by Gao (20), in which the ideal two-step reaction was depicted as shown in Scheme 1. Our modification was made to improve the reliability of the synthesis. First, Michael addition of MA to DETA gives intermediate 1 or 2 at room temperature. Although it is inevitable that amidation reaction happens between MA and DETA, Michael addition reacts faster at the lower temperature (3). Gradually increasing temperature drives intermediate 1 or 2 to achieve the h-PAMAM by intermolecular reaction, including repeated Michael addition and amidation. We usually carried out this step in rotary evaporator because the methanol, products of amidation and solvent, can be fast removed under vacuum and high temperature. The purification step via precipitation in diethyl ether can effectively erase the unreacted monomer or low molecular weight products to narrow the molecular weight distribution. The h-PAMAM showed good solubility in polar solvents, i.e., methanol, ethanol and poor solubility in diethyl ether and acetone.

We applied the viscosity method to investigate the reaction procedure because the viscosity of hyperbrached polymers may roughly reflect and be proportional to their molecular weight (1), just as linear polymers. In Figure 2, the inherent viscosity values gradually increased as the temperature was raised from 60°C to 140°C, which indicated that the molecular weight was larger and larger. The viscosity reached the maximum and remained constant when the temperature was at 140°C for another two hours. The constant viscosity at this temperature showed the reaction had finished and the polymers grew as large as they could. However, the longer reaction time was still utilized during this process, because the high temperature and vacuum could remove the impurities, such as solvents, monomers and very low molecular weight oligomers.

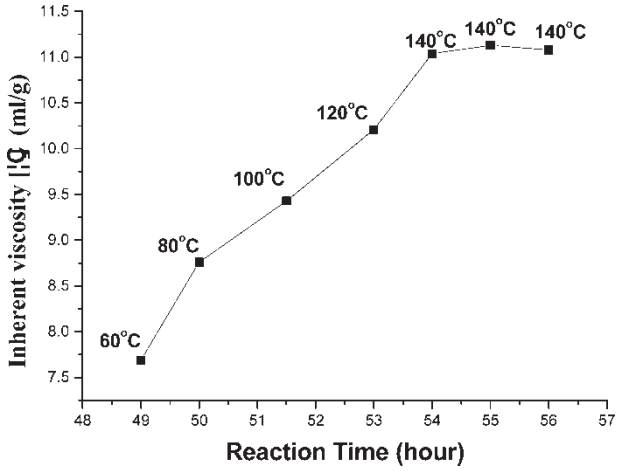


Fig. 2. The inherent viscosity of the product as a function of reaction time.

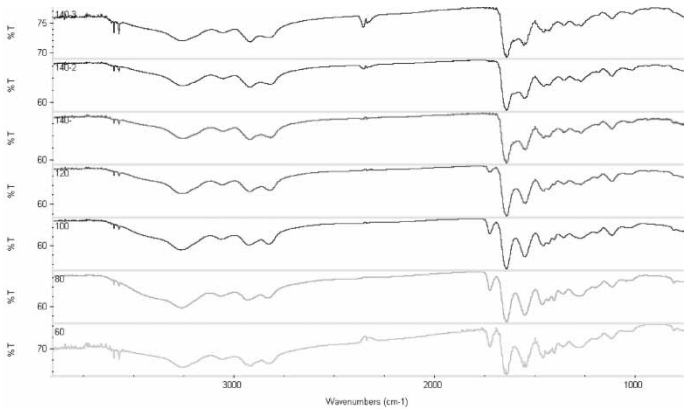


Fig. 3. FTIR spectra of h-PAMAM at different reaction temperature. (From bottom to top: 60°C, 80°C, 100°C, 120°C, 140°C (1 h),140°C (2 h), 140°C (3 h)).

FTIR is another easy and effective way to study the reaction mechanism of h-PAMAM. Figure 3 consisted of FTIR curves of intermediates at different reaction temperatures. We found the peak at 1730 cm^{-1}, assigned to C═O (3, 15) of MA, became smaller gradually and even disappeared as the temperature was increased from 60°C to 140°C. Meanwhile, the peaks at 1650 cm^{-1} and 1558 cm^{-1}, assigned to –CONH- of h-PAMAM (3, 15), became stronger and stronger. These two trends showed that the intermediate 1 or 2 were gradually reacted with each other to grow into the final h-PAMAM. Little changed in the FTIR curves in the following 2 h at 140°C, indicating that h-PAMAM had already formed and the extended time should not lead to a bigger polymer. The FTIR measurement corroborated the viscosity test quite well to prove the growth of h-PAMAM.

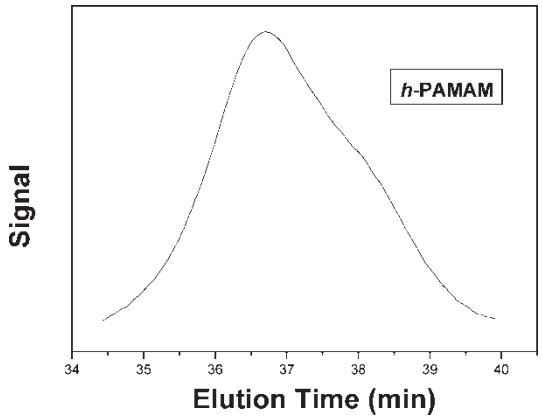


Fig. 4. Molecular weight of tertiary the amine terminated h-PAMAM measured by GPC.

The molecular weight of primary amine terminated h-PAMAM could not be analyzed directly by gel permeation chromatography (GPC) because the polymer absorbed to the column (18, 29). This problem was overcome by changing the primary amine to tertiary amine in the terminal groups. GPC results (Figure 4) showed that the molecular weight of tertiary amine terminated h-PAMAM was about 4.5×10^3 and the molecular weight polydispersity was 1.20. Clearly, the characterization of this polymer demonstrated that h-PAMAM was quite comparable to the G2 or G3 PAMAM dendrimer. The calibration of degree of branching (DB) was attempted from ^{1}H-NMR and ^{13}C-NMR, but failed because of the symmetry and extremely complicated structure of h-PAMAM. The peaks of linear, branching and terminal groups were heavily overlapped and could not be easily distinguished. Such difficulties in determining the DB of hyperbranched polymers were also met by other research groups (29).

Our modified method takes advantage of forming hyperbranched architectures in one single step and can be carried out in large quantities. The widely commercial monomers MA and DETA are easy to obtain commercially and not necessary to synthesize like other methods (18, 19). We also manage to adjust the feed ratio between the MA and the DETA for the polymer synthesis to achieve different molecular weight of the h-PAMAM. The final products have different inherent viscosity, from sticky liquid to wax. The viscosity data in Table 1 show that the h-PAMAM, made by the feed molar ratio (MA/DETA) of 1.2:1, have the largest viscosity. Other h-PAMAM samples have the lower viscosity, and if self crosslinking occurred, the polymer cannot be well dissolved in any solvents. So in this paper we have focused our work on the h-PAMAM obtained from MA/DETA mixture with 1.2:1 feed molar ratio.

3.2 Fluorescence Properties of h-PAMAM

Strong fluorescence emissions were observed from our h-PAMAM system under acidic conditions. In Figure 5, the

Table 1. Inherent viscosity of h-PAMAM with different feed ratios

Item	H1	H2	H3	H4	H5	H6
Feed weight ratio (g) (MA/DETA)	17.2/20.6	20.6/20.6	25.2/20.6	29.4/20.6	34.4/20.6	42/20.6
Feed molar ratio (MA:DETA)	1:1	1.2:1	1.5:1	1.75:1	2:1	2.5:1
Inherent viscosity mL/g	10.4	11.03	X	X	X	8.15

Note: X means the polymer was crosslinked and can not be dissolved in any solvents.

Fig. 5. Left tube: strong fluorescence phenomenon of h-PAMAM in aqueous solution under UV lamp; right tube: pure water.

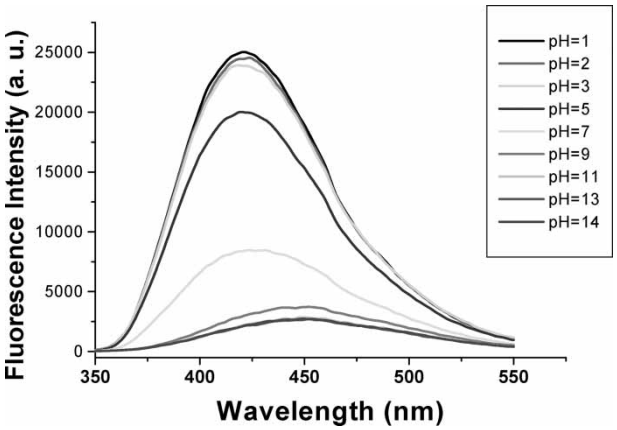


Fig. 7. Fluorescence emission spectra of h-PAMAM at different pH values. (from top to bottom: pH increased; excitation at 336 nm.).

strong blue light was observed from 5 mg/mL acidic h-PAMAM solutions (in left tub) under UV lamp irradiation. Furthermore, the photoluminescence phenomenon can last for several months without any changes. Excitation and emission fluorescence spectra of h-PAMAM at pH 1 are represented in Figure 6. The h-PAMAM had two excitation peaks at 254 nm and 336 nm in Figure 6A, and an emission peak at 425 nm in Figure 6B. In acidic G2 PAMAM solutions, Wang (26) found two excitation peaks at 260 nm and 340 nm, and one emission peak at 415 nm, but the fluorescence intensity is lower than that of h-PAMAM. Based on the similar excitation and emission fluorescence spectra of two polymers, the data generated from the h-PAMAM compounds should be helpful to explain the intrinsic fluorescence properties of PAMAM.

The h-PAMAM showed a significant pH-dependent fluorescence property (Figure 7) as PAMAM dendrimer (26). At pH $>$ 9, the fluorescence emission intensity had a little change. However, as the pH was reduced from 9 to 3, the rapid increase intensity of fluorescence emission took place. The fluorescence intensity reached maximum at pH 3, and was scarcely changed even when the pH was reduced to 1. In addition, the emission peak position of h-PAMAM was blue-shift from the basic to acidic conditions (Figure 8). The emission peaks were located at around 450 nm and had little change above pH 9. When the pH was reduced from 9 to 3, the peaks shifted to lower wavelength from 450 nm to 425 nm. When the pH decreased blow 3, the emission peak was still at 425 nm. At the same process, emission peak positions of PAMAM dendrimers had little changes (26). Although this difference between the two polymers in the fluorescence property could not explained, these data demonstrated that reducing the pH values is a simple and effective method to make the fluorescence intensity of PAMAM or h-PAMAM increase. The possible reason is suggested that the stronger hydrogen bonding and the charge-charge repulsion in low pH range which make the PAMAM and h-PAMAM structure more rigid and co-planar are thought to improve the fluorescence efficiency (26). Lee (25) reported the $(NH_4)_2S_2O_8$-treated PAMAM with observed blue emission, and offered the possible reason as oxidation reaction of functional groups

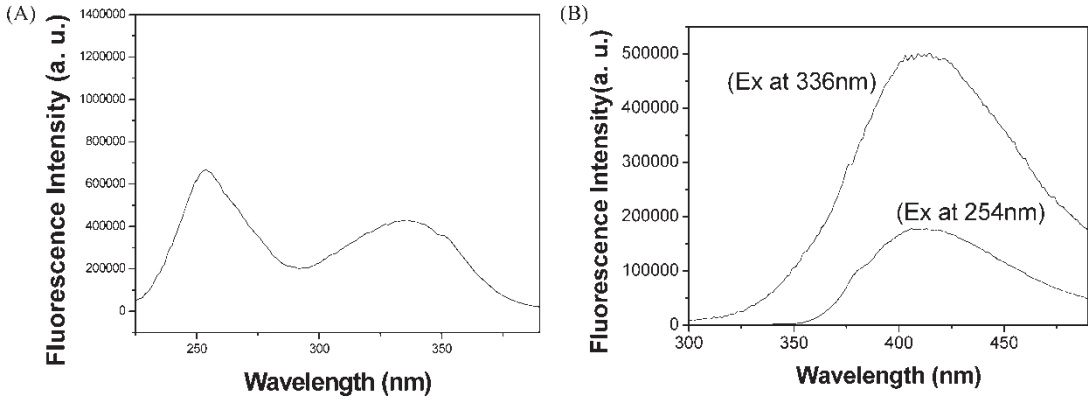


Fig. 6. Fluorescence spectra of h-PAMAM at pH 1. (A) Excitation spectra emitted at 450 nm, (B) Emission spectra excited at 336 nm and 254 nm.

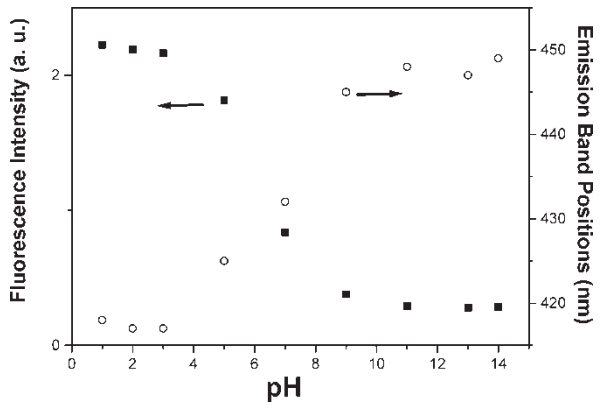


Fig. 8. pH-dependent fluorescence intensity (■) and emission band positions (o) of h-PAMAM in the pH range from 1 to 14.

taking place along the PAMAM branches. However, in our viewpoint, the pH is still another main factor because the decomposition of $(NH_4)_2S_2O_8$ can reduce the pH of the solutions (30).

Interestingly, when the pH was reduced, the longer fluorescence lifetime of h-PAMAM became dominated (Figure 9). While the lifetime of G2.5 and G3.5 carboxyl terminated PAMAM is around 1.3 ns and 4.22 ns, respectively [8d], the lifetimes of h-PAMAM, around 5 ns and 15 ns, are much longer. The longer lifetime of h-PAMAM is possibly due to hyperbranched polymer's imperfect and more complicated architecture. The different terminal groups and concentrations of solutions can also affect the fluorescence lifetime (27–28). The pH values also greatly influenced the fluorescence quantum yields of h-PAMAM (quinine sulfate in 0.1M sulfuric acid as standard (26)), which increased from 4.0% at pH 14 to 4.9% at pH 7, to 11.1% at pH 5, to 17.1% at pH 1. The relatively low quantum yields were possibly due to a weak n-π^* transition in amide groups (24). The n-π^* excited state with long lifetime has a great chance of inner-system crossing taking place, e.g. collision, or other external quenching mechanism (27–28).

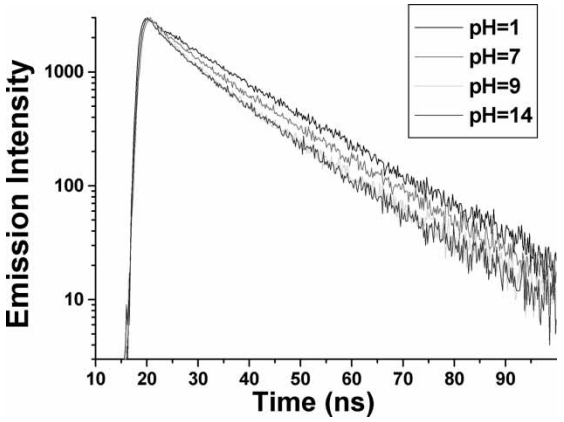


Fig. 9. Fluorescence lifetime of h-PAMAM under different pH conditions.

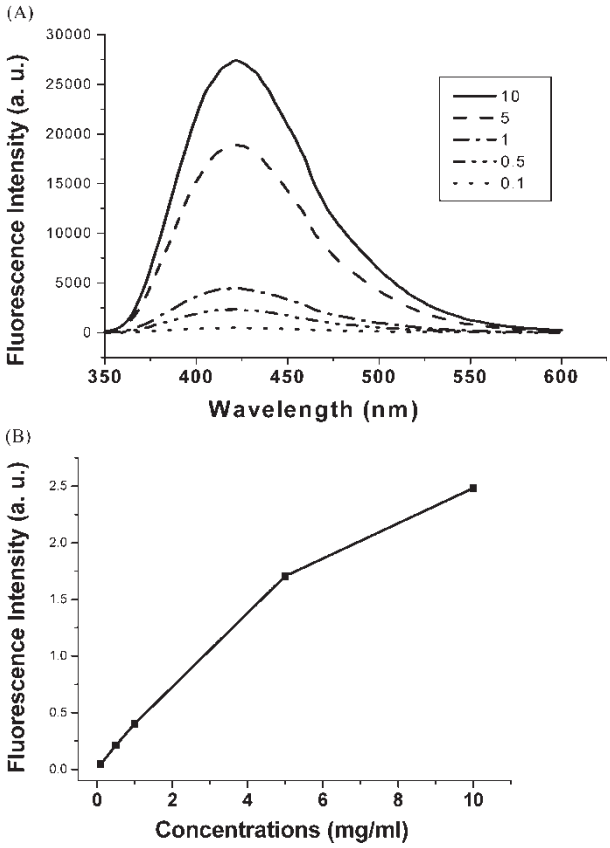


Fig. 10. (A) Fluorescence intensity of h-PAMAM at different concentrations. (0.1 mg/mL, 0.5 mg/mL, 1 mg/mL, 5 mg/mL, 10 mg/mL; excitation at 336 nm and emission at 421 nm). (B) Integral fluorescence intensity of h-PAMAM at different concentrations (0.1 mg/mL, 0.5 mg/mL, 1 mg/mL, 5 mg/mL, 10 mg/mL; excitation at 336 nm and emission at 421 nm).

The fluorescence intensity is related in linear fashion to the concentration of the aqueous h-PAMAM solution in Figure 10. When the concentrations changed from 0.1 mg/mL to 10 mg/mL, the fluorescence intensity was enhanced gradually. The absence of shift of emission peak positions means the same fluorescent moiety at different concentrations of h-PAMAM (Figure 10A). At low concentrations, we cannot directly observe the blue light emitted from the solution under the UV lamp. Wang (26) also mentioned that the concentration is a critical condition for the visible blue light observation. The concentrations-dependent fluorescence properties may be attributed to the increasing amount of the fluorophores. Normally the fluorescence emission intensity will increase with more fluorophores.

The fluorescence properties of h-PAMAM were also influenced by solvents (Figure 11). The same concentrations of h-PAMAM (5 mg/mL) show the stronger emission intensity in water or ethanol than that in dimethyl formamide or chloroform. Water and ethanol, compared with dimethyl formamide or chloroform, are more polar and easier to form hydrogen bonding, which support the structure of h-PAMAM and

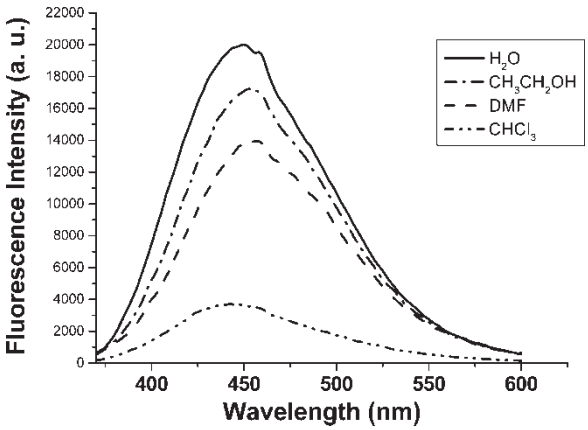


Fig. 11. Fluorescence emission spectra of h-PAMAM at different solvents (from top to bottom: Water, Ethanol, DMF, and Chloroform, excitations at 347 nm and emissions at 447 nm).

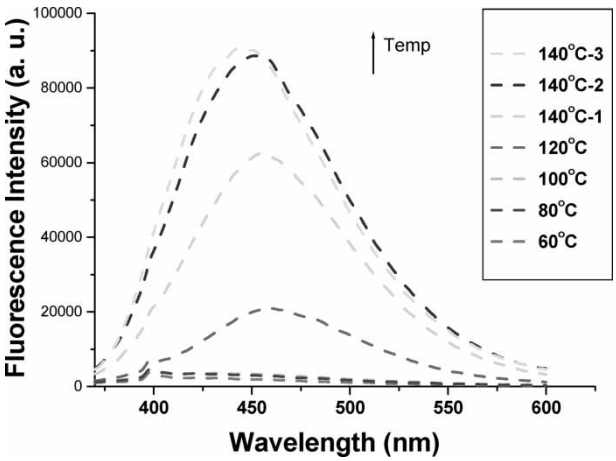


Fig. 13. Fluorescence emission spectra of h-PAMAM at different reaction temperature. (From bottom to top: 60°C, 80°C, 100°C, 120°C, 140°C-1, 140°C-2, 140°C-3, excitation at 357 nm).

then make it rigid. As mentioned before, a more rigid structure will reasonably lead to higher fluorescence performance.

Figure 12 shows the fluorescence emission spectra produced from the carboxyl, tertiary amine, and primary amine terminated h-PAMAM at the same concentrations and acid conditions. The fluorescence peak positions were similar but the intensities were different. The primary amine terminated h-PAMAM showed the strongest fluorescence emission while the carboxyl terminated h-PAMAM in the middle and the fluorescence intensity of the tertiary amine terminated h-PAMAM was the lowest. Although the emission intensity of h-PAMAM depended on the terminal groups, the emission peak positions were at the similar wavelength. This indicated that the backbone of h-PAMAM was the fluorescence center and played the most important role in the fluorescence properties of h-PAMAM. Wang (26) also achieved similar conclusions that it is the backbone not terminal group as origins of the fluorescence of PAMAM.

We measured the fluorescence emission intensity of intermediate products at different temperatures and times (Figure 13). At the lower reaction temperatures (from 60°C to 100°C), the emission intensity was quite low. As the temperature continued rising higher, the fluorescence emission became stronger and stronger. The emission intensity reached the max value and remained almost constant when the temperature was held at 140°C for 2 h. These results indicated that the high molecular weight h-PAMAM is favorable for the striking fluorescence property. It was reported that the emission intensity of G4 PAMAM was stronger than that of G2 PAMAM (26). In our case, there may be one similar reason that the more fluorescent moieties in local position are easy to show stronger fluorescent property when the molecular weight increases. Figure 14 showed the emission intensity of h-PAMAM before and after purification by diethyl ether. Purified h-PAMAM samples showed much stronger emission intensity than that of the unpurified because

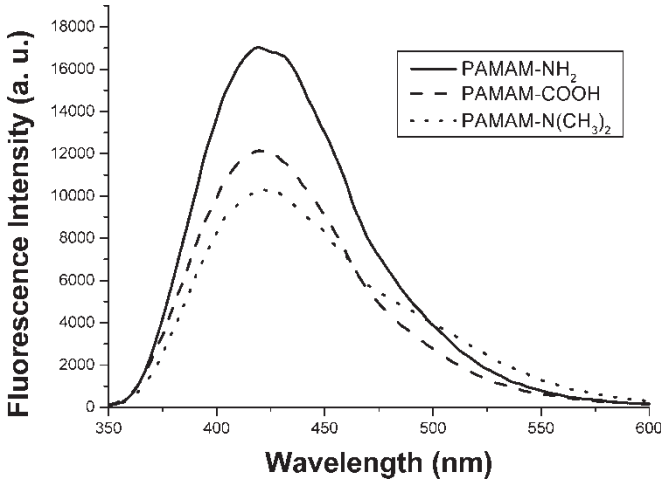


Fig. 12. Fluorescence emission spectra of h-PAMAM with different terminal groups.

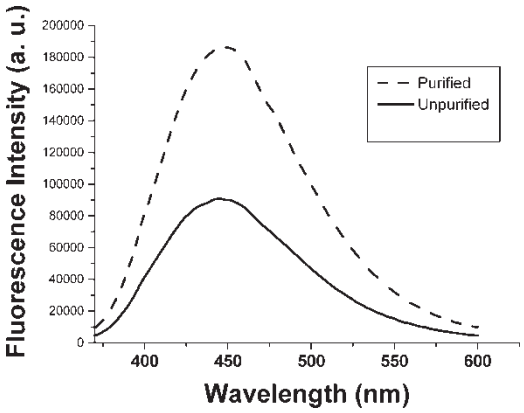


Fig. 14. Fluorescence emission spectra of h-PAMAM after purified (dashed line) and before purified (solid line) (Excitation at 357 nm).

purification can remove low molecular weight h-PAMAM and impurities which may lower the emission intensity as quenchers.

4 Conclusions

We described a simple one-pot step method to successfully prepare hyperbranched poly (amido amine) as analogues of PAMAM dendrimers. Such hyperbranched polymers have similar chemical and physical properties to G2 and G3 PAMAM dendrimers. Interestingly, the h-PAMAM can emit strong observable blue light under acidic conditions. Such exciting fluorescence properties can be influenced by pH values, solvents, concentrations and terminal groups. Although we did not give satisfied explanations, it could be confirmed that the stronger fluorescence emission was from more rigid structures. Also, the fluorescence center may be induced by the n-π^* transition from the various amido groups along the h-PAMAM branches.

5 Acknowledgements

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