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ORIGINAL CONTRIBUTION

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Immobilization of cationic polymer particles having active ester groups onto solid surfaces

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Abstract Monodispersed cationic polymer particles with sulfonium groups and active ester groups at their surfaces were prepared by emulsifier-free emulsion copolymerization of styrene (ST) with a water-soluble active ester monomer, methacryloyloxyphenyldimethylsulfonium methylsulfate (MAPDS). The cationic polymer particle monolayers were fabricated on unmodified and aminated glass plates by electrostatic interactions and chemical reactions, respectively. The polymer particles were immobilized onto unmodified glass plates at relatively regular intervals in the absence of electrolytes, and the morphology of particle monolayers on the glass plates was changed with solid content of latex, electrolyte and cationic surfactant concentration. The polymer particles were immobilized onto aminated glass plate as aggregates by controlling the pH of latex and electrolyte concentration. Remaining active ester groups of the particle monolayers were confirmed to react easily with primary amino compounds.

Keywords Cationic polymer particle · Polymer particle monolayer · Electrostatic interaction · Chemical reaction · Active ester group

Introduction

Preparation of monodispersed functional polymer particles has been established over the past few decades by emulsifier-free emulsion copolymerization, dispersion copolymerization and seeded polymerization [1, 2, 3, 4, 5, 6, 7]. Particularly, emulsifier-free emulsion copolymerization is known to be the most effective method for preparing the polymer particles with submicron sizes in diameter. The use of the polymer particles in an aqueous medium for applications such as affinity chromatography, drug delivery and clinical test have been demonstrated in several publications [8, 9, 10]. Recently, fabrication of the polymer particles onto solid substrates has received much attention, because of the potential applications to several devices such as catalysts, diagnostics, biosensors, multiple electrodes and novel optical materials. When the particle monolayers are fabricated, the driving forces between the polymer particles and solid substrates such as electrostatic interactions, surface tension, van der Waals forces, steric interactions and capillary forces play key roles. In fact, novel immobilization techniques for fabrication of particle monolayers have been reported. Fabrication of polyacrolein particle monolayers onto modified polyethylene and glass surfaces by covalent immobilization has been demonstrated by S. Margel's and S. Slomkowski's groups [11, 12, 13, 14]. Electrostatic interactions between carboxylated latex particles and charged surfaces were employed by B. Tieke's groups, and the morphology of the particle monolayers was examined [15, 16].

The synthesis and characterization of novel cationic polymer particles (P(ST-*co*-MAPDS) particles) with a high surface density of sulfonium groups and active ester groups by emulsifier-free emulsion copolymerization of styrene (ST) with methacryloyloxyphenyldimethylsulfonium methylsulfate (MAPDS) have been described in previous publications (Fig. 1) [17, 18]. The active ester groups at the surface of the particles have a high reactivity with primary amino compounds. We have found that P(ST-*co*-MAPDS) particles are well immobilized onto hydrophobic surfaces through hydrophobic interactions [19]. In this paper, the morphology of P(ST-*co*-MAPDS) particle monolayers onto unmodified glass plates through electrostatic interactions and onto aminated glass plates treated with 3-aminopropyltriethoxysilane (APTES) through chemical reactions was observed by a scanning electron microscopy (SEM), factors affecting the morphology of the particle monolayers were investigated. The reactivity of remaining active ester groups on the particle monolayers was also examined.

Experimental

Materials

ST was purchased from Wako Pure Chemical Industries, Ltd. and purified by distillation under reduced pressure in a nitrogen atmosphere. MAPDS was prepared according to the method described in our previous paper [17].

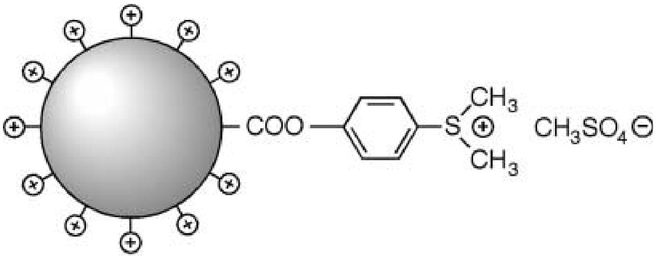


Fig. 1. Structure of P(ST-*co*-MAPDS) particles

2,2'-Azobis(2-amidinopropane) dihydrochloride (V-50, reagent grade from Wako Pure Chemical Industries, Ltd.) was employed as received. 3-Aminopropyltriethoxysilane (APTES) and dansylhydrazine were purchased from Tokyo Kasei Kogyo Co., Ltd. Glass plates (10 mm×26 mm×1.0 mm) used in this study were cleaned with boiling HNO_3 solution for 1 h, washed with deionized water, and dried in vacuum. Cationic surfactants of *n*-hexyltrimethylammonium bromide (HTAB), *n*-octyltrimethylammonium bromide (OTAB), *n*-dodecyltrimethylammonium bromide (DTAB) and *n*-tetradecyltrimethylammonium bromide (TTAB) received from Tokyo Kasei Kogyo Co. and *n*-cetyltrimethylammonium bromide (CTAB) obtained from Wako Pure Chemical Industries, Ltd. were employed after purification by recrystallization.

Preparation of P(ST-*co*-MAPDS) particles

P(ST-*co*-MAPDS) particles were prepared by emulsifier-free emulsion copolymerization of ST (320 mmol) with MAPDS (3.2 mmol) at 60 °C with V-50 (3.2 mmol) as an initiator, as described elsewhere [18]. The polymer particles were purified by centrifugation and washing with deionized water several times. The number-average diameter, d_n, the standard deviation, σ, and the coefficient of variation, C_v were determined using a SEM (JEOL, JSM-5310). Surface charges of the polymer particles were measured by colloidal titration using potassium polyvinyl sulfate as a titrant.

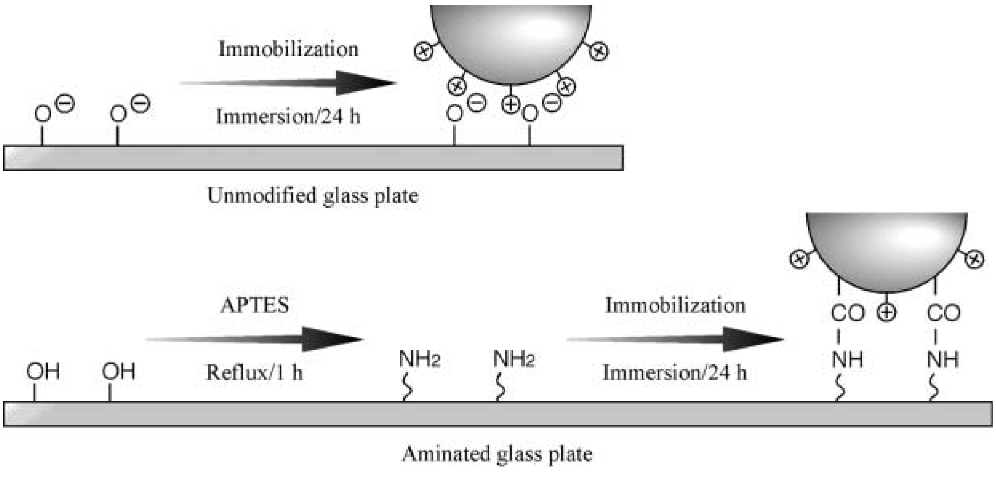
Amination of glass plates

Glass plates were aminated with toluene solutions of APTES at 110 °C for 1 h, followed by washing with ethanol and drying in vacuum [20, 21]. Characterization of aminated glass plates treated with APTES was carried out using an X-ray photoelectron spectroscopy (Shimadzu, ESCA-1000).

Immobilization of P(ST-*co*-MAPDS) particles

Unmodified or aminated glass plates were immersed into P(ST-*co*-MAPDS) latex dispersions at 25 °C for 24 h, taken out of the dispersions, and washed in deionized water by ultrasonic cleaning for 5 min to remove weakly and/or physically bound particles. Figure 2 shows schematic representation of immobilization of the polymer particles onto glass and aminated glass plates. The morphology of the particle monolayers was observed by a SEM and the coverage of glass plates with the polymer particles was determined using the following equation (1):

Fig. 2. Schematic representations of immobilization of P(ST-*co*-MAPDS) particles onto unmodified or aminated glass plates



$$\text{Coverage}(\%) = \frac{\text{Total perspective area of polymer particles on unit area}}{\text{Unit area of solid plate}} \times 100 \qquad (1)$$

Patterning using P(ST-*co*-MAPDS) particles

Fabrication of patterned polymer particle monolayer onto aminated glass plates was carried out using photoresist methods as follows: aminated glass plates were spin-coated using resist solution (Nippon Zeon Co., Ltd.; ZPP1700PG) (first: 500 rpm/5 s; second: 3,000 rpm/3 s) to form photoresist films (thickness, 1.5 μm). The plates were irradiated through a photomask using a mercury lamp (19.0 mW/cm^2) for 10 s. The irradiated glass plates were developed in alkaline solution (MIF developing solution obtained from Fuji Photo Film Co., Ltd.) for 60 s. After rinse in water, the photopatterned plates were dried in air at 20 °C. The photopatterned plates were immersed into latex dispersions (0.25 wt%) containing 100 mM sodium chloride (NaCl) for 24 h, taken out of the dispersion, and washed in 1.0 M aqueous potassium hydroxide solutions and in water by ultrasonic cleaning for 5 min. The morphology of the particle monolayers was observed by a SEM.

Reactivities of active ester groups on P(ST-*co*-MAPDS) particle monolayers

P(ST-*co*-MAPDS) particles monolayers on aminated glass plates were immersed into 10 mM methanol solutions of dansylhydrazine as a fluorescence probe for 3 h, taken out of the solutions, and washed in methanol. When exposed to excitation at wavelength of 340 nm, the fluorescence intensity near wavelength of 460 nm was examined.

Results and discussion

Preparation of P(ST-*co*-MAPDS) particles

P(ST-*co*-MAPDS) particles with ca. 220 nm in diameter can be obtained in high yield (ca. 96%). The values of σ and C_v were 5.8 and 2.6, respectively, which indicate the formation of well-defined polymer particles with narrow distribution of particle size. The surface charges of the polymer particles were determined to be 13.0 μeq/g-latex by colloidal titration. The occupied area for one positive charge was calculated to be ca. 60 Å^2, which indicates that the polymer particles have a high positive surface charge density originating from MAPDS and V-50.

Immobilization of P(ST-*co*-MAPDS) particles onto unmodified glass plates

Unmodified glass plates were immersed into P(ST-*co*-MAPDS) latex dispersion (0.025 wt%) at 25 °C for 24 h. Figure 3 shows SEM photograph of the polymer particles immobilized on the glass plate. The polymer particles are homogeneously immobilized onto the glass plates at relatively regular intervals, though some small aggregates are also observed. Such formation of regular array is due to the strong electrostatic repulsion between the cationic polymer particles. The relationship between latex concentration and the coverage of unmodified glass plates with P(ST-*co*-MAPDS) particles is shown in Fig. 4. The coverage gradually increases with an increase in the latex concentration.

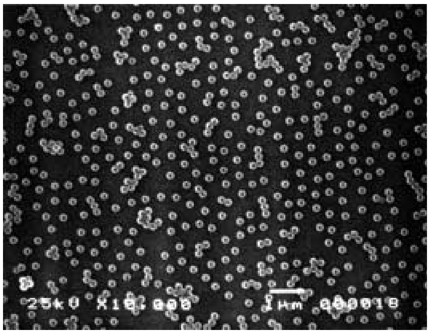


Fig. 3. SEM photograph of P(ST-*co*-MAPDS) particles immobilized on unmodified glass plates: [Latex], 0.025 wt%

The influence of electrolytes on the particle immobilization was investigated in order to control the morphology of P(ST-*co*-MAPDS) particle monolayer. Unmodified glass plates were immersed into P(ST-*co*-MAPDS) latex dispersions (0.025 wt%) containing NaCl or sodium iodide (NaI) as electrolytes. The relationship between electrolyte concentration and the coverage is shown in Fig. 5. The coverage drastically increases with an increase in the NaCl concentration. This can be ascribed to the decrease in the electrostatic repulsion between the polymer particles. In the latex dispersions containing 400 mM NaCl, however, the coverage suddenly decreases. This could be caused by the flocculation of the polymer particles in aqueous solution. Similarly as observed in the presence of NaCl, the coverage increases with an increase in the NaI concen-

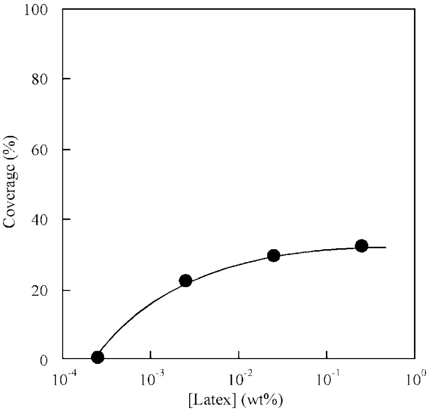


Fig. 4. Coverage of unmodified glass plate with P(ST-*co*-MAPDS) particles against latex concentration

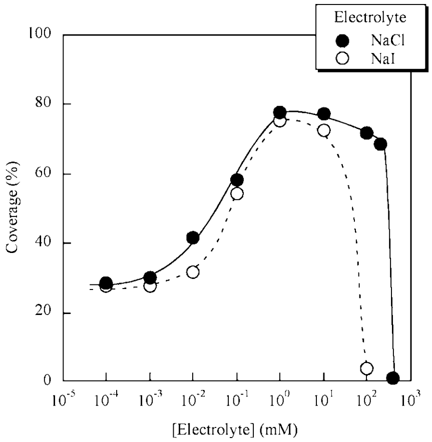
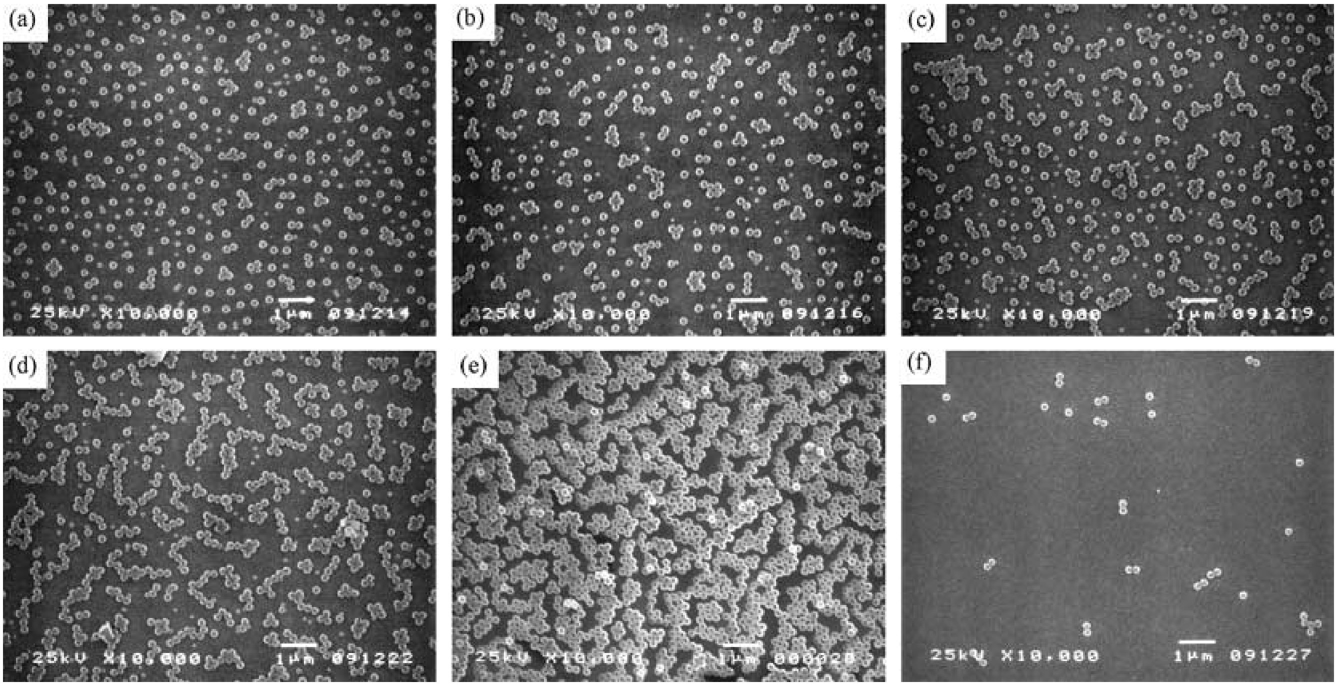


Fig. 5. Coverage of unmodified glass plate with P(ST-*co*-MAPDS) particles against electrolyte concentration: [Latex], 0.025 wt%

tration and greatly decreases at NaI concentration above 100 mM, compared with that for the latex dispersions using NaCl as the electrolyte. Such greater effect of NaI may result from ion exchange of methylsulfate ion ($CH_3SO_4^-$) by soft iodide anion at the surface of P(ST-*co*-MAPDS) particles. Figure 6 shows the SEM photographs of P(ST-*co*-MAPDS) particle monolayers on unmodified glass plates at different NaCl concentrations. The polymer particles are immobilized onto the glass plates at relatively regular intervals up to 0.1 mM NaCl concentration, though the number of small aggregates increases with increasing the NaCl concentration. At NaCl concentrations above 0.1 mM, larger aggregates are predominately immobilized. At 400 mM NaCl, only a few polymer particles are immobilized. The coverage is largest at 1.0 mM NaCl under the conditions.

To get a better insight of the surface interactions between polymer particles and glass plates, particle immobilization onto the glass plates was carried out in the presence of cationic surfactants with various alkyl chains. Unmodified glass plates were immersed into P(ST-*co*-MAPDS) latex dispersions (0.025 wt%) containing HTAB, OTAB, DTAB, TTAB or CTAB of 0.75 mM lower than their critical micelle concentrations. Strong dependence of the coverage on the alkyl chain length of cationic surfactants is observed as shown in Fig. 7. Polymer particles are densely assembled at the coverage of about 80% as aggregates onto the glass plates in the presence of HTAB, OTAB or DTAB. On the contrary, no particles are immobilized for the case of TTAB and CTAB (coverage ≈0%). This can be ascribed to the adsorption of cationic surfactants onto the polymer particles and the glass plates. The surfactants with long alkyl groups are well adsorbed onto the solid surfaces to form hemi-micelles, admicelles, bilayer and other complicated assembled structures dependent on the relative strength of the interactions of the ionic groups and hydrocarbon components of the surfactants with the substrates [22, 23]. In the case of HTAB, OTAB

Fig. 6a–f. SEM photographs of P(ST-*co*-MAPDS) particles immobilized on unmodified glass plates against NaCl concentration: [Latex], 0.025 wt%; [NaCl], **a** 0.0001 mM, **b** 0.001 mM, **c** 0.01 mM, **d** 0.1 mM, **e** 25 mM, **f** 400 mM



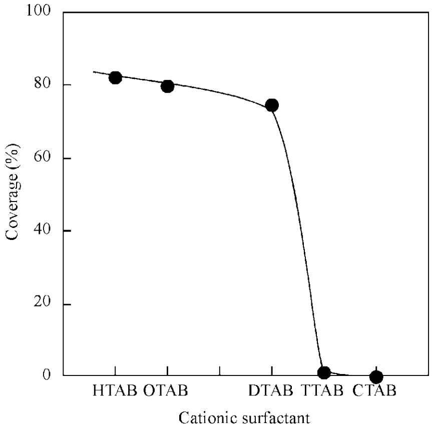


Fig. 7. Coverage of unmodified glass plate with P(ST-*co*-MAPDS) particles against cationic surfactant: [Latex], 0.025 wt%; [Surfactant], 0.75 mM

and DTAB, these surfactants are electrostatically adsorbed onto the glass surface to form monolayers orienting the hydrophobic alkyl chains toward to aqueous phase. Thus, P(ST-*co*-MAPDS) particles would be effectively immobilized onto the glass plates by hydrophobic interactions, as reported previously [19]. On the other hand, the surfactants bearing long alkyl groups, such as TTAB and CTAB, are adsorbed onto both the glass surface and polymer particles, resulting in a reduction of the hydrophobic interaction in contrast with the short-alkylated surfactants. This would make the particle immobilization onto the glass plates through hydrophobic interaction unfavorable.

Immobilization of P(ST-*co*-MAPDS) particles onto aminated glass plates

The surface composition of glass plates aminated with APTES was carried out using XPS analysis. The peak at 402.3 eV is assigned to the amino groups on the glass plate.

Aminated glass plates were immersed into P(ST-*co*-MAPDS) latex dispersions (0.25 wt%) of pH adjusted by hydrochloric acid and sodium hydroxide at 25 °C for 24 h. Figure 8 shows SEM photographs of polymer particle monolayers fabricated on aminated glass plates against pH of latex. When aminated glass plates were immersed at pH 3.0, the coverage is low (ca. 5%). This would be due to the electrostatic repulsion between the polymer particle and the protonated amino groups (–NH_3^+) on glass plates. At pH 11.0, the polymer particles are immobilized on the aminated glass plates as aggregates through the reaction of active ester groups on the polymer particles with amino groups on the aminated glass plate. The relationship between pH of latex and the coverage is shown in Fig. 9. The coverage drastically increases up to ca. 80% with an increase in pH of latex above pH 9.0.

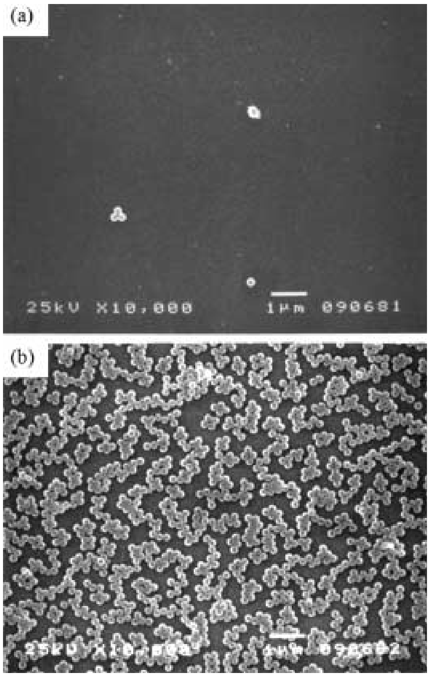


Fig. 8a, b. SEM photographs of P(ST-*co*-MAPDS) particles immobilized on aminated glass plates against pH of latex: [Latex], 0.25 wt%; **a** pH 3.0, **b** pH 11.0

Aminated glass plates were immersed into P(ST-*co*-MAPDS) latex dispersions without pH adjustment (pH 6.2) containing NaCl as an electrolyte in order to suppress the electrostatic repulsion between polymer particle and the protonated amino groups (–NH_3^+) on the glass plates. The relationship between NaCl concentration and coverage is shown in Fig. 10. For both 0.025 and 0.25 wt% latex dispersions, the coverage

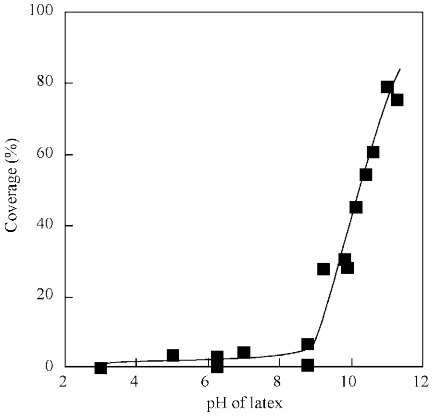


Fig. 9. Coverage of aminated glass plate with P(ST-*co*-MAPDS) particles against pH of latex: [Latex], 0.25 wt%

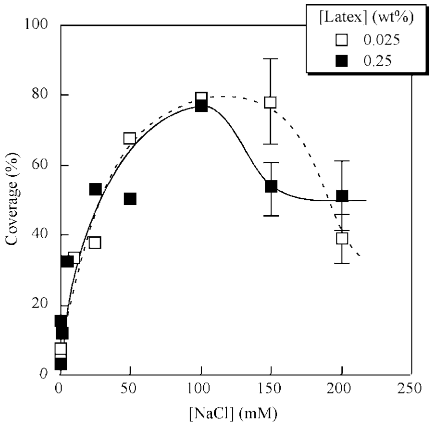


Fig. 10. Coverage of aminated glass plate with P(ST-*co*-MAPDS) particles against NaCl concentration: Latex, pH 6.2

greatly increases with an increase in the NaCl concentration. For latex dispersions containing NaCl above 100 mM, however, the coverage decreases. The decrease in the coverage is smaller than that for unmodified glass plates shown in Fig. 5. Figure 11 shows SEM photographs of polymer particle immobilized on the aminated glass plates. When aminated glass plates were immersed into electrolyte-free latex dispersions, the coverage is low. In the latex dispersion containing 100 mM NaCl, the coverage increases up to ca. 80% and the aggregates of polymer particles are immobilized onto the aminated glass plates.

Patterning using P(ST-*co*-MAPDS) particles

Fabrication of patterned polymer particle monolayer onto solid substrates has been investigated [24, 25, 26]. Patterned assembling of polymer particles onto the aminated glass plates was carried out using photoresist methods. Figure 12 shows SEM photographs of P(ST-*co*-MAPDS) particles immobilized on photopatterned aminated glass plates at the magnification of (a) 5,000× and (b) 10,000×. The polymer particles are successfully patterned onto aminated glass plate.

Reactivities of active ester groups on P(ST-*co*-MAPDS) particle monolayers

Reactivities of remaining active ester groups of P(ST-*co*-MAPDS) particle monolayers on the aminated glass plates were examined by the reaction of active ester groups on the immobilized polymer particles with dansylhydrazine as fluorescence probe. Figure 13 shows fluorescence spectra of immobilized P(ST-*co*-MAPDS) particle monolayers on aminated glass plates before and after the reaction with dansylhydrazine. The peak at 460 nm is assigned to the dansylhydrazide on the

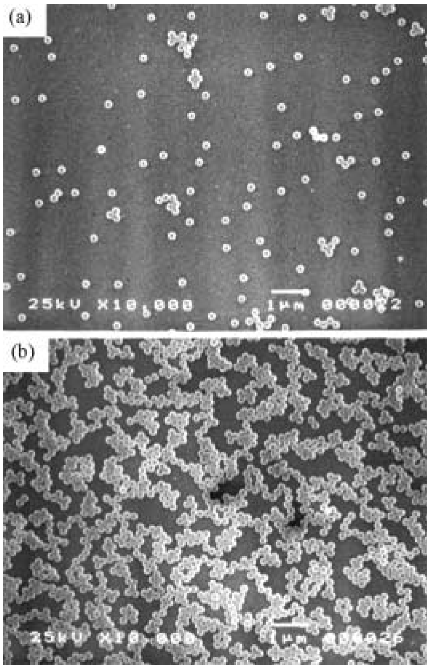


Fig. 11a, b. SEM photographs of P(ST-*co*-MAPDS) particles immobilized on aminated glass plates against NaCl concentration: [Latex], 0.025 wt%; pH 6.2; [NaCl], **a** 0 mM, **b** 100 mM

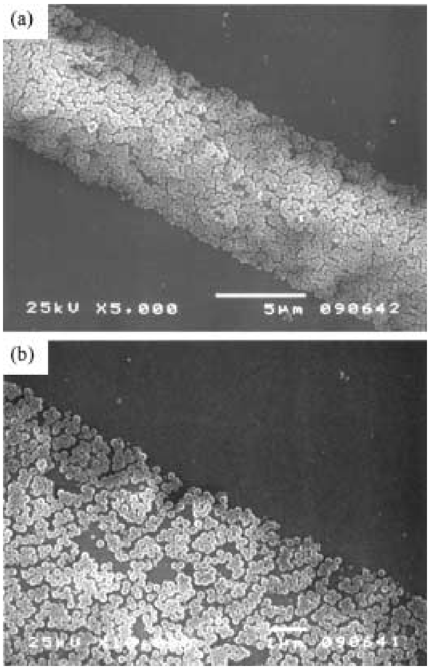


Fig. 12a, b. SEM photographs of P(ST-*co*-MAPDS) particles patterned on aminated glass plates: [Latex], 0.25 wt%; [NaCl], 100 mM; Magnification, **a** 5,000×, **b** 10,000×

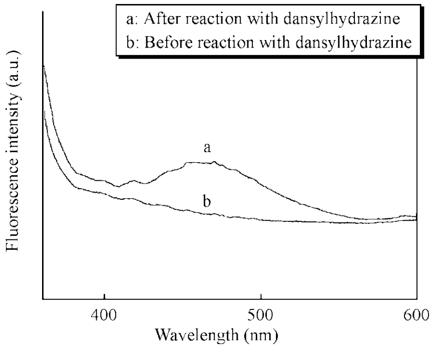


Fig. 13a, b. Fluorescence spectra of immobilized P(ST-*co*-MAPDS) particle monolayers on aminated glass plate **a** after reaction with dansylhydrazine, and **b** before reaction with dansylhydrazine

aminated glass plates, indicating that particle monolayer at the surface of aminated glass plates retain the reactivity of active ester groups. Thus, primary amino compounds can be introduced to the particle monolayer.

Conclusions

It was found that P(ST-*co*-MAPDS) particle monolayers are fabricated on unmodified and aminated glass plates by electrostatic interactions and chemical reactions, respectively. P(ST-*co*-MAPDS) particles were immobilized onto unmodified glass plates at relatively regular intervals in the absence of electrolytes. The morphology of particle monolayers on the glass plates was dependent on solid content of latex, electrolyte and cationic surfactant concentration. However, in the case of aminated glass plates, the polymer particles were immobilized as aggregates by controlling pH of latex and electrolyte concentration. Primary amino compounds could be easily introduced to the particle monolayers. Thus, the particle monolayers fabricated in this study would be useful for further functionalizations by taking advantage of remaining active ester groups.

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