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ORIGINAL CONTRIBUTION

Synthesis and characterization of poly(ethyleneimine) dendrimers

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Abstract Poly(ethyleneimine) (PEI) dendrimers up to the third generation (G3) were prepared by a divergent synthesis method from an ethylenediamine (EDA) core. The amine terminals were bonded with vinylbromide by a Michael addition reaction. Then, the bromide terminals were converted to amine groups using a Gabriel amine synthesis method. PEI dendrimers displayed pH-dependent luminescence, and their emission intensities at pH 6 increased over time. Fluorescence intensities also increased with increasing dendrimer generation from G1 to G3. Air-bubbling in aqueous solutions of dendrimers made to incorporate detectable amount of oxygen in dendrimers. EDA also behaved similarly in luminescence and oxygen incorporation.

Keywords Poly(ethyleneimine) dendrimer · Synthesis · Divergent synthesis · Michael addition reaction · Gabriel amine synthesis · Characterization · Fluorescence

Introduction

There has been growing interest among researchers about highly branched polymers such as dendrimers because of their unique properties and applications in various fields [1–4]. The physicochemical properties of dendrimers are sometimes in sharp contrast to the behavior of linear polymers because of their explicit symmetry and high density of functional terminals. Experimental works have reported various properties of dendrimers in connection with chemical structure, chemical character, size, and shape of dendrimers [5–11].

Examples of typical dendrimers are poly(amido amine) (PAMAM) dendrimers and poly(propyleneimine) (PPI) dendrimers. Because the former dendrimer has amide groups in spacer moieties but the latter dendrimer does not have them, the properties of dendrimers, such as solvent penetration and guest doping, are different from each other in dependence upon a different chemical structure [11, 12]. The properties were also compared between PAMAM dendrimes with a different spacer length [13]. In comparison, the interest in physicochemical properties of PPI dendrimers inspired us to study poly(ethyleneimine) (PEI) dendrimers, which have a shorter alkyl spacer than PPI dendrimer. Poly(ethyleneimine) is one of the commercially available and valuable cationic polyamines and has been widely used as an efficient drug carrier, a gene delivery system [14], and an adhesive component for wood [15]. However, there is no report on a synthesis of PEI dendrimers.

In this work, we synthesize PEI dendrimers and examine their characters. Although both divergent [16–18] and convergent [19, 20] synthetic methods have been utilized to produce well-defined nanostructures, we selected a divergent procedure from an ethylenediamine (EDA) core through a route of Gabriel amine synthesis, different from the synthesis of PPI dendrimer [21, 22]. The properties of a model compound, EDA, was also examined in comparison with those of PEI dendrimers.

Experimental part

Materials

EDA, vinylbromide, phthalimide, hydrazine hydrate, hydrochloric acid, and solvents were purchased from

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Aldrich Chemical Co., USA or Tokyo Kasei Kogyo Co. Ltd., Japan and were used without further purification.

Michael addition reaction (G_n Br)

Michael addition reaction was carried out as follows: EDA or amine-terminated PEI dendrimer (Gn PEI) was dissolved into 20 cm^3 of methanol, and a methanol solution of vinylbromide was added dropwise. The mixture was stirred in the dark at room temperature for 2 days. The solvent and excess vinylbromide were removed under vacuum to give a light yellow oily product.

G1 Br: Reactants: EDA (0.6 g, 10 mmol), vinylbromide (4.24 cm^3, 85 mmol). Yield: 80%. Fourier transform-infrared (FTIR; neat): 3,356, 3,287, 3,181, 2,932, 2,858, 1,602, 1,454, 1,041 cm^{-1}. Elemental analysis (FW 484) ($C_{10}H_{20}N_2Br_4$): calculated C24.79, H4.13, N5.78; observed C25.08, H4.0, N5.70. Proton nuclear magnetic resonance (^{1}H NMR; 500 MHz, CDCl$_3$, δ): 1.21–1.39 (s, 12 H), 2.71–2.75 ppm (d, 8 H). **G2 Br**: Reactants: G1 PEI (0.92 g, 10 mmol), vinylbromide (3.5 cm^3, 85 mmol). Yield: 70%. FTIR (neat): 3,354, 3,285, 3,180, 2,930, 2,859, 1,600, 1,453, 1,040 cm^{-1}. Elemental analysis (FW 1087) ($C_{26}H_{52}N_6Br_8$): calculated C28.70, H4.82, N7.72; observed C29.00, H4.72, N7.80. ^{1}H NMR (500 MHz, CDCl$_3$, δ): 2.30–2.42 (m, 20 H), 2.84 (d, 16 H), 3.34 ppm (d, 16 H). **G3 Br**: Reactants: G2 PEI (0.57 g, 0.1 mol), vinylbromide (1.7 cm^3, 0.16 mol). Yield: 65%. FTIR (neat): 3,353, 3,285, 3,180, 2,932, 2,859, 1,601, 1,455, 1,042 cm^{-1}. Elemental analysis (FW 2288) ($C_{58}H_{116}N_{14}Br_{16}$): calculated C30.45, H5.11, N8.57; observed C31.10, H5.25, N8.60. ^{1}H NMR (500 MHz, CDCl$_3$, δ): 2.34–3.21 (m, 52 H), 2.80–2.90 (d, 32 H), 3.42 ppm (d, 32 H).

Gabriel amine synthesis (G_n Ph)

Gabriel amine synthesis was carried out as follows: Gn Br was dissolved into 30 cm^3 of dimethylformamide (DMF), and phthalimide was added. The mixture was heated in an oil bath at 80 °C for 6 h . After the reaction was completed, the product was precipitated as white solid in water. It was further purified by recrystallization from a mixture of ethylacetate/acetone and dried at 25 °C till constant weight.

G1 Ph: Reactants: G1 Br (1.21 g, 5 mmol), phthalimide (2.94 g, 20 mmol). Yield: 75%. FTIR (KBr): 2,932, 2,858, 1,671, 1,507, 1,438, 1,390, 1,258, 1,089 cm^{-1}. Elemental analysis (FW 752) ($C_{42}H_{36}N_6O_8$): calculated C67.02, H4.78, N11.17; observed C67.0, H4.80, N12.0. **G2 Ph**: Reactants: G2 Br (1.0 g, 0.1 mol), phthalimide (1.17 g, 0.8 mol). Yield: 80%. FTIR (KBr): 2,931, 2,859, 1,670, 1,504, 1,440, 1,390, 1,259, 1,090 cm^{-1}. Elemental analysis (FW 1617) ($C_{90}H_{84}N_{14}O_{16}$): calculated C66.82, H5.23, N12.12; observed C67.0, H5.40, N12.32. **G3 Ph**: Reactants: G2 Br (0.45 g, 0.1 mol), phthalimide (0.7 g, 0.16 mol). Yield: 78%. FTIR (KBr): 2,930, 2,859, 1,670, 1,505, 1,439, 1,390, 1,258, 1,089 cm^{-1}. Elemental analysis (FW 3347) ($C_{186}H_{180}N_{30}O_{32}$): calculated C66.73, H5.42, N12.55; observed C66.85, H5.58, N12.68.

Synthesis of amine-terminated PEI dendrimer (G_n PEI)

Amine synthesis was carried out as follows: Gabriel Gn Ph was mixed with hydrazine hydrate and hydrochloric acid, and the reaction mixture was refluxed for 6 h and cooled. The solid product (phthalylhydrazide) was filtered off, and the amine product was isolated by neutralization and vacuum distillation.

G1 PEI: Reactants: G1 Ph (1.50 g, 10 mmol), hydrazine hydrate (2.0 cm^3, 40 mmol). Yield: 85%. FTIR (neat): 3,361, 3,292, 2,943, 2,805, 1,576, 1,464, 1,375, 1,316, 1,184, 1,073 cm^{-1}. ^{1}H NMR (500 MHz, D$_2$O, δ) 2.10–2.21 (s, 4 H), 2.32–2.50 (d, 8 H), 2.60–2.70 ppm (d, 8 H). **G2 PEI**: Reactants: G2 Ph (1.61 g, 0.1 mol), hydrazine hydrate (3.0 cm^3, 0.1 mol). Yield: 90%. FTIR (neat): 3,356, 3,294, 2,946, 2,866, 2,804, 1,581, 1,469, 1,382, 1,314, 1,184, 1,072 cm^{-1}. ^{1}H NMR (500 MHz, D$_2$O, δ) 2.10–2.45 (m, 20 H), 2.55–2.65 (d, 16 H), 2.70–2.90 ppm (d, 16H). **G3 PEI**: Reactants: G2 Ph (1.67 g, 5.6 mmol), hydrazine hydrate (3.0 cm^3, 0.1 mol). Yield: 80%. FTIR (neat): 3,358, 3,288, 2,940, 2,866, 2,804, 1,581, 1,463, 1,382, 1,308, 1,185, 1,073 cm^{-1}. ^{1}H NMR (500 MHz, D$_2$O, δ) 2.20–2.50 (m, 52 H), 2.60–2.85 (m, 32 H), 2.90–3.10 ppm (m, 32H).

Measurements

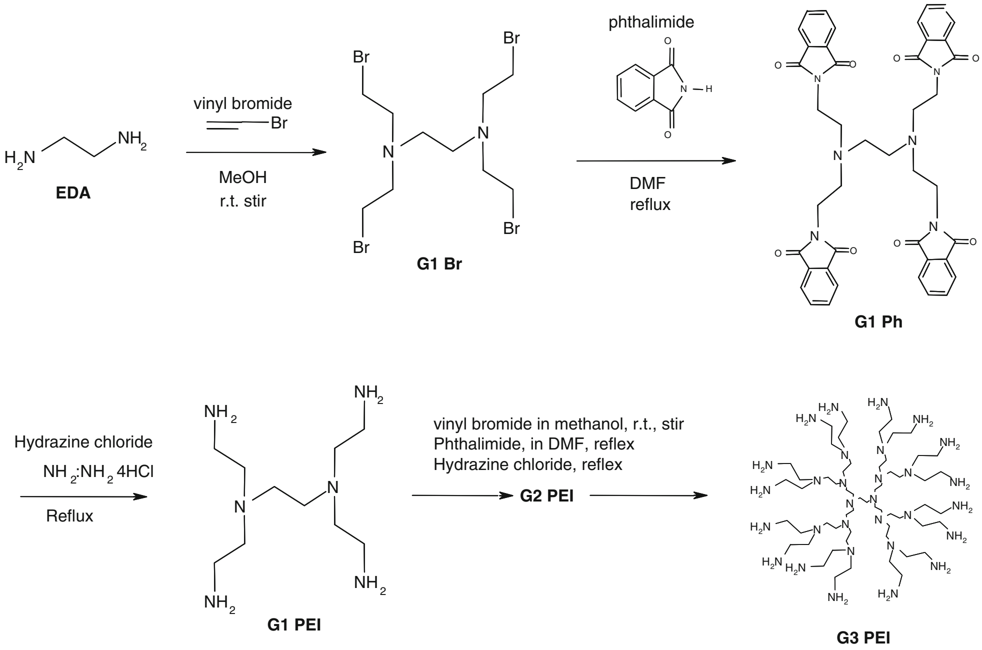
Elemental analysis was performed on a LECO CHN-900C analyzer. Fourier transform-infrared absorption spectra were recorded using a Bio-Rad FTS 575C spectrometer equipped with a cryogenic mercury cadmium telluride detector. KBr pellets of powders were prepared. Fluorescence spectra were measured on a Hitachi F-4010 model. Solutions were prepared by dissolving EDA or dendrimer (0.7 mM) in water. Nuclear magnetic resonance spectra were recorded for a deuterated aqueous solution (1 mg cm^{-3}) of EDA or dendrimer on a JEOL JNM-L500. All the measurements were carried out at room temperature.

Results and discussion

Synthesis of poly(ethyleneimine) dendrimers

PEI dendrimers were synthesized using EDA as a core. On the growth step of generation, Michael addition reaction was used for alkylation and followed by Gabriel amine synthesis to produce amine-terminated dendrimers. PEI

Scheme 1 Synthesis pathway of PEI dendrimers from ethylenediamine



dendrimers up to G3 with amine terminals were prepared. The general synthesis pathway is presented in Scheme 1. The condition of reactions and the characterization of products are described in the "Experimental section" and listed in Table 1.

The comparative FTIR absorption spectra of G1 Br, G1 Ph, and G1 PEI are shown in Fig. 1. A spectrum of G1 Br revealed methylene stretching bands at 2,932 and 2,858 cm^{-1}, a methylene bending band at 1,454 cm^{-1} and a C–Br stretching band at 946 cm^{-1}. Additional bands at 3,356, 3,287, 3,181, 1,602, and 1,041 cm^{-1} were assigned to nonevapolated water [23] but not to G1 Br. In a spectrum of G1 Ph, there were bands from methylene groups at 2,932, 2,858, and 1,438 cm^{-1} and a band of a C=O stretching mode of phthalimide groups at 1,671 cm^{-1}. Other bands at 1,600–1,000 cm^{-1} were ascribable to ring structure of phthalimide groups. A spectrum of G1 PEI displayed amine stretching bands at 3,361 and 3,292 cm^{-1}, methylene stretching bands at 2,943 and 2,805 cm^{-1}, and bending bands of amine and methylene at 1,576 and 1,464 cm^{-1}. Spectra of Gn Br, Gn Ph, and Gn PEI (n=2, 3) revealed IR absorption bands similar to corresponding G1 homologs.

The comparative ^{1}H NMR spectra of Gn PEI in D_2O are given in Fig. 2. A spectrum of G1 PEI displayed signals of methylene protons of a core at 2.10–2.21 ppm and signals of methylene protons of dendrimer arms at 2.32–2.50 and 2.60–2.70 ppm. Similarly, a ^{1}H NMR spectrum of G2 PEI presented signals from a core and adjacent carbons at 2.10–2.45 ppm and signals from arms at 2.55–2.65 and 2.70–2.90 ppm. For G3 PEI, signals at 2.20–2.50 ppm are ascribed to protons of a core and adjacent carbons, and signals at 2.60–2.85 and 2.90–3.10 ppm are to protons of terminal arms of a dendrimer. ^{1}H NMR spectra of Gn Br (n=1–3) displayed a similar behavior to corresponding Gn PEI homologs, although the chemical shifts were slightly different.

Table 1 Elemental analyses of EDA, Gn PEI, and their air-injected products

Compound FW (NH_2 group, N atom)	Neat calculated	Neat observed	Air-injected observed	Air-injected evaluated (composition formula)
EDA $C_2H_8N_2$ (2, 2)			C19.10 H7.43 N20.70 (O52.77)	$C_{2.1}H_{9.8}N_{2.0}O_{4.3}$ ($C_2H_8N_2$+2.1O_2)
G1 PEI $C_{10}H_{28}N_6$ 232 (4, 6)	C51.72 H12.06 N36.20	C51.08 H12.20 N36.70	C25.06 H6.02 N18.10 (O50.82)	$C_{9.7}H_{27.7}N_{6.0}O_{14.7}$ ($C_{10}H_{28}N_6$+7.4O_2)
G2 PEI $C_{26}H_{68}N_{14}$ 576 (8, 14)	C54.13 H11.08 N33.99	C54.25 H11.20 N34.15	C27.12 H5.40 N17.13 (O50.35)	$C_{26.0}H_{61.7}N_{14.0}O_{36.2}$ ($C_{26}H_{68}N_{14}$+18.1O_2)
G3 PEI $C_{58}H_{148}N_{30}$ 1266 (16, 30)	C55.03 H11.78 N33.19	C55.00 H12.08 N33.25	C28.02 H6.00 N16.20 (O49.78)	$C_{58.0}H_{148.1}N_{28.9}O_{77.4}$ ($C_{58}H_{148}N_{30}$ + 38.7O_2)

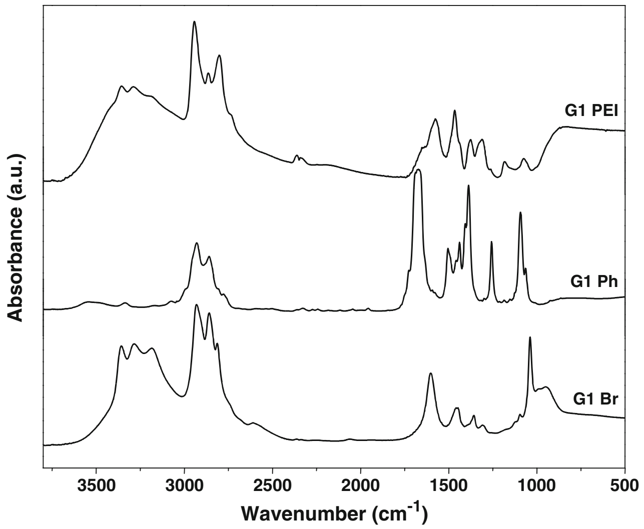


Fig. 1 Comparative FTIR absorption spectra of *G1 Br*, *G1 Ph*, and *G1 PEI*

The results of IR absorption and ^{1}H NMR spectra confirmed the successful synthesis of dendrimers. Such verification was also obtained from elemental analysis. The observed values were in good consistency with calculated ones for all series of Gn Br, Gn Ph, and Gn PEI, as seen in the "Experimental section" and Table 1.

Fluorescence behavior

Recently, some research groups have discovered fluorescence emission from PAMAM dendrimers [24–31]. It has been confirmed that the fluorescence emission from the fourth generation PAMAM dendrimer was strongly dependent on pH [32, 33]. The strong fluorescence was detected for a PPI dendrimer and hyperbranched poly(amino ether)s as well [32–34].

Gn PEI in the present work also emitted fluorescence in water, and their emission bands (440, 450, and 470 nm for G1, G2, and G3 PEI, respectively) red-shifted with generation. The fluorescence emission was pH dependent, and similar fluorescence behavior was observed even for EDA in water. Figure 3 shows the plot of emission intensity against pH of a solution. Fluorescence intensities of Gn PEI and EDA increased with decreasing pH from 7, while there were little changes in the intensities at the pH range of 14 to 7. It was seen that increasing the generation from G1 to G3 increased slightly the emission intensity, and the lowest intensity was observed for EDA. The maximum intensity was obtained at pH 2 for all dendrimers and EDA. It was clarified that the protonated dendrimers and EDA at acidic conditions showed the pronounced emission intensity.

Figure 4 presents plots of emission intensities at pH 6 against time. The intensity increased slowly until 5 days, and afterwards, it showed the accelerated rise. This means that the ageing process proceeds slowly at an initial stage, and over time it is enhanced, although such ageing effect is weaker for lower generation of Gn PEI and weakest for EDA.

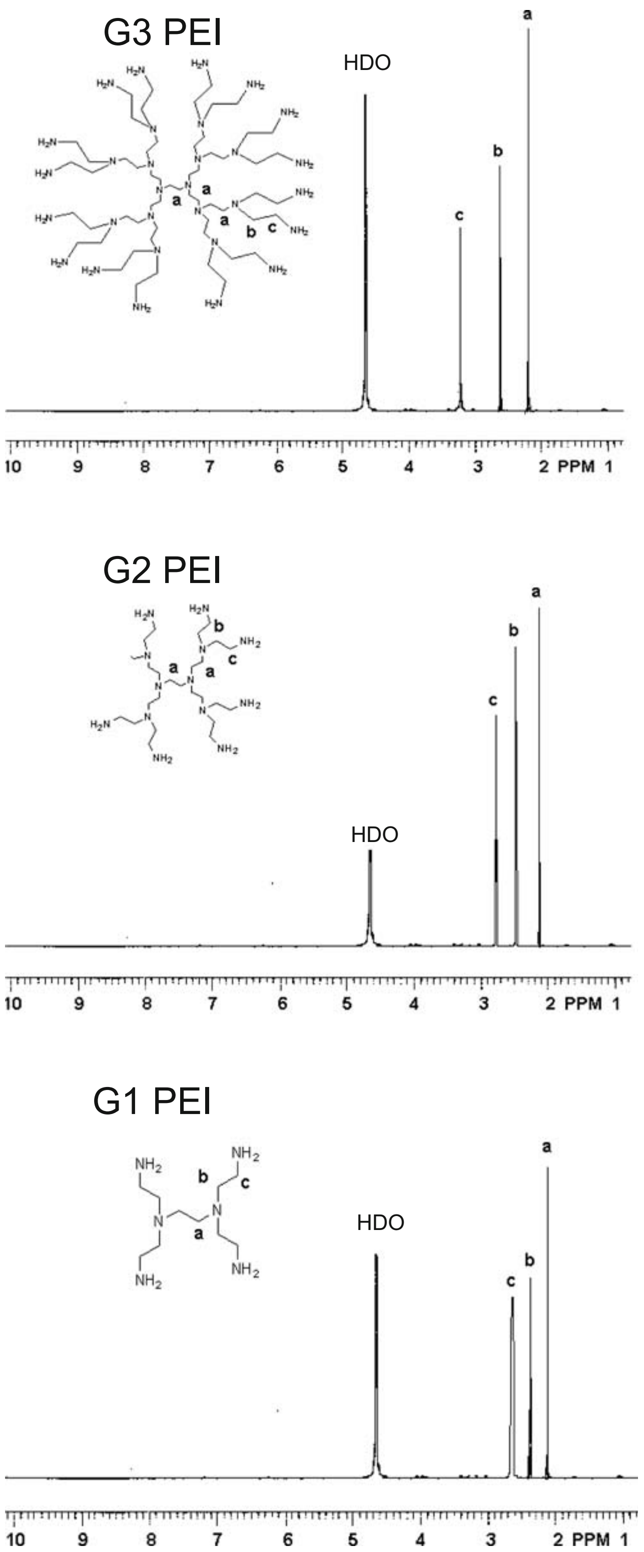


Fig. 2 Comparative ^{1}H NMR spectra of Gn PEI in D_2O

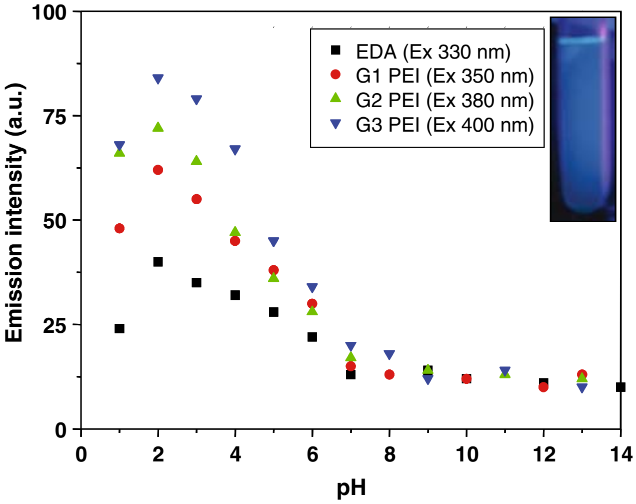


Fig. 3 pH-dependent emission intensities of EDA and Gn PEI. Emission bands of EDA, G1 PEI, G2 PEI, and G3 PEI were at 430, 440, 450, and 470 nm, respectively. *Inset*: an illuminated photograph of an aqueous 0.7 mM solution of G3 PEI at pH 2. The solution was excited by a 4.5 W UV lamp

The fluorescence behaviors of PEI dendrimers are alike previous observation for PAMAM and PPI dendrimers [32, 33]. It should be noticed that the fluorescence behavior is scarcely dependent on spacer groups and lengths like amide, propyl, and ethyl groups. It should be surprised that EDA also presents fluorescence. It can be focused that EDA has amine groups, although it is a small core molecule of PEI dendrimers.

Injection of air in dendrimers

Blue photoluminescence with high quantum yield has been achieved upon oxidation of PAMAM dendrimers with ammonium persulfate [31] or air-bubbling [37]. Therefore, in the present work, the effect of air-bubbling for PEI dendrimers is compared to that for EDA. The injection of air in EDA and dendrimers was carried out as follows: EDA (0.60 g, 10 mmol) or Gn PEI (G1: 0.46 g, 20 mmol; G2: 1.14 g, 20 mmol; G3: 1.26 g, 10 mmol) was dissolved in 10 cm^3 of water, air-bubbled for 5 min, and kept at room temperature. The solution turned yellowish after 1 week. The air-injected product was recovered by evaporating water, and the obtained white solid was further characterized.

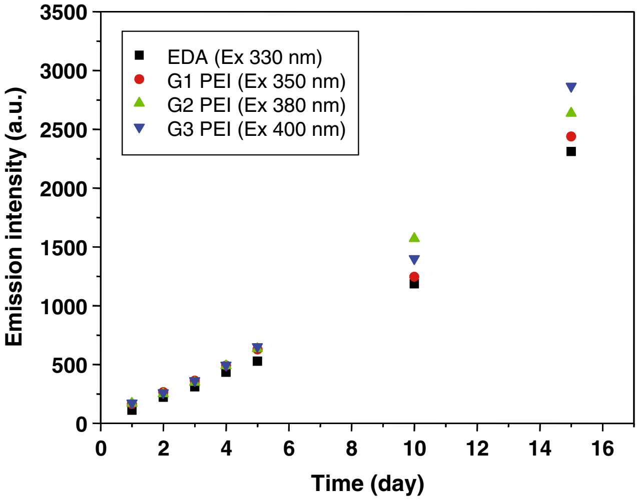


Fig. 4 Time-dependent emission intensities of EDA and Gn PEI at pH 6. Emission bands of EDA, G1 PEI, G2 PEI, and G3 PEI were at 430, 440, 450, and 470 nm, respectively

The elemental analyses of air-injected PEI dendrimers (Gn PEI-Ox) and EDA (EDA-Ox) were carried out in comparison with neat compounds, as listed in Table 1. The contents of C, H, and N atoms of air-bubbled Gn PEI and EDA were only half of neat compounds corresponding. Supposing that the rest is oxygen, composition formula of each compound was evaluated and listed in Table 1. It can be noticed that the estimated composition formulas of air-bubbled compounds are in consistency with those of neat compounds including additional oxygen atoms. If oxygen in air-bubbled compounds is O_2 molecules, the content of O_2 is comparable to the number of N atom in EDA and is 1.3 times of N atom in Gn PEI.

As described above, the effect of air-bubbling drastically appeared on elemental analysis data. O_2 comparable to or more than the number of amine was incorporated in dendrimers and EDA. It can be interpreted that O_2 interacts with nitrogen in amine groups. It is known that amines after oxidation form heterocyclic organic compounds, and finally, they are decomposed into methane, nitrogen, and carbon dioxide gases [35, 36]. Otherwise, amines are oxidized to the corresponding carboxylic acid through aldehyde [37]. Mechanism and rate of the decomposition depend on the type of catalyst and temperature. In the present procedure, only the air-bubbling was carried out, and no hard conditions like using of catalyst or heating were imposed. The situation of oxygen atoms in dendrimer and the relation of oxygen atoms with luminescence are less well on the present state, and the resolution of such subjects is in progress.

Conclusion

Amine-terminated PEI dendrimers up to third generation were synthesized using EDA as a core by a divergent synthesis method. A strong fluorescence from PEI dendrimers was observed under acidic conditions. Emission intensities increased with increasing dendrimer generation from G1 to G3 and time. To consider the effect of oxygen on fluorescence of PEI dendrimers, air-bubbling was carried out in the aqueous solutions. Detectable amounts of oxygen were incorporated in dendrimers. It was an amazing result that EDA also behaved similarly to

PEI dendrimers on fluorescence properties and oxygen incorporation. The present results give us a clue on the clarification of the luminescence mechanism, which probably involves the interaction of oxygens with amine groups.

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References

1. Fréchet JMJ, Hawker CJ (1996) In: Allen G (ed) Comprehensive polymer science, 2nd suppl. Elsevier Science, Pergamon, Oxford
2. Fischer M, Vögtle F (1999) Angew Chem Int Ed 38:884
3. Imae T (2003) In Esumi K, Ueno M (ed) Structure-performance relationships in surfactants: 2nd Edn, Revised and Expanded. Surfactant Science Series volume 112, Marcel Dekker, 525
4. Tomalia DA (2005) Chimica OGGI-Chem Today 23:41
5. Zeng F, Zimmerman SC (1997) Chem Rev 97:1681
6. Imae T, Funayama K, Aoi K, Tsutsumiuchi K, Okada M, Furusaka M (1999) Langmuir 15:4076
7. Percec V, Holerca MN (2000) Biomolecules 1:6
8. Adronov A, Frechet JM (2000) Chem Commun 1701
9. Lee I, Athey BD, Wetzel AW, Meixner W, Baker JR Jr (2002) Macromolecules 35:4510
10. Hedden RC, Bauer BJ (2003) Macromolecules 36:1829
11. Funayama K, Imae T, Aoi K, Tsutsumiuchi K, Okada M, Furusaka, M, Nagao M (2003) J Phys Chem B 107:1532
12. Imae T (2004) In: Nalwa HS (ed) Encyclopedia of nanoscience and nanotechnology. American Scientific, 3, p 685
13. Ujihara M, Mitamura K, Torikai N, Imae T (2006) Langmuir 22:3656
14. Godbey WT, Wu KK, Mikos AG (1999) J Control Release 60:149
15. Li K, Geng X, Simonsen J, Karchesy J (2004) Int J Adhesion Adhesive 24:327
16. Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S, Roeck J, Ryder J, Smith P (1986) Macromolecules 19:2466
17. Aoi K, Motoda A, Ohno M, Tsutsumiuchi K, Okada M, Imae T (1997) Polymer J 31:1071
18. Aoi K, Motoda A, Okada M, Imae T (1997) Macromol Rapid Commun 18:945
19. Hawker CJ, Fréchet JMJ (1990) J Am Chem Soc 112:7638
20. Wooley WKL, Hawker CJ, Fréchet JMJ (1991) J Am Chem Soc 113:4252
21. Wörner C, Mülhaupt R (1993) Angew Chem Int Ed Engl 32:1306
22. de Brabander-van den Berg EMM, Meijer EW (1993) Angew Chem Int Ed Engle 32:1308
23. Ikeda Y, Imae T, Hao J, Iida M, Kitan T (2000) Langmuir 16:7618
24. Pistolis G, Malliaris A, Paleos CM, Tsiourvas D (1997) Langmuir 13:5870
25. Jockusch S, Ramirez J, Sanghvi K, Nociti R, Turro NJ, Tamalia DA (1999) Macromolecules 32:4419
26. Wade DA, Torres PA, Tucker SA (1999) Anal Chim Acta 397:17
27. Sideratou Z, Tsiouras D, Paleos CM (2000) Langmuir 16:1766
28. Richter-Egger DL, Tesfai A, Tucker SA (2001) Anal Chem 73:5743
29. Varnavski O, Ispasoiu RG, Balogh L, Tomalia D, Goodson T III (2001) J Chem Phys 114:1962
30. Larson CL, Tucker SA (2001) Appl Spectrosc 55:679
31. Lee WI, Bae Y, Bard AJ (2004) J Am Chem Soc 126:8358
32. Wang D, Imae T (2004) J Am Chem Soc 126:13204
33. Wang D, Imae T (2007) J Colloid Interface Sci 306:222
34. Wu D, Liu Y, He C, Goh SH (2005) Macromolecules 38:9906
35. Kirchner K, Merget N, Schmidt C (1974) Chem Ing Tech 46:661
36. Patai S (Ed) (1982) The chemistry of amino, nitroso and nitro compounds and their derivatives, part 2. Patai series, Wiley, New York
37. Blaschko H (1953) Enzymic Brit Med Bull 9:146