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Enhanced fluorescence and thermal sensitivity of polyethylenimine modified by Michael addition

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ABSTRACT

Polyethylenimine (PEI) with enhanced fluorescence and thermal sensitivity was achieved by Michael addition of divinylsulfone (DVS) and *N*-isopropylacrylamide (NIPAm) respectively. The fluorescence enhancement is quantitatively studied by tailoring the amount of DVS, the medium pH, the substituting acrylates, the substrate polymers with different type amine groups, and the substrate molecular weights. The results suggested that different amine groups affected the performance of fluorescence with quantum yields varied from 0.340 for primary amine (NH_2-) to 0.090 for tertiary amine (N(−C)$_3$), and further to 0.049 for secondary amine (-NH-). It was also found that the fluorescence enhancement was attributed to the specific molecular structure of DVS-substituted product. The fluorescence moiety is believed to involve a proton-transfer process and have O_2-dependent and pH-sensitive fluorescence properties. From the current study, it can be expected that the accurate prediction of the O_2-dependent fluorescence would be dependent on molecular orbital calculation of the small amine molecules (oxidized with O_2) and their DVS-substituted products.

1. Introduction

Novel fluorescent reagents attract a great deal of research interest due to their potentially wide applications in imaging and display, analysis and sensing, biomedical diagnosis and therapy [1–3]. Many well developed fluorescence regents, e.g., fluorescent proteins and quantum dots, contribute significantly to the development of biochemistry and polymer science [4,5]. Fluorescence probing has now become an important technique even enabling the study of single molecule dynamic [6].

Organic fluorescent reagents generally contain an aromatic or conjugated fluorophore in their molecular structure [7]. However, an abnormal fluorescence phenomenon was recently noticed in oxidized dendrimers and hyperbranched polymers with nitrogen (N) atom as branch site (without any conjugated fluorophore), whose molecular structures are shown in Scheme 1. Imae et al. studied the strong fluorescence from hydroxy- (OH-) terminated, carboxylate-terminated, amine- (NH_2-) terminated poly(amido amine) (PAmAm) dendrimers, and NH_2- terminated poly(propyleneimine) (PPI) dendrimer, where PAmAm of 2 generations (G2) showed excitation peaks (ExPk's) at 260/340 nm and an emission peak (EmPk) at 415 nm, G4 showed ExPk's at 250/390 nm and an EmPk at 450 nm, and PPI G5 showed an ExPk at 430 nm and an EmPk at 465 nm. They assumed that the backbone of the dendrimer played the key role in forming the novel fluorescent center [8]. Wu et al. studied the fluorescence from hyperbranched poly(amino ester)s (PAmEs's), and assumed that the fluorescence is an inherent property of hyperbranched PAmEs rather than being caused by oxidation, although oxidation by $(NH_4)_2S_2O_8$ or exposure to air strongthened the fluorescence. The OH- terminated PAmEs has an ExPk at 395 nm and an EmPk at 475 nm; the NH_2- terminated PAmEs has an ExPk at 375 nm and an EmPk at 470 nm; the double OH-terminated PAmEs has an ExPk at 370 nm and an EmPk at 455 nm. The OH- terminated PAmEs oxidized by $(NH_4)_2S_2O_8$ has a quantum yield (Q) of 0.035. Meanwhile no convincing change in the chemical structures could be detected by ^{1}H and ^{13}C NMR spectra [9]. Jayamurugan et al. reported that OH- terminated poly(propyl ether imine) (PPEIm) dendrimers of 1–5 generations absorbed in the region of 260–340 nm in methanol and aqueous solutions. Excitation of the dendrimer solution led to an emission at 390 nm. Lifetime measurements showed at least two species responsible for the emission. The presence of air did not affect the emission profiles, nor did the aging for prolonged periods. They suggested that the anomalous emission profile might arise from the tertiary amine (N(−C)$_3$) interacting with the oxygen in ether bond [10]. Although all the amines in PPI, PAmEs, and PPEIm are mainly tertiary amines,

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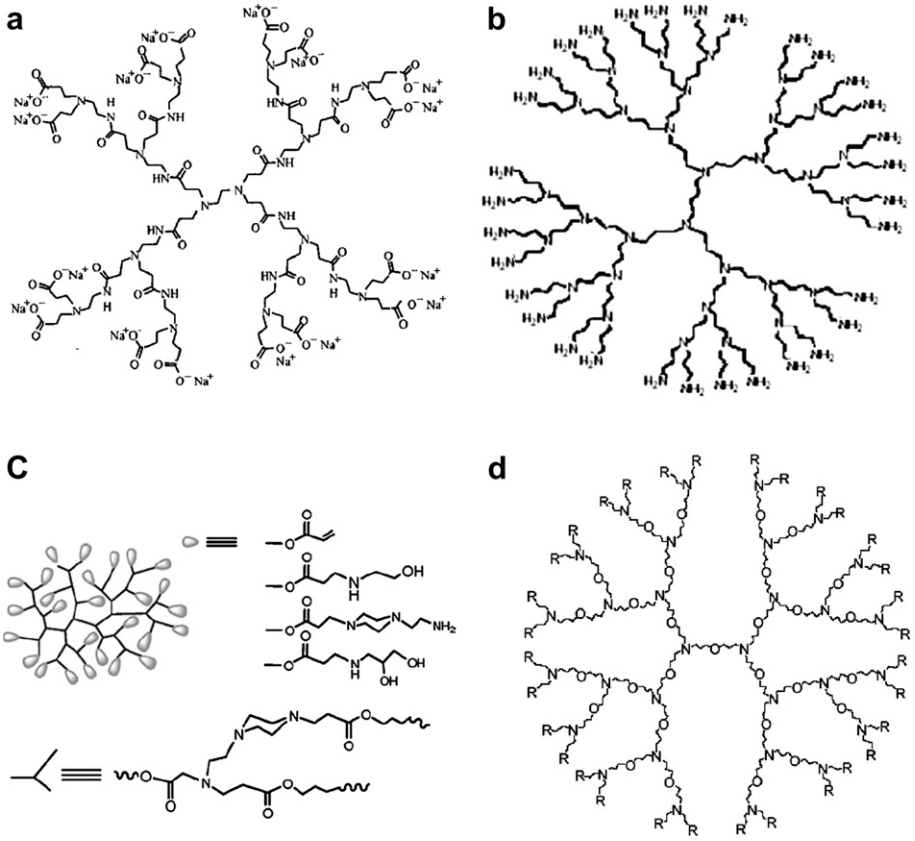


Scheme 1. Molecular structures of PAmAm (a), PPI (b), PAmEs (c), and PPEIm (d).

their fluorescence peaks are much different between each other, due to the sensitivity of fluorescence property to chemical environments, as envisioned from the fluorescent proteins [4,5]. The mechanism of this abnormal fluorescence has not been well explored yet. Its final resolve needs an accumulation of more research data and detailed analysis.

The abnormal fluorescence from branched polyethylenimine (PEI) was also reported by a few groups [11,12]. PEI is a versatile polymer of wide interest due to its many important applications, such as excellent delivery vehicle of DNA [13], component of layer-by-layer assembling composite films [14,15], basic units of supermolecular nanocapsules and thermotropic liquid crystals [16,17], template modulating the deposition of metal particles [18–20], and stabilizer for nanoparticles and nanotubes [21,22]. Its active NH_2- and secondary amine (-NH-) groups allow facile modifications by amidation, substitute reaction, Michael addition, and redox radical polymerization. In order to improve the PEI performance in DNA delivery, various modifications of PEI by grafting substitution and copolymerization have been studied [23–25]. In a few cases, fluorescent imaging reagents were also incorporated in the PEI/DNA complex [26]. In fact, PEI produces a weak fluorescence by itself upon oxidation. Chen et al. firstly reported the fluorescence quantum yield and lifetime of PEI, linear polyethylenimine (LPEI), and their methylation derivatives [11]. However, their fluorescence quantum yields are quite low (less than 0.1) even after an oxidation treatment by $(NH_4)_2S_2O_8$. Later on, thermal sensitive PEI derivatives have also been reported by Chen et al. and Han et al. [12,27,28], where amidation reactions in organic solvents were employed. In Han's report, the PEI fluorescence was improved over 10 times by a hydrophobic graft of cyclohaxane carboxylic amide, but no quantum yield was quantitively determined [12].

Here, we present an interesting fluorescence enhancement and a thermal sensitization of PEI by a facile Michael addition in aqueous medium. Through quantitive measurements of the fluorescence property (the fluorescence quantum yield of PEI is increased up to 0.345), the possible fluorescence mechanism and its dependence on molecular weights and structures are discussed in depth, which will help to understand the abnormal fluorescence from all N-bearing molecules and to promote the PEI applications.

2. Experimental section

2.1. Materials

All the reagents were purchased from Aldrich Co. and used as received, including branched polyethylenimine (PEI) ($M_w = 25{,}000$ by LS, $M_n = 10{,}000$ by GPC, polydispersity index (PDI) = 2.5, NH_2-/-NH-/$N(-C)_3$ = 33/40/27), linear polyethylenimine (LPEI, M_w = 250,000, PDI = 1.4), polyallylamine (PAA, M_w = 17,000, PDI = 1.2), divinylsulfone (DVS, m = 118), 1,6-hexanediol bisacrylate (HBA, m = 226), hydroxyethyl methacrylate (HEMA, $m = 130$), N-isopropylacrylamide (NIPAm, m = 113), diethylenetriamine (DETA, m = 103), ethylene diamine (EDA, $m = 60$), ammonia solution (32 wt.% in water), n-butyl amine ($m = 73$), fluorescamine ($m = 278$), and deuterium oxide (D_2O). In all the syntheses, deionized water was used.

2.2. Synthesis

All of the modifications of polymers or small molecules containing amine groups were achieved in their aqueous solutions of suitable concentrations that were mixed with the predetermined amount of DVS (or acrylates) under magnetic stirring (at room temperature unless otherwise stated).

PEI hydrogel was prepared by adding crosslinker DVS 0.20 g (or HBA 0.50 g) into 10 wt.% PEI solution 10.0 g. The solution of PEI modified with DVS was prepared by adding predetermined amount of DVS into 2.5 wt.% PEI solution 10.0 g. The solution of PEI modified with NIPAm (or HEMA) was prepared by adding predetermined amount of NIPAm (or HEMA) into 2.5 wt.% PEI solution 10.0 g at 60 °C for 12 h. The solution of PAA (or LPEI) modified with DVS was prepared by adding predetermined amount of DVS into 0.5 wt.% PAA solution 10.0 g (or 0.5 wt.% LPEI solution 10.0 g just after heat melting and cooling to room temperature to prevent the LPEI crystallization and precipitation).

2.3. Characterization

UV–Vis absorbance spectra and fluorescence spectra of sample solutions were recorded by a Perkin Elmer UV/Vis spectrometer Lambda 18 and a Perkin Elmer fluorescence spectrometer LS55 in quartz cells. Unless otherwise stated, all the spectra were recorded in aqueous solution of pH 9.0. ^{1}H NMR spectra were recorded by 64 repetitions on a Varian Inova AS 500 spectrometer in D_2O. The thermal-induced phase transition of polymers in solution were monitored by a Malvern Zetasizer Zetaplus equipped with a 10 mW He–Ne laser (633 nm), operating at an angle of 90° and constant temperatures.

3. Results and discussion

3.1. Fluorescence enhancement of PEI substituted by DVS

When the Michael addition reaction between PEI and DVS was employed to prepare a hydrogel, an unexpected strong fluorescence was observed under UV irradiation. In a typical trial, gelation occurred quickly in 10 s. The transparent hydrogel quickly produced a light purple color (see Fig. 1). This color gradually disappeared, and an evident yellowing occurred at the interfaces of air/gel and gel/stirrer. This yellowing gradually extended into the gel bulk from the two interfaces. As shown in Fig. 1, the yellowing extended quite deeply in 8 h, and a surprisingly strong fluorescence appeared at the yellow sections under UV irradiation. This phenomenon is quite interesting since there is no conjugated fluorophore in this system. In comparison, while 1,6-hexanediol bisacrylate (HBA, of equivalent mole to DVS) was added instead of DVS, gelation occurred at a lower speed (in about 15 min). And this gel always remained as a transparent colorless gel, without any visible fluorescence in the subsequent aging period (image is not shown here). Therefore, the strong fluorescence from PEI-DVS gel must depend on the DVS structure and the air diffusion into the gel. This fluorescent phenomenon is related to those reported for dendrimers containing amine branches, which show the analogous appearance and may help to understand the fluorescence mechanism. Generally, the fluorescence intensity from dendrimers containing amine branches depends on oxidation process. It has been reported that superoxide anion radicals can be produced in dimethyl sulfoxide (DMSO) aqueous solution, with a high yield at a high pH [29]. This superoxide anion radical from sulfones may explain the rapid yellowing process at the air/gel and gel/stirrer interfaces and the visible intense fluorescence by oxidation. However, when DMSO was dropped into PEI aqueous solution, no fluorescence enhancement was observed in the aging period. The fluorescence enhancement seems primarily depending on the DVS reaction rather than the oxidation reaction.

In order to obtain quantitative results of the fluorescence, PEI solution at a lower concentration (2.5 wt.%, 10.0 g) was mixed with DVS (1# –10 mg, 2# –50 mg, and 3# –150 mg, corresponding mole ratio PEI/DVS = 68/1, 14/1, and 4.6/1) to produce soluble PEI-DVS products that were ready for spectral measurements. Their reaction completed very quickly in a few minutes, as judged from a ^{1}H NMR measurement. Because gelation or precipitation was not observed in these mixtures (except for a slight gel in 3#, which was removed by a centrifugation.), the products were believed to be intramolecular crosslinked PEI. The molecular structures of PEI and PEI-DVS are illustrated in Scheme 2. In the product solutions, gradually increasing fluorescence was noticed under UV lamp. After being stirred for 24 h in air, their fluorescence became stabilized or saturated. Their UV–Vis absorbance spectra and fluorescence spectra were measured to calculate their fluorescence quantum yields (Q's), with fluorescamine in ethanol solution of n-butyl amine as a standard ($Q = 0.230$) [30,31]. As shown in Fig. 2, excited at 380 nm, all the PEI-DVS solutions produce a strong and wide EmPk at around 500 nm; they give a shoulder peak around 350 nm in UV–Vis spectra, and two ExPk's at 260/380 nm; Their relative quantum yields (Q's) are 0.200, 0.304 and 0.345. It is noticed that

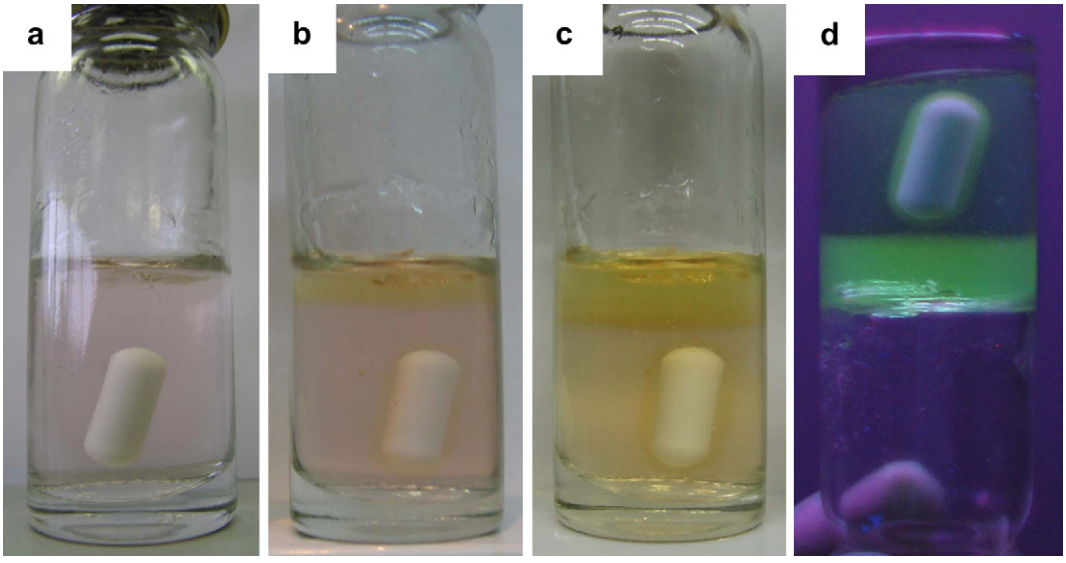
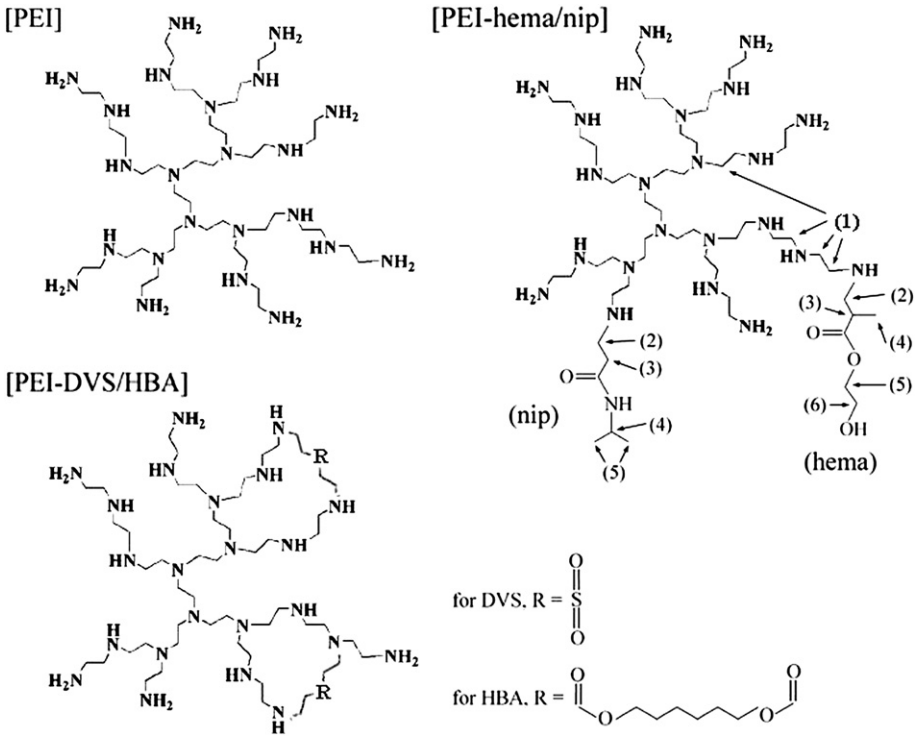


Fig. 1. Images of PEI hydrogels crosslinked by DVS at different reaction periods: 1 min (*a*), 1 h (b), 8 h (c is under daylight, and d is under a UV lamp of 8 W Philips TL05).



Scheme 2. Chemical structures of PEI, PEI-DVS, PEI-HBA, PEI-hema and PEI-nip.

aged pure PEI solution has an EmPk at 465 nm ($Q = 0.076$) and a main ExPk at 350 nm with a weak shoulder at 270 nm. Mirror-image rule violation is manifest in the single emission peak relative to the double excitation peaks. Seemingly there are two excitation ways leading to an emission, as reported for 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and PAmAm [8,32]. The Q increment upon DVS substitution (or intramolecular crosslink) is remarkable, with a maximum over fourfold. The resulted fluorescence yields are much higher than those reported by Chen et al. who enhanced the PEI fluorescence by decreasing pH, chemical methylation and oxidation at the amine groups [11]. We could ascribe this fluorescence enhancement to the crowded structure in PEI-DVS products or the specific sulfone group at this stage.

The pH effect on the PEI-DVS2 was investigated. As shown in Fig. 3, the EmPk blue-shifts with pH decreasing, and the Q value has a maximum at pH 11, which decreases to 0.304 at pH 9, 0.165 at pH 6, and 0.137 at pH 2. This PH sensitivity suggests the inclusion of a convergent intramolecular proton-transfer process in the fluorescence moiety, as reported for DHICA [32]. The proton-transfer between amine groups and oxygen-related defects has also been proposed by Carlos to explain the emission from amine-functionalized organosilica hybrids [33–35]. It is well known that the branched PEI contains three kinds of amines: primary (NH_2-), secondary (-NH-) and tertiary ($N(-C)_3$) amines. The p*K*a value of primary amine is around 9, secondary amine around 8 and the tertiary amine around 6–7 [36]. At pH 11, all the primary, secondary and tertiary amines are in the disassociated state, which allow their easy oxidation in air as envisaged for the dopamine solution [37,38] and more fluorescent moieties than at a lower pH. Additionally, the moieties at different pH's may possess different energy levels based on molecular orbital model as calculated for DHICA [32], resulting in a higher quantum yield at pH 11 than at a low pH. The increase in both quantity and quantum yield of fluorescent moiety leads to the remarkable visible fluorescence in the PEI-DVS products at a high pH. This pH effect is consistent to that of grafted PEI reported by Han [12].

The PEI solution (2.5 wt.%) was also mixed with HBA (100 mg, mole ratio PEI/HBA = 14/1). After reaction, the resultant solution (microgel was removed by centrifugation) was fully aged (for a week) before a spectroscopic measurement. As shown in Fig. 3, the PEI-HBA has an EmPk and two ExPk's analogous to those of PEI-DVS. Although having a clear absorbance peak at 360 nm in its UV spectrum, its fluorescence is very weak, with a low quantum yield of 0.062 analogous to that of pure PEI. Therefore, the fluorescence enhancement in PEI-DVS is a special effect from the DVS substitution rather than from the crowded structure of intramolecular crosslink. In order to avoid the possible contribution from conjugated impurities, the PEI-DVS2 and PEI-HBA solutions were transferred into NMR tubes, dried and dissolved in D_2O, measured with ^1H NMR as shown in Fig. 4. The typical signals 2.45–2.80 for PEI C–H adjacent to the N atoms are clear in both the spectra of PEI-DVS2 and PEI-HBA. The C–H of reacted DVS gives peaks at 2.95–3.25. The reacted HBA produces peaks at 2.25–2.45 (HC–C=O), 2.75–3.00 (HC–N), 3.45–3.60 (HC–O), and 1.20–1.55 (C–CH2–C). Evidently, there is not any unsaturated -CH = CH- signal in both spectra.

It is noticed that three types of amine groups coexist in most of the PEI and PEI derivatives. Do they contribute equally to the fluorescence? If there is a difference, it can be clarified by model polymers with only one type of amine groups. Here, another two linear polymers were tried: PAA with only NH_2- groups and LPEI with only -NH- groups. They were treated with DVS in different mole ratio: PAA/DVS = 4.7/1, LPEI/DVS = 11/1. The products have different amino types: NH_2-/-NH- = 2.7/2 for PAA-DVS, -NH-/N $(-C)_3$ = 9/2 for LPEI-DVS. Their spectra data are shown in Fig. 5. The PAA or PAA-DVS have an absorbance peak at 255 or 335 nm, an ExPk at 310 nm and an EmPk at 448 nm. The quantum yield of PAA or PAA-DVS is calculated as 0.336 or 0.097. It is surprising that pure PAA has a high Q value and the DVS treatment seriously reduces its Q value. As to LPEI or LPEI-DVS, they have a shoulder peak around 355 nm in UV–Vis spectra. Their fluorescence spectra give an ExPk

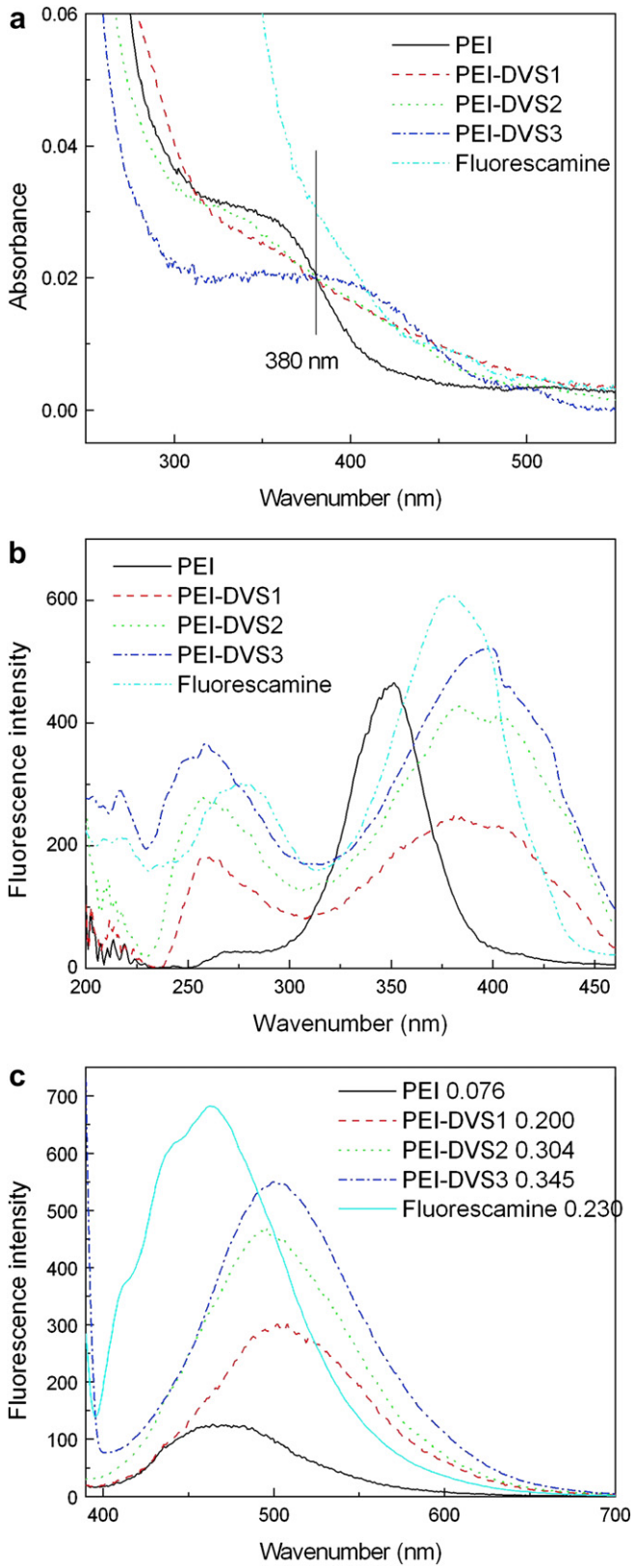


Fig. 2. UV–Vis absorbance spectra (a), fluorescence excitation (b) and emission (c) spectra of PEI, PEI-DVS and fluorescamine solutions (all are excited at 380 nm).

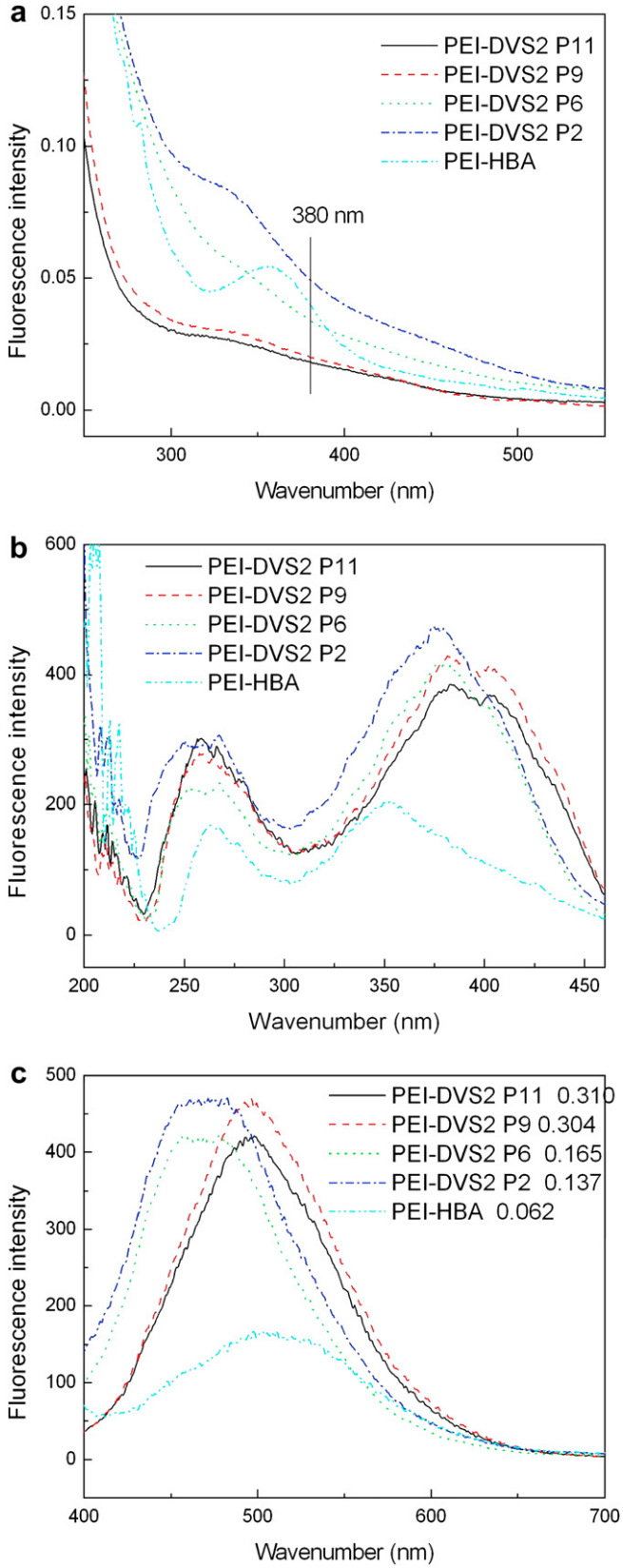


Fig. 3. UV–Vis absorbance spectra (a), fluorescence excitation (b) and emission (c) spectra of PEI-DVS2 at different pH values and PEI-HBA in aqueous solutions (all are excited at 380 nm).

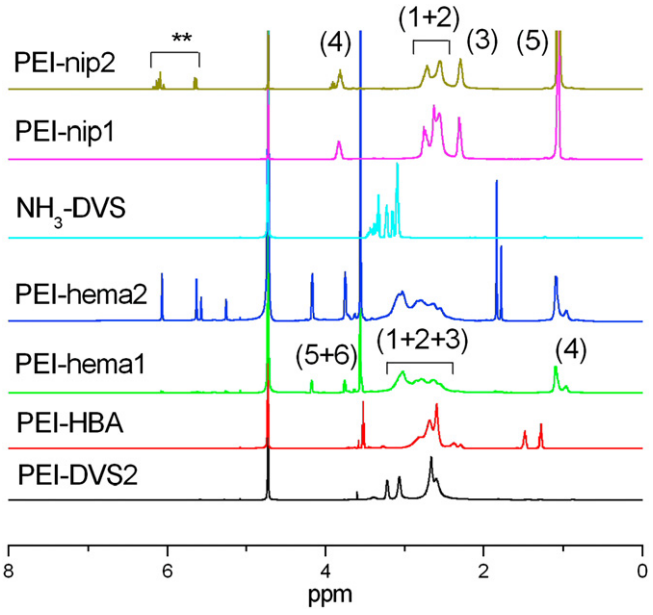


Fig. 4. ^{1}H NMR spectra of PEI-DVS2, PEI-HBA, PEI-hema1, PEI-hema2, NH_3-DVS, PEI-nip1, and PEI-nip2 in D_2O.

at 355 nm and an EmPk at 490 nm, with a quantum yield 0.049 or 0.268. Here the enhancement of fluorescence by DVS treatment is over fivefold, similar to the case of branched PEI. The Q values of LPEI and PEI are in a same magnitude, although they have different amino types and chain structures. Stiriba reported that LPEI emits even more strongly than branched PEI, as excited at 360 nm in methanol solution [11]. All these results suggest the irrelevance of fluorescence to dendritic structure. And their Q values are in the sequence NH_2- > -NH-. The fluorescence enhancement by DVS substitution is confirmed only for -NH-; while for NH_2-, an opposite change is obtained.

Then how about the N(−C)$_3$ group? In order to obtain the fluorescence property of a polymer with only tertiary amine groups, PEI was substituted with an acrylate monomer − HEMA, which gave a graft product PEI-hema (as illustrated in Scheme 2). Two products of different graft degree - PEI-hema1 and PEI-hema2 - were measured by ^{1}H NMR in D_2O, as shown in Fig. 4. The signals from PEI CH_2 (1#) and HEMA (2#CH_2 and 3#CH) protons overlap at 2.4–3.2. The peaks at 0.85–1.15 are ascribed to the HEMA CH_3 group. In the spectrum of PEI-hema2, the peaks at 5.5–6.2 are due to the CH_2=C of unreacted HEMA residue in the solution. Accordingly, the graft/PEI mole ratios for PEI-hema1 and PEI-hema2 are calculated from the peak areas of (1# + 2# + 3#) H and 4#H, as HEMA/EI = 0.74 and 1.0 respectively, which means that the H atoms on PEI amine groups are completely substituted in PEI-hema2 and there are only tertiary amine groups. Their optical spectra data are shown in Fig. 5. PEI-hema1 or PEI-hema2 has an ExPk at 355 nm and an EmPk at 490 nm. Their Q values are 0.053 and 0.090 respectively, in a same level to the original PEI, PEI-HBA and LPEI. The Q values of secondary and tertiary amines are slightly different: N(−C)$_3$ (0.090) > -NH- (0.049).

In order to clarify the effect of molecular weight on fluorescence, small molecules $NH_3 \cdot H_2O$, EDA, and DETA were also treated by DVS in predefined mole ratio: NH_3/DVS = 100/1, EDA/DVS = 2/1, DETA/DVS = 2/1, and fully aged for a week. DVS and $NH_3 \cdot H_2O$ reacted quickly and produced a saturated compound (whose ^{1}H NMR spectrum is shown in Fig. 4). This compound gives a stable purple color, with an absorbance peak at 310 nm, and a very weak fluorescence Q = 0.049 (see Fig. 6). In comparison, after DVS treatment,

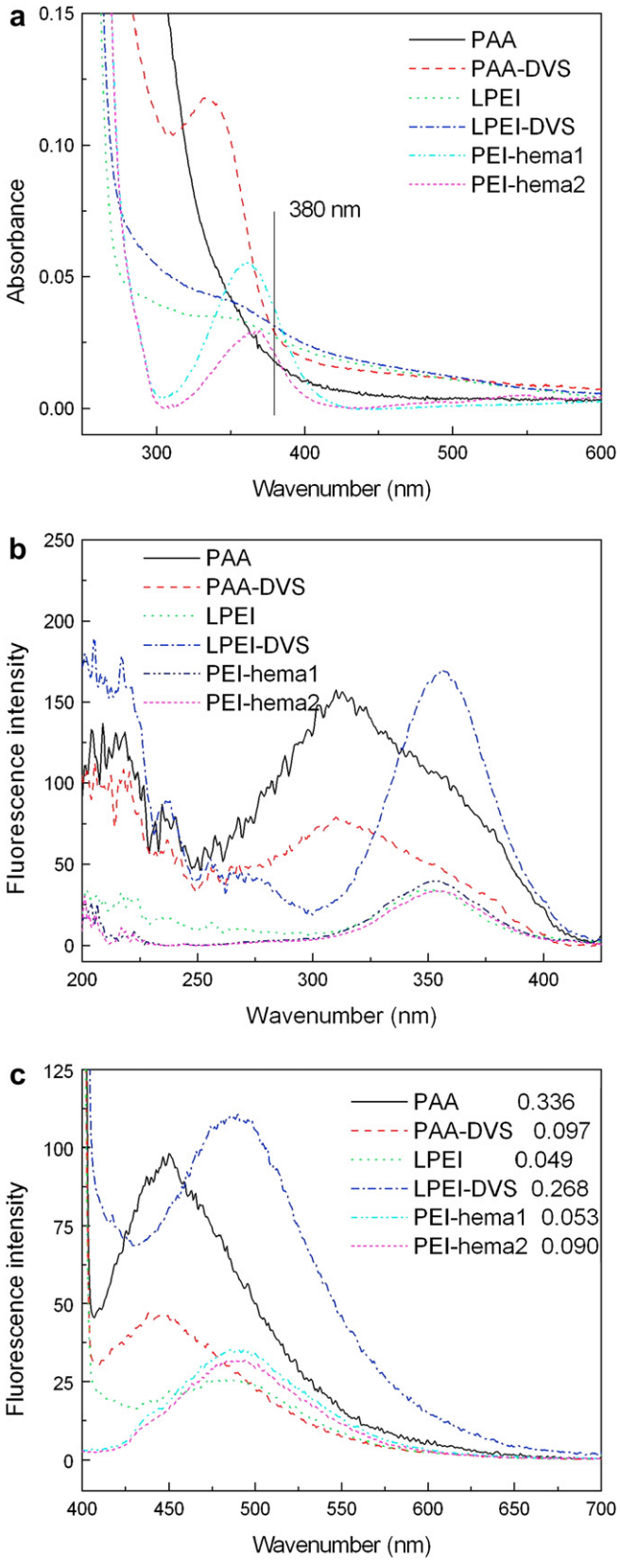


Fig. 5. UV–Vis absorbance spectra (a), fluorescence excitation (b) and emission (c) spectra of PAA, PAA-DVS, LPEI, LPEI-DVS, PEI-hema1, and PEI-hema2 in aqueous solutions (all are excited at 380 nm).

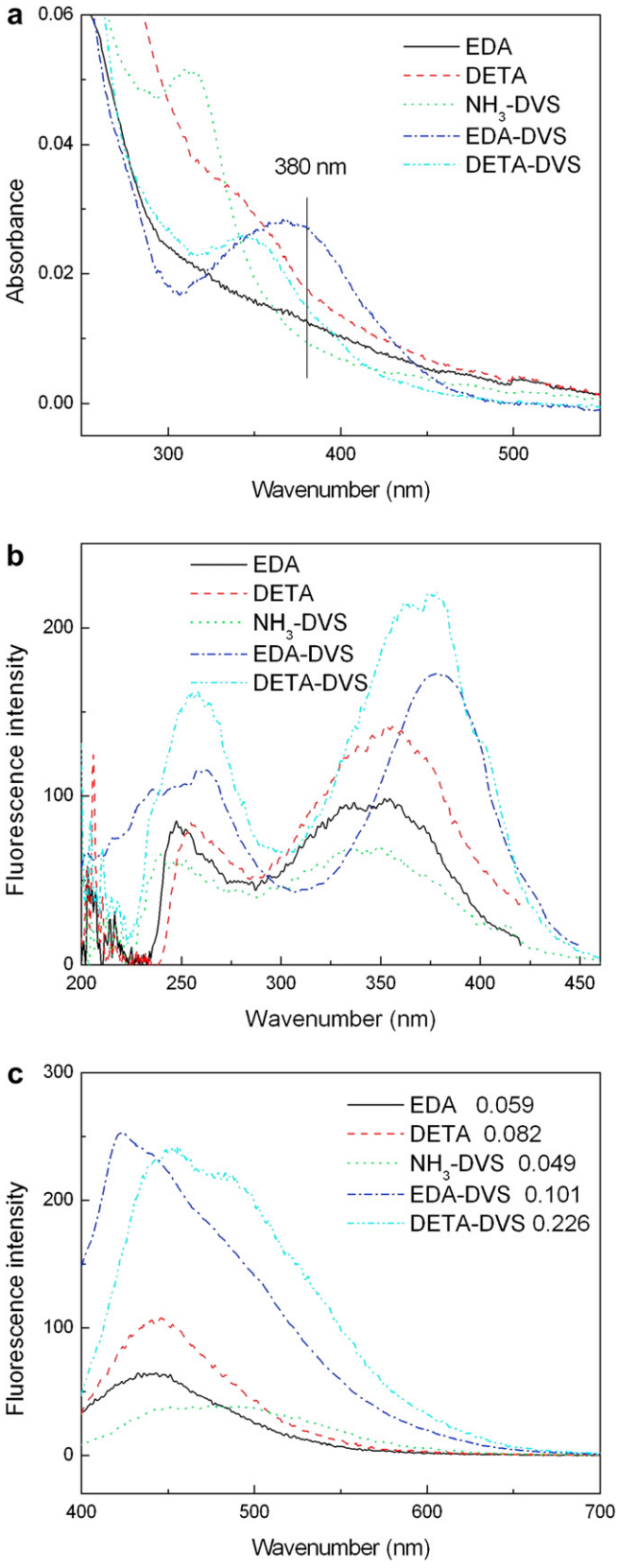


Fig. 6. UV–Vis absorbance spectra (a), fluorescence excitation (b) and emission (c) spectra of small molecules and their derivatives by DVS substitution in aqueous solutions (all are excited at 380 nm).

EDA and DETA show a UV absorbance peak at 370 and 345 nm, double ExPk's at 260/360 nm and an EmPk at 430 and 450 nm. Their fluorescence yields are calculated as 0.101 and 0.226 respectively. Without DVS treatment, the original EDA and DETA show only a weak shoulder absorbance at around 350 nm, double ExPk's at 250/355 nm and an EmPk at 445 nm, with low Q values of 0.059 and 0.082 respectively. Here, the pure amine molecules give a stronger fluorescence with the increase of molecular weight; and DVS can largely improve the fluorescence yield from -NH- group rather than NH_2- group. These small molecules allow a theoretical quantum calculation to investigate their molecular orbital energy levels [32], and explain the fluorescence features from different amines and the fluorescence enhancement by DVS substitution.

The molecular structure of DETA-DVS was monitored by ^1H NMR in D_2O, which results are shown in Fig. 7. This reaction between DETA and DVS is very fast: in 5 min after mixing, the typical signals of DVS at 6.3–7.0 totally disappear, and the intensity ratio of peaks at 2.71 and 2.64 for DETA changes remarkably, with their positions kept constant. This is due to the increase of C–H near secondary N atoms. The peaks of C-H adjacent to O=S=O appear at 3.0–3.5. Surprisingly, they appear as 3 peaks: 3.43, 3.26 and 3.12. Their ratio changes with aging, and finally only the peak at 3.12 (after 7 days) retains. In this period, the fluorescence intensity increases with time, without any signal of unsaturated group in ^1H NMR data. When the sample is acidified by adding 37% HCl, its signal of C–H adjacent to O=S=O splits into several peaks at 2.83, 2.95, 3.03, and 3.26. Interestingly, an aromatic signal at 7.21 appears too, indicating a possible rearrangement of the molecules into an aromatic compound, although the rearrangement mechanism is unsure. In addition, the solution fluorescence is seriously weakened, suggesting the irrelevance of the possible aromatic rearrangement to the fluorescence enhancement.

3.2. Thermal sensitivity of PEI substituted by NIPAm

Inspired by the thermal sensitivity of poly(NIPAm) in aqueous solution, NIPAm was used to substitute PEI, giving a product PEI-nip (as illustrated in Scheme 2). Two graft products - PEI-nip1 and PEI-nip2 - were measured by ^1H NMR in D_2O, as shown in Fig. 4. The signals 2.4–2.9 for C–H adjacent to the N atoms increase with the

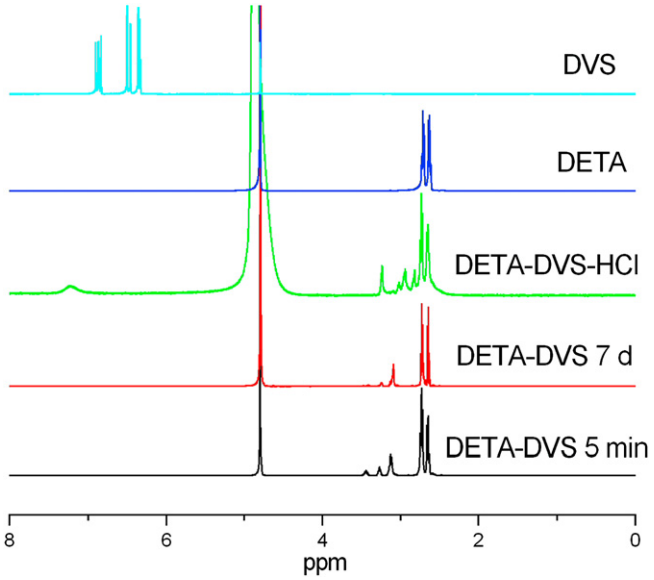


Fig. 7. ^1H NMR spectra of DETA, DVS, and their reaction product (DETA-DVS) aged for 5 min or 7 days, and that being finally acidified by HCl in D_2O.

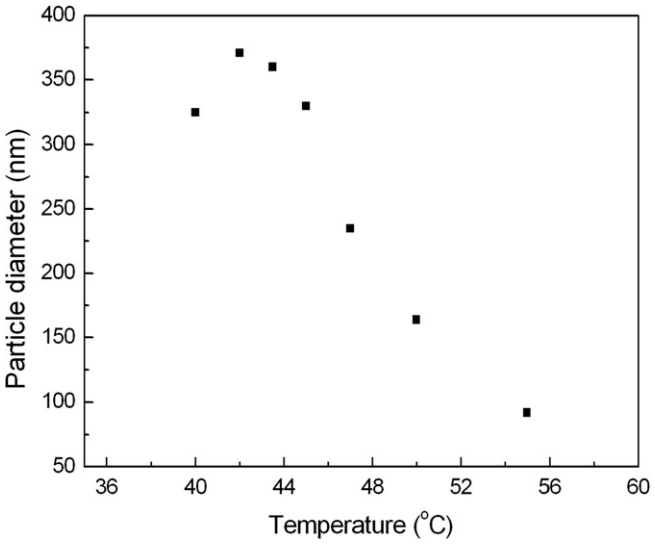


Fig. 8. Size of PEI-nip2 particles resulted from the phase separation above its LCST in aqueous solution.

substitution. The peak at 2.3 is ascribed to the C—H adjacent to the C═O of graft NIPAm (nip). In the spectrum of PEI-nip2, the peaks at 5.5–6.2 are due to the CH_2═CH of unreacted NIPAm in the solution. Accordingly, the graft/PEI mole ratios for PEI-nip1 and PEI-nip2 are calculated from the peak areas of (1# + 2#) H and 3#H, as nip/EI = 0.60 and 1.0 respectively. Their fluorescence Q values are in a same level to that of the original PEI, 0.087 for PEI-nip1 and 0.067 for PEI-nip2 (whose spectra are not shown here).

Although the NIPAm graft on PEI did not enhance the PEI fluorescence, it imparted a thermal sensitivity to PEI in aqueous solution. The PEI-nip1 aqueous solution has a lower critical solution temperature (LCST) of 55 °C, above which a double layered liquid is formed with upper water and lower viscous PEI-nip1. The PEI-nip2 aqueous solution has a LCST of 40 °C, above which PEI-nip1 aggregation particles are obtained and characterized by a Zetasizer. With the temperature increasing, the fine particles condense into smaller size, from 370 nm at 42 °C to 90 nm at 55 °C (Fig. 8). A few sensitive PEI derivatives have also been reported by Chen and Han, which were terminated by isobutyric amide or cyclohexane carboxylic amide groups, and showed similar LCST's that decreased with the substitution increasing [12,27,28]. The influence of PEI molecular weight, PEI concentration, pH value, and salt concentration on their LCST's were also studied by Chen. These PEI derivatives with thermal sensitivity and fluorescence may find potential applications in bio-related applications.

4. Conclusions

The Michael addition has been proved as an effective method to modify PEI in its aqueous solution. When PEI was modified by DVS substitution, a strong fluorescence depending on oxidation process was noticed. The quantum yield of this fluorescence increased with the amount of DVS substitution, and decreased with the decreasing pH from 11 to 2. This fluorescence enhancement is attributed to the specific molecular structure there-formed rather than to the crowded structure of intramolecular crosslink, since the HBA substitution did not give the analogous enhancement of PEI fluorescence. From three polymers with only respective NH_2-, -NH-, and N(—C)$_3$ groups, the quantum yields of the three amine groups were estimated as 0.340, 0.049, and 0.090 respectively. The fluorescence enhancement by DVS substitution was also effective for small molecules. Although the exact mechanism is unclear at this stage, we believe that this O_2-dependent fluorescence could be accurately explained by the quantum calculation of the small amine molecules (oxidized with O_2) and their DVS substitution structures. The perspective theoretical work will also uncover the fluorescence mechanism of previous amine-functioanlized dendrimers and organosilicas. Furthermore, PEI with thermal sensitive behavior was also obtained by the NIPAm substitution. All these novel PEI derivatives would largely promote the PEI applications.

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