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Preparation and characterization of a new polyamide containing ethidium bromide

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Summary

Conventional solution polycondensation of adipoyl chloride with ethidium bromide provided a new polyamide containing ethidium bromide in a polymer main chain. The polyamide was characterized by TGA, DSC, solubility, and absorption and fluorescent spectroscopy. A Good optical-quality film was obtained from HFIP solution of the polyamide. The polyamide showed blue-shift in the absorption band and high fluorescent intensity compared with ethidium bromide.

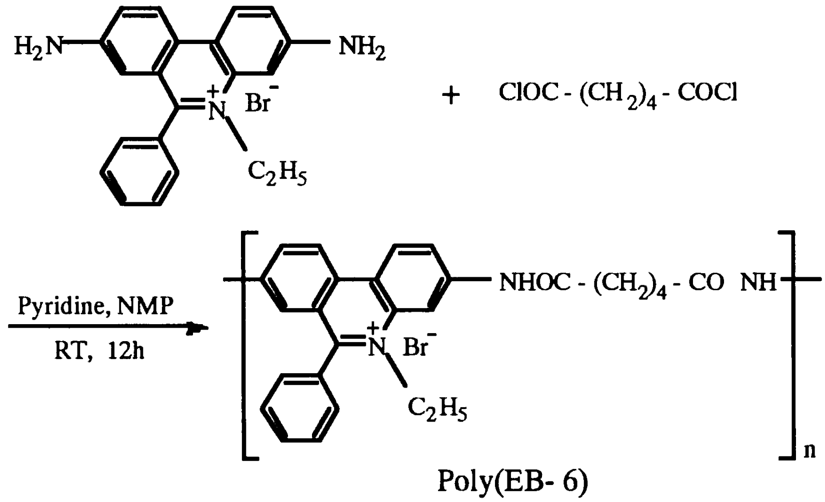
Introduction

Polyamides represent a class of high performance polymers usually formed by the reaction of diamines and diacylchloride. These polymers have found use in a wide variety of applications since they possess many desirable characteristics, such as good processability, good mechanical properties, good thermal stability, and chemical resistance [1, 2].

Ethidium bromide is a polycyclic aromatic molecule binding to deoxyribonucleic acid (DNA) by intercalating itself between the base pairs of the double helix [3, 4]. Recently, ethidium bromide intercalated within DNA matrix showed high quantum efficiencies of photoinduced electron transfers [5, 6]. Although ethidium bromide has been investigated for many years, no example of a polymer containing ethidium bromide in the main chain is known. Therefore, it is of interest in the photochemical and electrochemical behaviors for a polymer containing ethidium bromide.

Polymer offer many advantages over small-molecule systems including enhanced processing capabilities and film quality, better control over morphology and molecular organization. It is quite likely that polyamides containing ethidium bromide will possess photo- and electrochemical properties as well as good processability and thermal stability. It is thus worthy exploring the feasibility of functionalizing ethidium bromide, introducing it high-performance polyamide system.

This paper describes the synthesis of the polyamide incorporating ethidium bromide unit in the polymer main chain by conventional low-temperature solution polycondensation as shown in Scheme 1 and reports on the characterization of the resulting polyamide. Some preliminary studies of the photochemical properties of the polymer in solution and cast film are discussed.



Scheme 1 Synthetic Route of Poly(EB-6)

Experimental

Materials

Adipoyl chloride was purchased from Tokyo Kasei Co. and purified by distillation under reduced pressure. Ethidium bromide was purchased from Aldrich Chem. Co. and used as received. N-methyl-2-pyrrolidone (NMP) was purified by distillation over calcium hydride. 2-Amino-2-hydroxymethyl-1,3-propanediol (Tris) and other reagents from Wako Pure Chem. Ind. were used as received.

Characterization

The NMR spectra was obtained at 300 MHz with a Varian VXR300. Fourier transform infrared (FTIR) was recorded on a Perkin Elmer System-2000 Fourier transform spectrophotometer (KBr pellets). Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were performed with Perkin Elmer thermal analyzers DSC-7 and TGA-7, respectively. The molecular weight was determined using Tosoh PS-8010 gel permeation chromatography (GPC) system with a combination of two 0.39 cm by 30 cm Tosoh TSK-GEL columns relative to poly(methyl methacrylate) standards. GPC analysis was carried out with HFIP (hexafluoro-2-propanol) containing 10 mM sodium trifluoroacetic acid as the eluent at a flow rate of 0.4 mL min^{-1}. The eluent was monitored by measuring absorbance at 214 nm. The absorption and fluorescence spectra of the solutions were measured with a Hitachi U-4000 spectrophotometer and a Jasco FP-777 spectrofluorometer, respectively. The film of the polyamide was cast on a quartz substrate from HFIP solution at concentration of 3 g L^{-1}.

Synthesis of Polyamide

Ethidium bromide (0.50 g, 1.2 mmol) and pyridine (0.29 g, 3.6 mmol) were dissolved in 40 mL of NMP. To the solution adipoyl chloride (0.22 g, 1.2 mmol) was added at 0 °C. The solution was allowed to warm to 25 °C and was stirred for 12 h. The viscosity of the solution became drastically viscous. The polymerization all proceeded in a homogeneous solution throughout the reaction. The reaction mixture was poured into

800 mL of a vigorously stirred methanol/water (1:1) solution and the precipitate was separated by filtration. The powder was washed several times with methanol, water, and then acetone and dried at 100 °C in a vacuum oven to constant weight. The polymer was isolated as 0.51 g (84%) of a reddish brown powder. The properties of poly(EB-6) are as follows: ^{1}H-NMR (HFIP-d_2, TMS, ppm):1.58-1.65 (t, 3H, —CH_3), 7.45-7.57 (s, 2H), 7.75-8.06 (m, 5H), 8.32-8.40 (s, 1H), 8.79-8.97 (dd, 2H), 9.06-9.14 (d, 1H) (aromatic hydrogen of ethidium bromide unit), 1.68-2.01 (t, 4H), 2.46-2.75 (dd, 4H) (adipoyl unit). Anal. Calcd for $C_{27}H_{26}NO_2Br$: C, 68.07%; H, 5.49%; N, 2.94%. Found: C, 68.01%; H, 5.58%; N, 2.87%.

Results and discussion

Synthesis of polyamide

Poly(EB-6) was synthesized from ethidium bromide with adipoyl chloride by low-temperature solution polycondensation as shown in Scheme 1. The polymerization was carried out in NMP at 25 °C for 12 h. Pyridine was used as a hydrogen chloride acceptor. The polymerization all proceeded in a homogeneous solution throughout the reaction. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) of poly(EB-6) were determined by GPC in HFIP to be 8,000 and 18,500, respectively, relative to poly(methyl methacrylate) standards. It is evident that the molecular weights are not so high. Poly(EB-6) was identified by means of IR and ^{1}H-NMR spectroscopies and elemental analysis. The FTIR spectrum of poly(EB-6) exhibited absorption bands at 3320 cm^{-1} (N—H) and 1642 cm^{-1} (C=O), characteristic of the amide group.

Characterization of polyamide

The thermogravimetric (TG) curve of poly(EB-6) are shown in Figure 1. A small weight loss was observed around 100 °C due to the loss of absorbed water. The initial and second decomposition temperatures were around 237 and 409 °C, respectively, in nitrogen. The initial temperature was in fair agreement with the decomposition temperature of ethidium bromide. The temperature of 10% weight loss (T_d) was at 265 °C. The glass transition and melting temperatures were not observed by DSC due to low decomposition temperature. The low thermal stability of poly(EB-6) compared with those of other reported thermally stable polyamides can be ascribed to ethidium bromide moiety.

Poly(EB-6) is soluble in NMP, HFIP, trifluoroethanol, *m*-cresol, trifluoroacetic acid, and water/HFIP (90/10 vol%). Although ethidium bromide is highly soluble in water and common organic solvents such as methanol and acetone, poly(EB-6) was not insoluble in the solvents.

The visible absorption spectra of poly(EB-6) and ethidium bromide in water/HFIP (90/10 vol%) containing 5 mM Tris buffer (pH 7.0) and 50 mM NaCl are shown in Figure 2. The π-π^* absorption band of poly(EB-6) appeared at a considerably shorter wavelength (λ_{max} = 430 nm) than that (λ_{max} = 484 nm) of ethidium bromide in water/HFIP (90/10 vol%). The observed blue-shift might be explained in terms of a lower electron donation of the amide group of poly(EB-6) compared with the amino group of ethidium bromide by reference to the empirical rule shown by Doub *et al.*[7, 8].

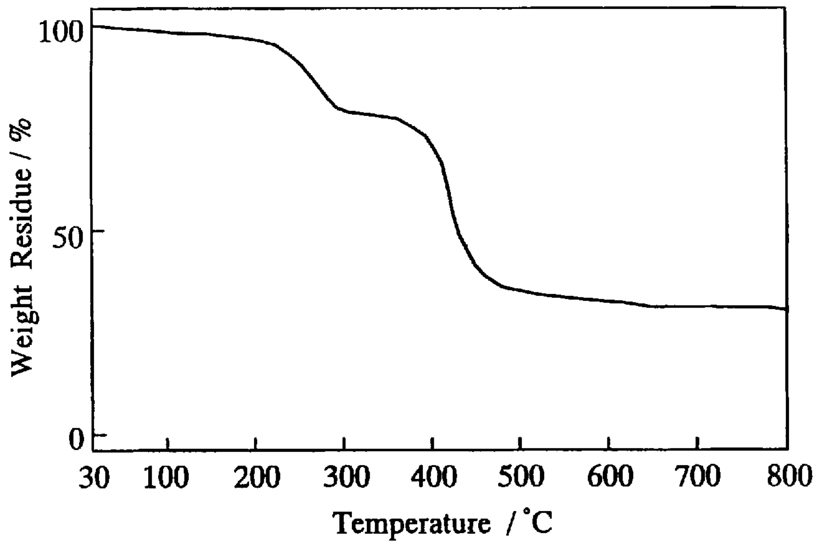


Figure 1 TG curve of poly(EB-6) at a heating rate 20 °C / min in nitrogen.

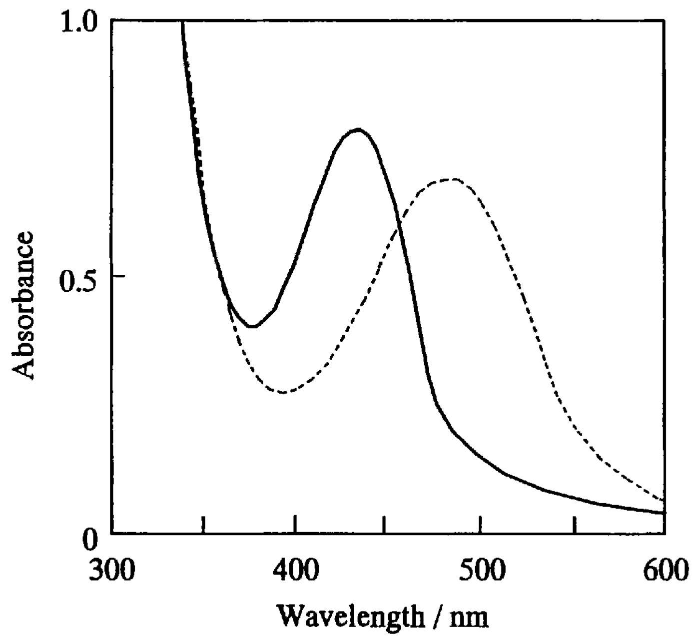


Figure 2 Absorption spectra for ethidium bromide (dotted line) and poly(EB-6) (solid line) in water/HFIP (90/10 vol%) containing 5 mM Tris buffer (pH 7.0) and 50 mM NaCl. The residual concentration of the ethidium bromide moieties is 110 μM.

Figure 3 compares fluorescence spectra of poly(EB-6) and ethidium bromide in water/HFIP (90/10 vol%) containing 5 mM Tris buffer (pH 7.0) and 50 mM NaCl. Ethidium bromide has a fluorescent band at 595 nm, whereas poly(EB-6) has a broad band at 511 nm and the intensity is much higher than that of ethidium bromide. This high fluorescent intensity could be caused by a decrease in radiationless deactivation due to reduced mobility of the ethidium bromide residue in high molecular weight compound [9].

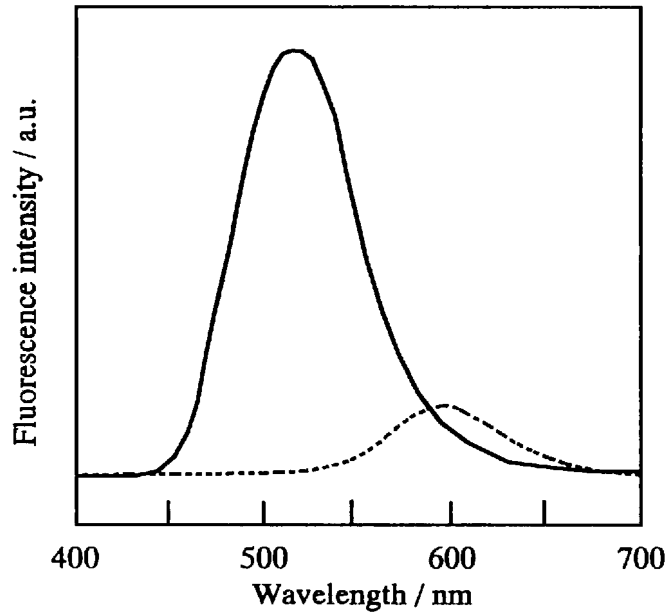


Figure 3 Fluorescence spectra for ethidium bromide (dotted line) and poly(EB-6) (solid line) in water/HFIP (90/10 vol%) containing 5 mM Tris buffer (pH 7.0) and 50 mM NaCl. The residual concentration of the ethidium bromide moieties is 11 μM. The excitation wavelength is 350 nm.

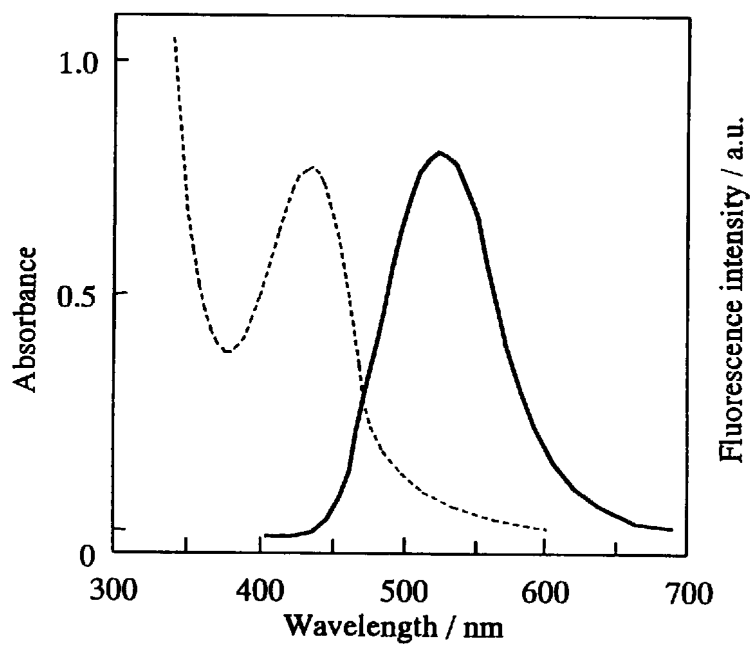


Figure 4 Absorption (dotted line) and fluorescence spectra (solid line) for the cast film of poly(EB-6) from HFIP solution. The film thickness is 0.6 μm. The excitation wavelength is 350 nm.

Figure 4 shows the absorption and fluorescence spectra of the cast film of poly(EB-6) from the HFIP solution. The absorption and fluorescence spectra of the cast film are similar to those of poly(EB-6) in the water/HFIP (90/10 vol%) solution. It turns out that the molecular weight (M_n = 8,000) is high enough to form high-quality film via casting method.

In conclusion, I have demonstrated that the polyamide containing ethidium bromide into the main chain can be synthesized by low-temperature solution polycondensation. The polyamide showed blue-shift in the absorption band and high fluorescent intensity compared with ethidium bromide. A good optical quality film was obtained from the polyamide. Multilayer thin film device can be fabricated with poly(EB-6) as the polycation and the polyanion such as poly(acrylic acid) via the layer-by-layer electrostatic self-assembly technique [10]. Further studies on the photophysical behavior and multilayer fabrication of the polyamide are currently underway.

Acknowledgments

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