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Short communication:

Preparation of fluorescent polymer particles by emulsion polymerization

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Abstract: Core-shell type fluorescent polymer particles were produced in multi-step emulsion polymerization. Cross-linking was introduced to the shell to suppress leakage of fluorescent dyes contained in the core particles. First, fluorescent polymer particles were synthesized by emulsion copolymerization of styrene (St) and butyl acrylate (BA) with an anionic surfactant (SE1025A) initiated by ammonium persulfate (APS) in the presence of pyrene. The concentrations of St, BA, SE1025A and APS were 550, 89, 8.2 and 5 mol/m³ water, respectively. Then the fluorescent particles were covered with cross-linked polymer by emulsion copolymerization of St, BA and divinylbenzene (DVB) with SE1025A emulsifier. In the polymerization, a mixture of St, BA, SE1025A and DVB was added dropwise to the reaction system. The average size of cross-linked fluorescent particles ranged from 50 to 70 nm. The fluorescence intensity of the particles prepared in a pyrene concentration range of 8 - 20 mol/m³ water increased linearly with pyrene concentration. Fluorescence intensities of the particles prepared with and without DVB were also measured. The cross-linked polymer formed by DVB addition drastically suppressed the intensity drop caused by dye leakage almost to one-third of the intensity drop measured for particles prepared without DVB addition.

Introduction

Polymer particles incorporating fluorescence dyes (fluorescent particles) have been used for a wide variety of applications, such as cell labelling, sensitive diagnostic reagents and blood flow measurements [1-3]. The fluorescent particles have been synthesized by various methods; physical adsorption is a popular method to attach fluorescent dyes to preformed polymer particles [4]. However, physical adsorption often meets the problem of easy desorption. To overcome this problem, copolymerization with reactive dyes has been developed to covalently bind dyes to polymer in

particles [5-7]. For example, addition of small amounts of dye-comonomer to the dispersion polymerization of styrene in ethanol led to fluorescent polystyrene particles with narrow size distribution, while addition of large amounts of the comonomer (≥ 1.0 wt.-%) led to polydisperse particles [8]. In an alternative approach, polymer particles were dyed *in situ* during the polymerization process [9-11]. Fluorescent polymer particles can be obtained by the simple procedure, but leakage of the dyes from the fluorescent particles can occur in a dispersive medium. Therefore, it is important to develop a technology for preventing fluorescent dyes from the leakage.

In the present work, we try to produce fluorescent polymer core particles with cross-linked shells to avoid the leakage of fluorescent dyes into a dispersive medium. Multi-step emulsion polymerization is adopted to form core particles and cross-linked shells. In the first step to prepare the core particles, the monomers of styrene (St) and butyl acrylate (BA) emulsified with the anionic surfactant SE1025A and initiated by ammonium persulfate (APS) are copolymerized in the presence of pyrene as a fluorescent dye. In the second step, to form cross-linked polymer shells in emulsion copolymerization of St and BA with divinylbenzene (DVB) and SE1025A emulsifier, a mixture of St, BA, SE1025A and DVB is added dropwisely to the reaction system. The effect of cross-linking the shell polymer on the leakage of the fluorescent dye is studied by measuring fluorescence spectra for the polymer particles obtained.

Results and discussion

Fig. 1 shows a transmission electron microscopy (TEM) image of polymer particles prepared at a pyrene concentration of 8.1 mol/m^3 and a DVB concentration of 480 mol/m^3 water. The polymer particles were spherical with an average size of 65 nm and a variation coefficient of the size distribution of 17%.

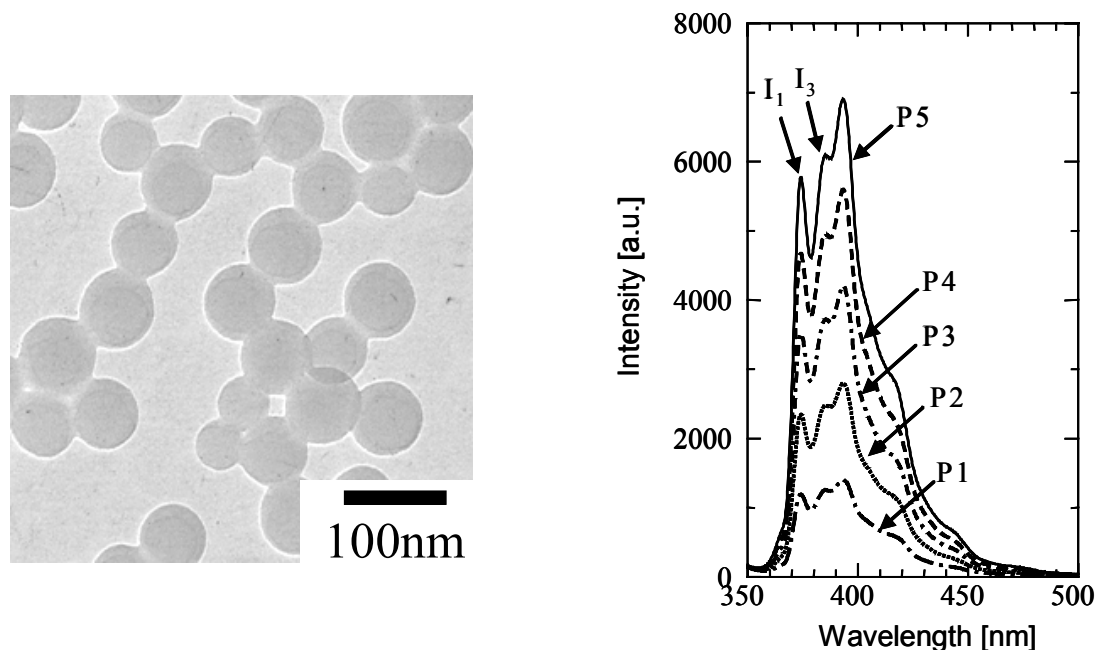


Fig. 1. TEM image of polymer particles prepared at a pyrene concentration of 8.1 mol/m^3 and a DVB conc. of 480 mol/m^3 water

Fig. 2. Fluorescence spectra of polymer particles prepared at different pyrene conc.: 4.0 (P1), 8.1 (P2), 12.0 (P3), 16.1 (P4) and 20.1 (P5) mol/m^3 water

Fig. 2 shows fluorescence spectra of the polymer particles prepared at a DVB concentration of 480 mol/m^3 water in a pyrene concentration range of $4.0 - 20.1 \text{ mol/m}^3$ water. Fluorescence spectra were measured after dialysis to remove non-reacted monomer. Each spectrum has three peaks at wavelengths of 375, 385 and 394 nm that were attributed to pyrene molecules [12]. It is known that the ratio of emission intensities of 375 nm (I_1) and 385 nm (I_3) depends on the dielectric constant of the disperse ion medium surrounding the pyrene molecules. The emission intensity ratios in Fig. 2 are well consistent with the specific value of 0.95 reported for pyrene incorporated into polystyrene [13] and very different from the value 1.9 reported for pyrene in water [14]. Since pyrene molecules are hydrophobic, it is likely that almost all the pyrene molecules added to the reaction system were incorporated into polymer particles. Fig. 3 shows the emission intensities I_1 in Fig. 2 plotted against pyrene concentration. Fluorescence intensity increased linearly with pyrene concentration.

Fig. 4 shows the relative decrease of fluorescence intensity before and after dialysis of fluorescent particles prepared at DVB concentrations of 0, 240 and 480 mol/m^3 and a pyrene concentration of 16.1 mol/m^3 water. The intensity of fluorescence of particles without DVB decreased by 8% during dialysis. Cross-linking with DVB concentrations examined drastically suppressed the relative intensity decrease almost to one-third of that of particles without DVB addition. Thus, cross-linkage in the shell was effective to suppress the leakage of fluorescent dye from the polymer particles.

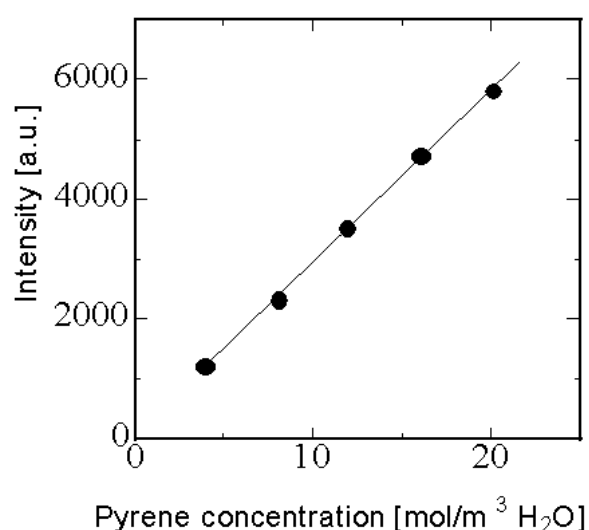


Fig. 3. Fluorescence intensity at a wavelength of 375 nm for the fluorescent particles prepared in a pyrene concentration range of 0 - 20.1 mol/m^3 water

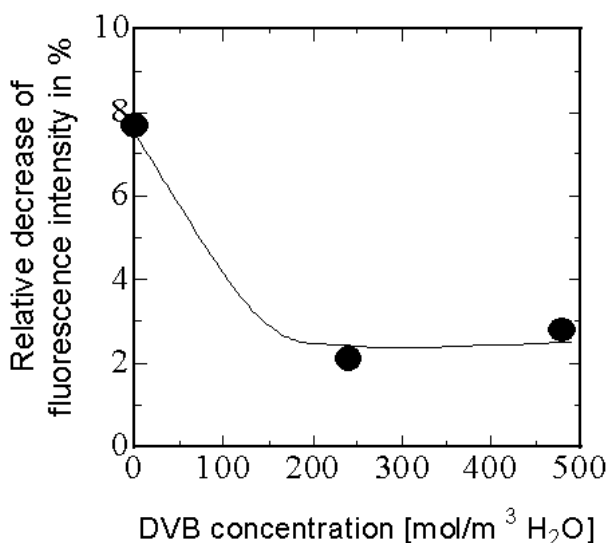


Fig. 4. Relative decrease of fluorescence intensity (I_1) by dialysis. The fluorescent particles were prepared in a DVB concentration range of 0 - 480 mol/m^3 water. The decrease in fluorescence was divided by the intensity before dialysis

Experimental part

Reagents

St (99.5%), BA (98%), DVB (55%), APS (98%) and pyrene (98%) were obtained from Wako Pure Chemical Industry (Osaka), and the anionic surfactant SE1025A (25%)

was purchased from Asahi Denka Co., Ltd. (Tokyo). The chemical structure of SE1025A is shown in Fig. 5. All chemicals were used as received. Water was deionized and distilled to have an electric resistance higher than 180 kΩ·m.

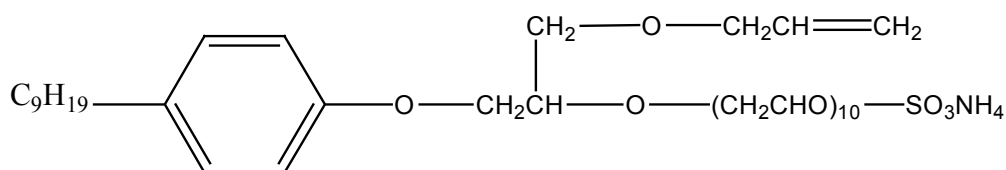


Fig. 5. Chemical structure of SE1025A

Polymerization

A typical example is described as follows: an emulsion composed of 4.0 g St, 0.8 g BA, 0.5 g SE1025A, 70 g water and a given amount of pyrene was added to a 300 ml four-neck flask equipped with an impeller, a reflux condenser, a thermometer and a N₂ inlet. The impeller was a 4-bladed 45° pitch paddle with a diameter of 5 cm. The emulsion to prepare fluorescent polymer particles was stirred under nitrogen atmosphere and heated up to 80°C. After 15 min stirring, a mixture of 0.4 g APS and 7.6 g water was added to the emulsion. The concentrations of St, BA, SE1025A and APS were adjusted to 550, 89, 8.2 and 5 mol/m³ water, respectively. At a reaction time of 30 min, an emulsion that consisted of 75.3 g St, 15.1 g BA, 1.5 g SE1025A, 80 g water and a given amount of DVB was added to the reactant emulsion. The dropwise addition was performed for 4 h, and stirring at 80°C was continued for the next 4 h to complete monomer conversion. The monomer conversion examined by measuring the polymer weight was confirmed to be higher than 95%.

Purification

A suspension of fluorescent particles formed in the polymerization mentioned above was purified via dialysis to remove non-reacted monomer. The suspension was charged into the cellulose tube (Spectrum Laboratories, molecular weight cut-off = 8000) and soaked in the deionized water for 1 week. The deionized water was replaced with new one per 24 h.

Characterization

Fluorescence spectra were measured with a fluorescence spectrometer (Hitachi, F-4500) equipped with right-angle geometry (90° collecting optics). The suspension of purified fluorescent particles was diluted 4000-fold before fluorescence measurement. The wavelength of 338 nm was used to excite the fluorescent particles. Particle size distributions of the polymer particles were determined with transmission electron microscopy (TEM) (Zeiss, LEO 912 OMEGA).

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