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ORIGINAL CONTRIBUTION

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# Novel self-organization of polymer particles on hydrophobic solid surfaces through hydrophobic interaction

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**Abstract** A novel technique of particle monolayer fabrication based on hydrophobic interactions in aqueous systems is described in this paper. When alkylated glass plates modified with various silane coupling agents were immersed in aqueous dispersions of submicron-sized polystyrene particles of cationic or anionic surface charges, cationic particle monolayers containing active ester groups were effectively formed at the plate surfaces, whereas no anionic particles were self-organized on the plate surfaces. The coverage of the plates with cationic particles and the morphology of the monolayers varied with the hydrophobicities of the particles and plates as well as with the ionic strength of the medium and temperature. For less hydrophobic methylated glass surfaces modified with methyltriethoxysilane, cationic particles were self-organized at relatively regular intervals, whereas they were self-organized in the form of aggregates for the more hydrophobic octadecylated glass plates treated with *n*-octadecyltriethoxysilane. Closely packed monolayers were fabricated by adjusting ionic strength and temperature. Fluorescence labelling of cationic particle monolayers was successfully accomplished by the reaction of remaining active ester groups on the monolayers with a fluorescence probe containing amino groups. Cationic particle monolayers were physically stabilized by heating above the glass transition temperature ($T_g$) of the particles.

**Keywords** Alkylated glass plate · Cationic particle · Anionic particle · Hydrophobic interaction · Fluorescence labelling

## Introduction

A variety of monodispersed colloid particles have been prepared and their applications have been studied over the last decades [1, 2, 3]. Recently, fabrication of highly ordered monolayers of the colloid particles in two dimensions has received much attention, because of their potential applications to several devices such as biosensors, biochips, multiple electrodes and novel optical materials [4, 5, 6, 7]. The formation of particle monolayers utilizing solvent evaporation, the Langmuir-Blodgett method and the electrophoretic deposition method has been investigated over the past few decades [8, 9, 10, 11, 12, 13]. Patterning of the assembled particles was realized by several methods [14, 15, 16]. Self-organization of colloid particles on substrates patterned with anionic and cationic regions through electrostatic and capillary forces has been reported [17]. These techniques for the formation of patterned structures are based on ionic adsorption onto solid substrates, and the utilization of chemical reactions between reactive polymer particles and substrates has also been reported.

Fabrication of polyacrolein particle monolayers, on oxidized polyethylene and glass surfaces modified with silane coupling agents containing amino groups, by covalent immobilization has been demonstrated [18, 19, 20]. We reported that cationic polystyrene particles having active ester groups at their surfaces were successfully immobilized at laminated glass plates treated with 3-aminopropyltriethoxysilane through chemical reaction [21, 22]. When such reactive polymer particles are used for fabrication of particle monolayers, further functionalizations are possible by taking advantage of remaining reactive groups of the monolayers.

We recently found a novel self-organization of polymer particles on hydrophobic solid surfaces such as polymer films and alkylated glass plates modified with silane coupling agents in aqueous systems [23]. By taking advantage of the results, we succeeded in the preparation of heterocoagulates of submicron-sized polymer particles on uncharged, cross-linked particles of micron size [24]. Although it is well known that surfactants and proteins are adsorbed on polymer particles through hydrophobic interaction [25, 26, 27, 28], self-organization of polymer particles at hydrophobic solid surfaces in aqueous systems has not been reported so far. The technique, based on hydrophobic interaction, would further extend the scope for the fabrication of particle monolayers in particular, which permits the use of polymeric materials as substrates. This paper reports the results on characteristic features of the formation and the structure of particle monolayers on alkylated glass plates in further detail.

## Materials and methods

*Materials* Styrene (ST) (Wako Pure Chemical) was purified by distillation under reduced pressure in a nitrogen atmosphere. Water-soluble, active ester monomer, 4-methacryloyloxyphenyldimethylsulfonium methylsulfate (MAPDS), was prepared according to the method described before [29]. Sodium *p*-styrenesulfonate (NaSS) purchased from Tokyo Kasei Kogyo was used after purification by recrystallization. 2,2'-Azobis(2-amidinopropane) dihydrochloride (Wako Pure Chemical) and ammonium peroxodisulfate (Kanto Chemical) were used without purification. Non-ionic surfactant, hexa(ethyleneglycol) mono *n*-dodecyl ether (C12EO6), was obtained from Nikko Chemicals. Glass plates (10×26×1.0 mm) used in this study were cleaned with boiling $HNO_3$ solution for 1 h, washed with water, and dried in vacuum at 100 °C. Methyltriethoxysilane and *n*-octadecyltriethoxysilane were obtained from Shin-Etsu Chemical and Tokyo Kasei Kogyo, respectively. Cationic surfactant, *n*-cetyltrimethylammonium bromide (CTAB), purchased from Wako Pure Chemical was employed after purification by recrystallization. Dansylhydrazine was used as received from Tokyo Kasei Kogyo.

*Synthesis of polymer particles* Cationic P(ST-*co*-MAPDS) particles containing sulfonium groups and active ester groups (Fig. 1a) were prepared by ST with MAPDS according to the procedure described elsewhere [22]. Anionic P(ST-*co*-NaSS) particles (Fig. 1b) were prepared by emulsifier-free emulsion copolymerization of ST with NaSS at 70 °C using ammonium peroxodisulfate as an initiator and sodium sulfate as an electrolyte. The resulting polymer particles were purified by centrifugation and washing with water several times. The surface charge number of the particles was measured by colloid titration.

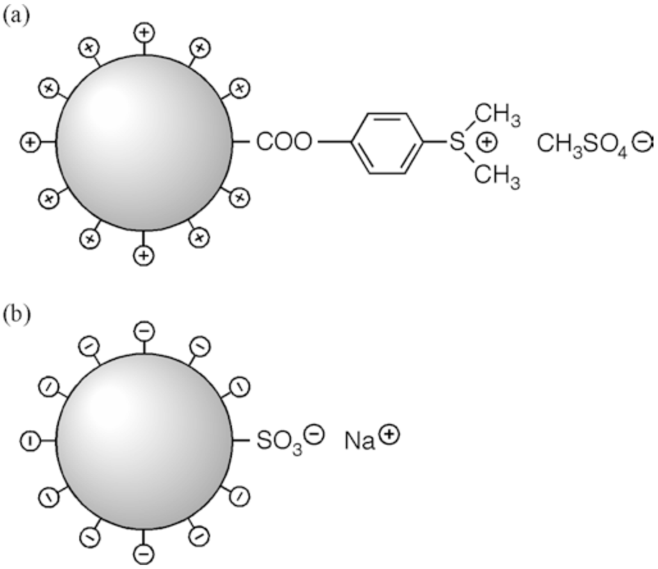

**Fig. 1a, b** Structures of **a** cationic poly(styrene-*co*-methacryloyloxyphenyldimethylsulfonium methylsulfate) [P(ST-*co*-MAPDS)], and **b** anionic poly(styrene-*co*- sodium *p*-styrenesulfonate) [P(ST-*co*-NaSS)] particles

*Estimation of hydrophobicity of polymer particles* The adsorption of C12EO6 as a non-ionic surfactant on the polymer particles was examined to characterize the hydrophobicity of the particle surfaces. The polymer particles were immersed in an aqueous solution of C12EO6 at 25 °C for 24 h and then the solution was filtered. The surfactant concentration in the filtrate was determined according to the colorimetric method[30], and the amount of adsorption was calculated.

*Preparation of alkylated glass plates* Freshly prepared glass plates were reacted with methyltriethoxysilane or *n*-octadecyltriethoxysilane (2.0 g) in purified toluene solutions (100 ml) for 24 h, followed by washing with ethanol and drying in vacuum. Surface characterization of the plates treated with the coupling agents was carried out by contact angle measurements of water.

*Self-organization of polymer particles on alkylated glass plates* Self-organization experiments were conducted as follows: alkylated glass plates were immersed into latex dispersion (10 ml) at a given concentration for 24 h, taken out of the dispersion, and washed in water by ultrasonic irradiation of 18 W at 42 kHz oscillation frequency for 5 min to remove weakly and/or physically bound particles. No change in the morphology of particle monolayers during ultrasonic irradiation was observed. The morphology of particle monolayers was observed by a scanning electron microscope (SEM) (JEOL, JSM-5310) and analyzed by determining the normalized surface coverage from SEM photographs. The coverage was calculated using Eq. 1:

$$\text{Coverage}(\%) = \frac{N}{N_{\max}} \times 100 \qquad (1)$$

where $N$ and $N_{\max}$ are the number of particles per unit area and the maximum number of particles with hexagonally closed packing, respectively.

*Fluorescence labelling of P(ST-co-MAPDS) particle monolayers* P(ST-*co*-MAPDS) particle monolayers on octadecylated glass plates modified with *n*-octadecyltriethoxysilane prepared in the presence of 10 mM NaCl were immersed in 10 mM methanol solutions of dansylhydrazine as a fluorescence probe for 3 h, taken out of the solutions, and washed in methanol. The fluorescence intensity near the wavelength of 460 nm was examined for the excitation at 340 nm.

*Physical stabilization of P(ST-co-MAPDS) particle monolayers* P(ST-*co*-MAPDS) particle monolayers on methylated glass plates treated with methyltriethoxysilane, prepared under the conditions of latex concentration of 0.025 wt% at 40 °C, were subjected to heat treatment at 110 °C above $T_g$ of the particles. The strength of the particle monolayer against peeling before and after heat treatment was examined using adhesive tape.

## Results and discussion

### Preparation of P(ST-*co*-MAPDS) and P(ST-*co*-NaSS) particles

The syntheses of cationic P(ST-*co*-MAPDS) and anionic P(ST-*co*-NaSS) particles by emulsifier-free emulsion copolymerization and their characteristics are listed in Table 1. Monodispersed cationic and anionic particles of almost the same size were obtained in high yields. The occupied surface areas for one charged group of cationic and anionic particles were calculated to be circa 70 and 100 Å$^2$, respectively, which means that the cationic particles have higher positive surface charge density, compared with that of the anionic particles.

In order to characterize the difference in hydrophobicity between anionic and cationic polystyrene particles, we carried out adsorption experiments of non-ionic surfactant, C12EO6, on the charged particles. The adsorption of non-ionic surfactants on cationic and anionic polymer particles has been demonstrated to be based on hydrophobic interaction [31]. Figure 2 shows adsorption isotherms of the surfactant on the cationic and anionic particles in water at 25 °C. The straight lines were obtained by plotting 1/adsorbed amount versus 1/equilibrium concentration of C12EO6, indicating that the adsorption isotherms are of the Langmuir type. By using this relationship, the adsorbed amounts of C12EO6 at saturation on cationic and anionic particles were estimated to be 5.49 and 3.50 μmol/m$^2$, respectively, and the equilibrium constants for adsorption were circa 90 and 73 mmol$^{-1}$, respectively. The results suggest more highly hydrophobic surfaces for the cationic particles.

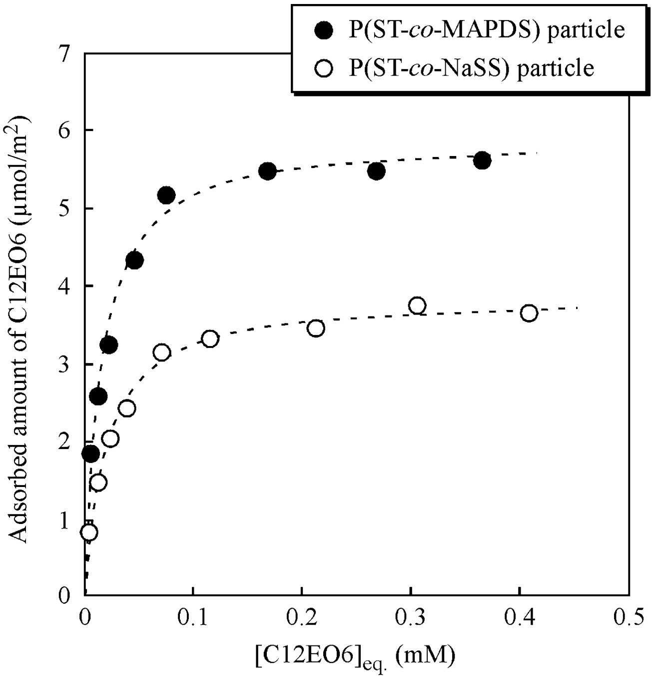

**Fig. 2** Adsorption isotherm of C12EO6 on polymer particles in water at 25 °C

### Surface characterization of alkylated glass plates

The average contact angles of water on alkylated glass plates with methyltriethoxysilane and *n*-octadecyltriethoxysilane were 80.3° and 95.4°, respectively, both higher

**Table 1** Preparation of polymer particles by emulsifier-free emulsion coplymerization, and their characteristics

| Latex code | Yield (%) | | Particle size[a] | | Surface charge (meq/g latex)[b] | Occupied area by a charged group (Å$^2$) |
|---|---|---|---|---|---|---|
| | Latex | Coagulum | $d_n$(nm) | $C_v$(%) | | |
| CS | 96.7 | 1.3 | 237 | 3.7 | +10 | 69.8 |
| AS | 99.0 | 0.2 | 225 | 1.7 | −4.2 | 96.8 |

[a] Determined by SEM: $d_n$ Number average diameter, $C_v$ coefficient of variation of particle size distribution
[b] Detrmined by colloid titration

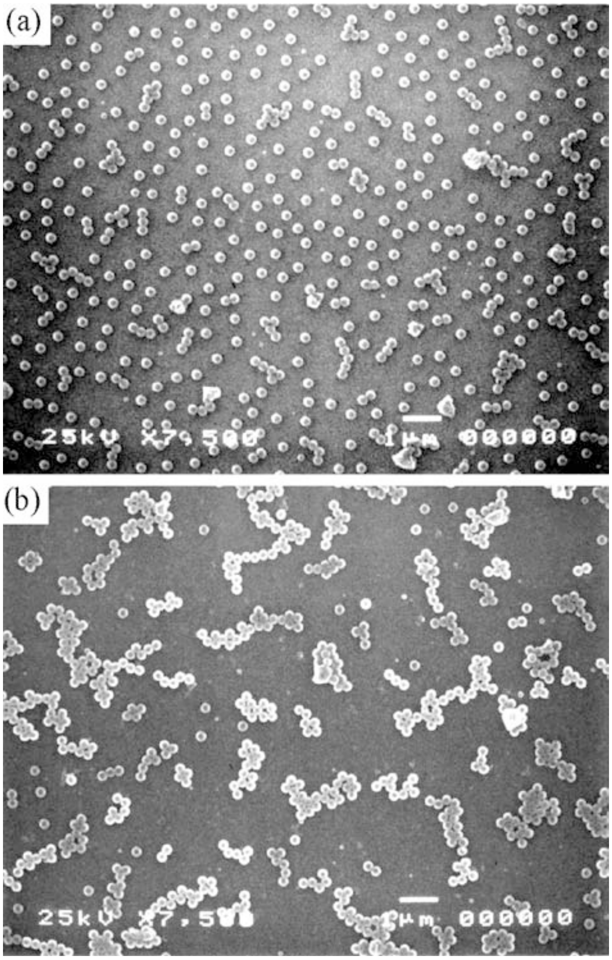

**Fig. 3a, b** Scanning electron microscope (SEM) photographs of P(ST-*co*-MAPDS) particles self-organized on alkylated glass plates at 25 °C: [latex], 0.025 wt%. **a** Methylated glass plate. **b** Octadecylated glass plate

than that (20.1°) for unmodified glass plates. Consequently, the hydrophobicity of the methylated glass plate would be lower than that of the octadecylated glass plate.

## Self-organization of polymer particles on alkylated glass plates

Alkylated glass plates were immersed into cationic and anionic latex dispersions of 0.025 wt% at 25 °C for 24 h. Figure 3 shows SEM photographs of cationic particles self-organized on alkylated glass plates. The cationic particle monolayers with relatively regular intervals are formed on less hydrophobic methylated glass plates modified with methyltriethoxysilane, which is termed "dispersed type" (Fig. 3a). Furthermore, the particles are also effectively self-organized on more hydrophobic octadecylated glass plates treated with *n*-octadecyltriethoxysilane as aggregates, which is termed "aggregated type" (Fig. 3b). In contrast, no anionic polymer particles are self-organized on either plate. This would be associated with a difference in the hydrophobicity between the cationic and anionic particles.

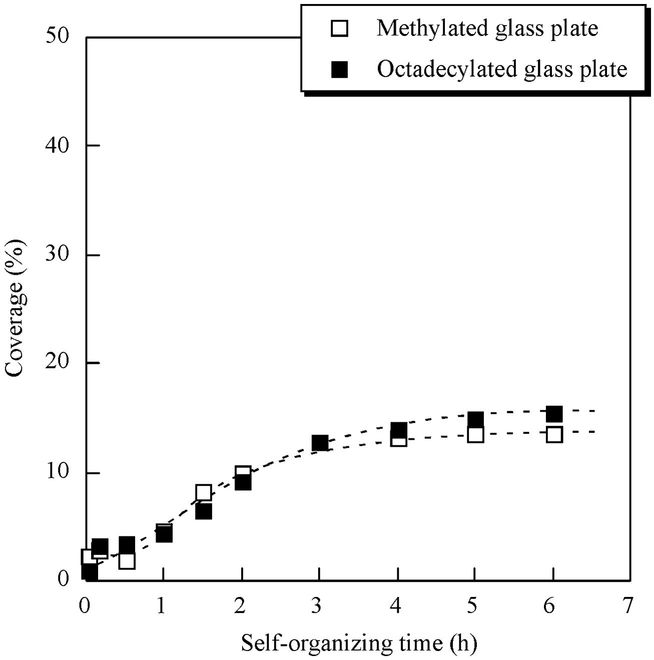

**Fig. 4** Self-organizing time dependence of coverage of alkylated glass plates with P(ST-*co*-MAPDS) particles: [latex], 0.025 wt%; temperature, 25 °C

The formation of cationic P(ST-*co*-MAPDS) particle monolayers was followed as a function of self-organizing time. Figure 4 shows the self-organizing time dependence of the coverage for methylated and octadecylated glass plates with cationic particles. For both alkylated glass plates, the coverage slowly increases with an increase in the self-organizing time, and then it becomes nearly constant beyond 3 h. The coverage for octadecylated glass plates is slightly higher than that for methylated glass plates at later stages.

The relationship between latex concentration and the coverage for methylated and octadecylated glass plates with cationic P(ST-*co*-MAPDS) particles is shown in Fig. 5, together with that for unmodified glass plates. The coverage for octadecylated glass plates is slightly higher at all latex concentrations, compared with that for methylated glass plates. As the latex concentration is increased, the coverage exhibits maxima for the alkylated glass plates, whereas it still increases at higher latex concentrations for unmodified glass plates. Such a difference in the coverage between the alkylated glass plates and unmodified glass plates, especially at higher latex concentrations, suggests that the interaction between the particle and the alkylated glass plate was different from

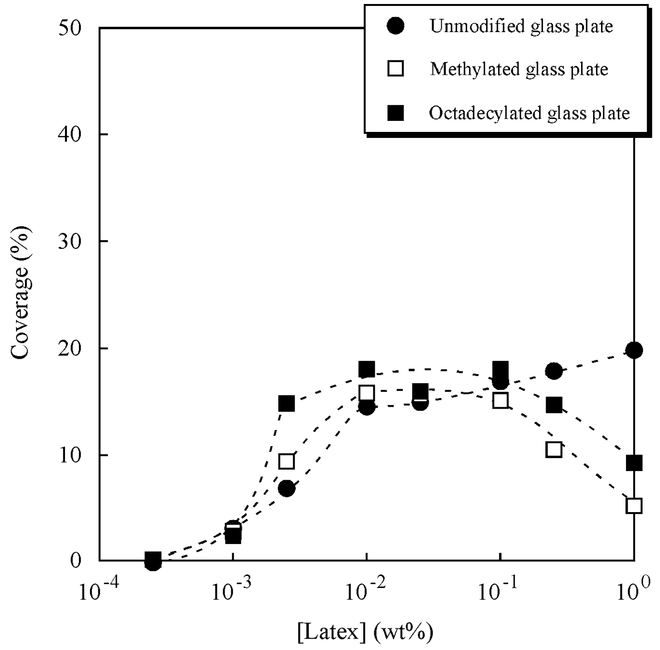

Fig. 5 Effect of latex concentration on coverage of unmodified and alkylated glass plates with P(ST-*co*-MAPDS) particles: temperature 25 °C

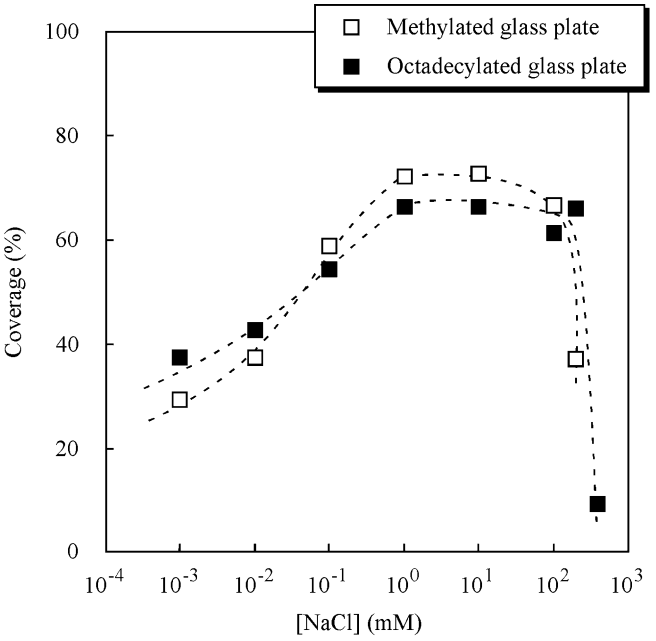

**Fig. 6** Effect of NaCl concentration on coverage of alkylated glass plates with P(ST-*co*-MAPDS) particles: [latex], 0.025 wt%; temperature 25 °C

the electrostatic interaction which is predominant for the self-organization of cationic particles on the unmodified glass plate.

Surface properties of the charged polymer particles are considered to be associated with the electric double layer, which is a function of ionic strength and temperature. Therefore, we investigated the effect of NaCl as an electrolyte on the particle monolayer formation. The effect of NaCl concentration on the coverage of methylated and octadecylated glass plates with cationic P(ST-*co*-MAPDS) particles is shown in Fig. 6. The coverage of alkylated glass plates with the particles greatly increases with increasing NaCl concentration. This result would be related to the electric double layer of the particles. The thickness of the electric double layer, the Debye length ($1/\kappa$), can be expressed as in Eq. 2:

$$\frac{1}{k} = \left( \frac{\varepsilon_r \varepsilon_0 RT}{4\pi F^2 \sum\limits_i C_i Z_i^2} \right)^{1/2} \qquad (2)$$

where $\varepsilon_r = \varepsilon/\varepsilon_0$ is the relative dielectric constant of solution ($\varepsilon$ the static permittivity of the solution and $\varepsilon_0$ the permittivity of a vacuum), $R$ the gas constant, $T$ the absolute temperature, $F$ the Faraday constant, $C_i$ the molar concentration of any ions, and $Z_i$ the valence of the ions. The Debye length decreases with increasing ionic strength, resulting in decreasing electrostatic repulsion between the particles and the increasing hydrophobicity of the particles. Thus, the coverage is strongly dependent on the hydrophobicity of the particles, which is related to the electrostatic repulsion. In latex dispersions containing more than 100 mM NaCl, however, the coverage suddenly decreases. The particles should be flocculated in aqueous solution of high ionic strength. Therefore, the degree of flocculation in the cationic latex dispersions was examined using the turbidity method [32]. The critical flocculation concentration of the cationic particles was 100 mM, which agrees with the concentration where the coverage begins to decrease. Therefore, the particles would be scarcely self-organized on the plates owing to the preferential flocculation of the particles in aqueous phase. The morphology of cationic particle monolayers on methylated and octadecylated glass plates in the presence of 10 mM NaCl is shown in Fig. 7. The cationic particles are self-organized in further aggregated form on both alkylated glass plates. SEM photographs of anionic P(ST-*co*-NaSS) particles self-organized on methylated glass plates in the presence of NaCl are shown in Fig. 8. Anionic particle monolayers are formed in the aggregated type on methylated glass plates only in the presence of 100 mM NaCl, though no particles are self-organized in the absence of NaCl. This may be due to the increase of hydrophobicity of the particles because of the compression of the electric double layer at the particle surfaces caused by the increase in ionic strength of the medium.

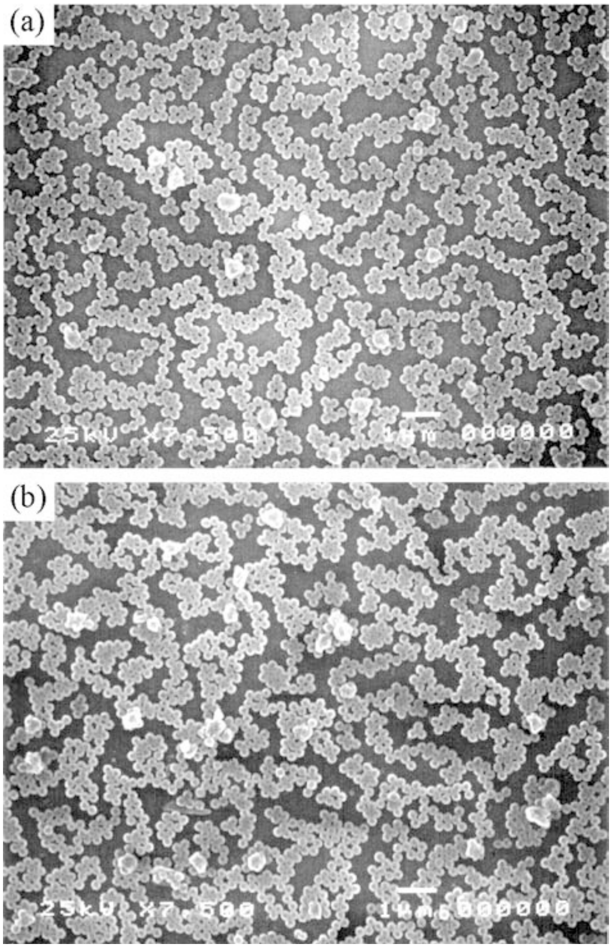

**Fig. 7a, b** SEM photographs of P(ST-*co*-MAPDS) particles self-organized on alkylated glass plates in the presence of NaCl: [latex], 0.025 wt%; temperature 25 °C; [NaCl], 10 mM. **a** Methylated glass plate. **b** Octadecylated glass plate

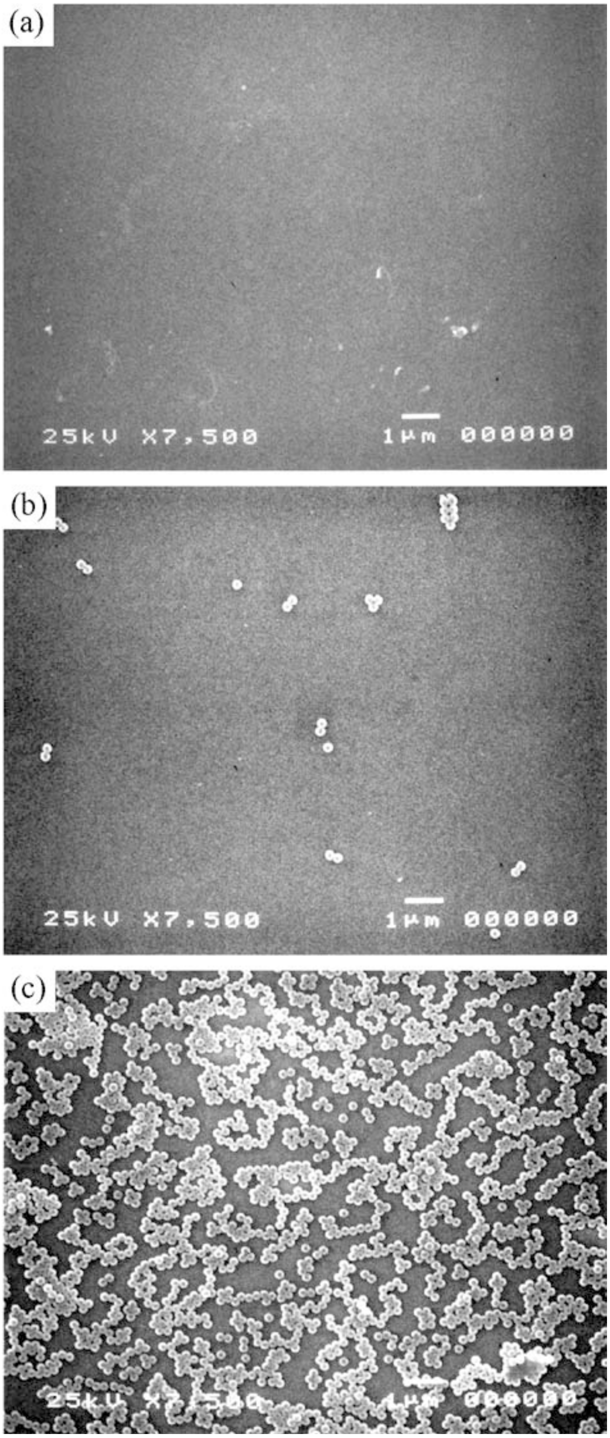

**Fig. 8a–c** SEM photographs of P(ST-*co*-NaSS) particles self-organized on methylated glass plates in the presence of NaCl: [latex], 0.025 wt%. **a** [NaCl] 0 mM, **b** [NaCl] 10 mM, **c** [NaCl] 100 mM

Equation 2 shows that $1/\kappa$ is also a function of temperature. Therefore, the influence of temperature on cationic P(ST-*co*-MAPDS) particle monolayer formation was examined. Figure 9 shows SEM photographs of cationic particles self-organized on methylated and octadecylated glass plates at 60 °C. For both methylated and octadecylated glass plates, the morphology of the dispersed type is observed at high temperature. Particularly, the morphology of particle monolayers on the more hydrophobic octadecylated glass plate changes from aggregated type to dispersed type with increasing temperature. The Debye length increases with increasing temperature of the aqueous solution through Eq. 2 and the permittivity of the aqueous medium decreases, resulting in increasing electrostatic repulsion between the particles. Thus, the particle monolayer of the dispersed type would be formed by the higher electrostatic repulsion between the particles and the lower hydrophobicity of the particles. Figure 10 shows the temperature dependence of the coverage of methylated and octadecylated glass plates with the cationic particles. The coverage of alkylated

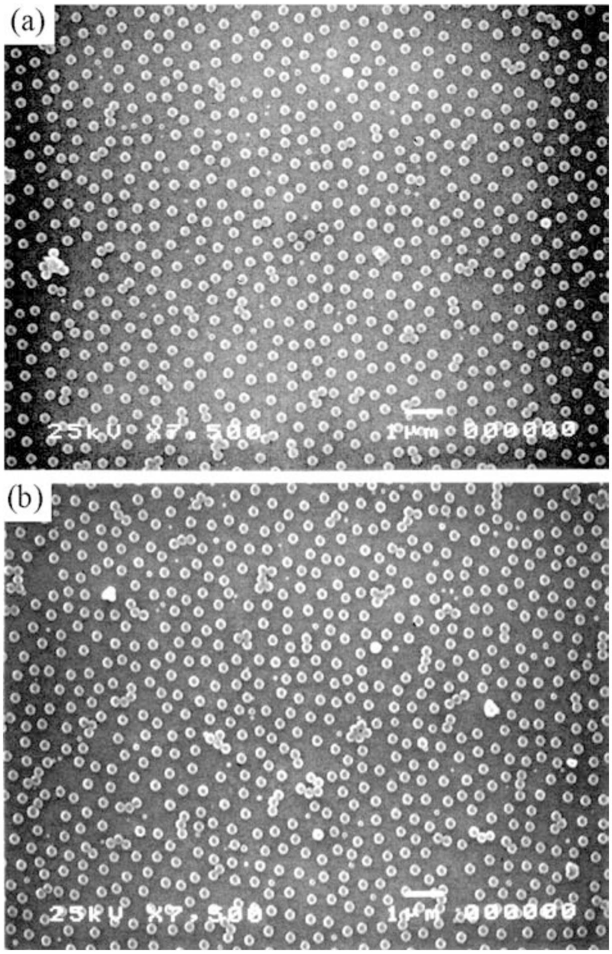

**Fig. 9a, b** SEM photographs of P(ST-*co*-MAPDS) particles self-organized on alkylated glass plates at 60 °C: [latex] 0.025 wt%. **a** Methylated glass plate. **b** Octadecylated glass plate

glass plates with the particles drastically increases with an increase in temperature more than 50 °C. Relatively ordered particle monolayers with a high particle density can be formed by adjusting temperature and ionic strength.

In general, the surface of polymer particles is hydrophobic, as shown by the adsorption of C12EO6. We previously reported that CTAB is also adsorbed on P(ST-*co*-MAPDS) particles [23]. The adsorption of CTAB would increase the surface charges of the particles. Hence, self-organization of the cationic particles on alkylated glass plates in the presence of CTAB was investigated. The effect of CTAB concentration on the coverage of octadecylated glass plates with the cationic particles in the presence of 50 mM NaCl is shown in Fig. 11. The particle monolayer of highest coverage (ca. 70%) is obtained at the higher ionic strength. However, the coverage begins to decrease at a CTAB concentration of about 1.0 μM and becomes close to zero at about 0.1 mM. This is ascribed to the decrease in the hydrophobicity of the particles as well as alkylated glass plates with increasing CTAB concentration, which is responsible for the decrease in the coverage of the plate with the particles.

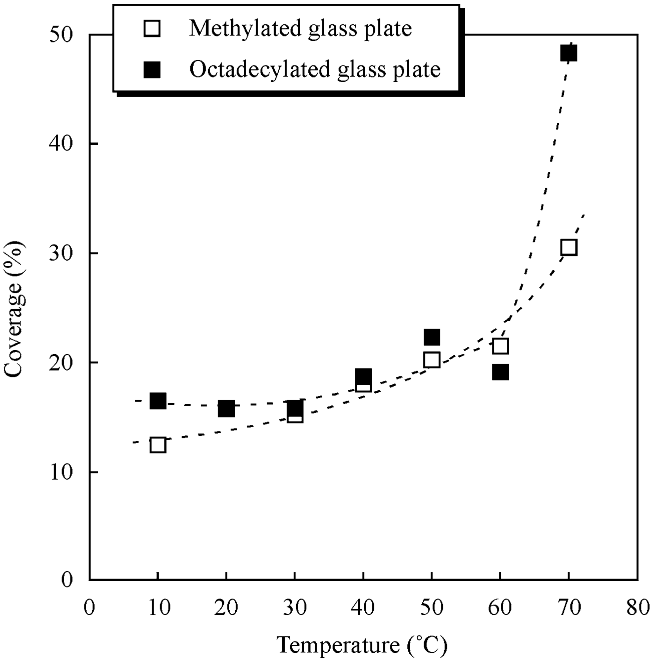

**Fig. 10** Effect of temperature on coverage of alkylated glass plates with P(ST-*co*-MAPDS) particles: [latex] 0.025 wt%

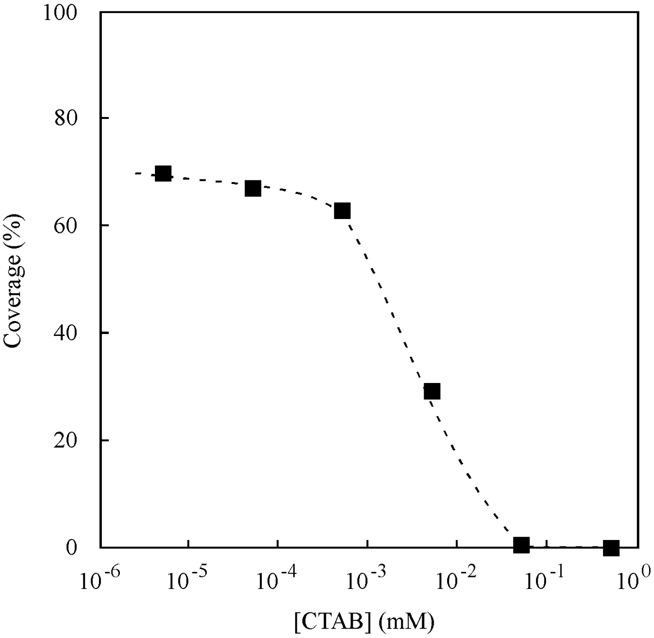

**Fig. 11** Effect of CTAB concentration on coverage of octadecylated glass plates with P(ST-*co*-MAPDS) particles: [latex] 0.025 wt%, temperature 25 °C, [NaCl] 50 mM

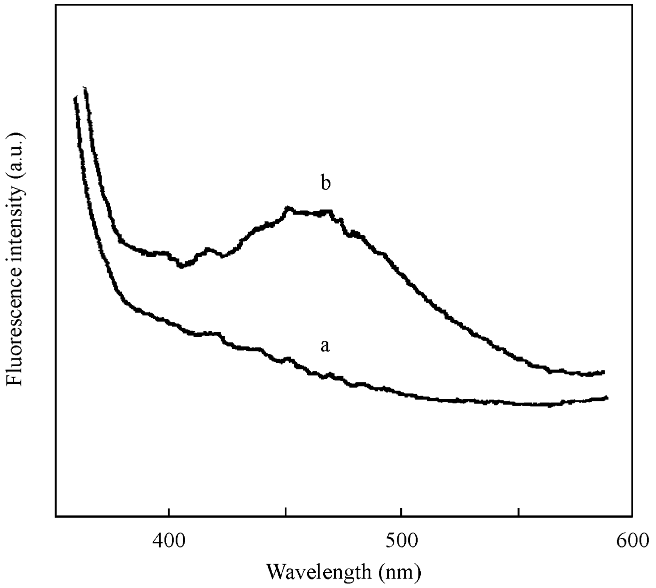

**Fig. 12** Fluorescence spectra of self-organized P(ST-*co*-MAPDS) particle monolayers on octadecylated glass plates *a* before reaction with dansylhydrazine, and *b* after reaction with dansylhydrazine. Self-organization, [latex] 0.025 wt%, temperature 25 °C, [NaCl] 10 mM

The results obtained here demonstrate that a main factor in the particle monolayer formation on alkylated glass plates is hydrophobic interaction. The morphology of dispersed and aggregated types would be chiefly concerned with the hydrophilic/hydrophobic balance of the particles and the plates, and the strength of hydrophobic interaction. In the case of less hydrophobic solid surfaces such as methylated glass plates, the formation of cationic particle monolayers of dispersed type occurs by electrostatic repulsion between the particles self-organized on the plate owing to low hydrophobic interaction between the particle and the plate. In contrast, the interaction between the particle and the more hydrophobic octadecylated glass surface is higher, resulting in the formation of the particle monolayer of aggregated type by cooperative action of the particle and the plate for coming particles.

## Fluorescence labelling of P(ST-*co*-MAPDS) particle monolayers

As cationic P(ST-*co*-MAPDS) particles used in this study have active ester groups on their surfaces, we carried out fluorescence labelling of the particle monolayer with dansylhydrazine as a fluorescent probe in order to confirm the reactivity of active ester groups. Figure 12 shows fluorescence spectra of cationic particle monolayers before and after the reaction with dansylhydrazine. When exposed to excitation at wavelength of 340 nm, no fluorescence peak at particle monolayers before the reaction with dansylhydrazine is observed, whereas the intensity at 460 nm increases after the reaction. This indicates that remaining active ester groups of particle monolayers on alkylated glass plates are still active towards primary amino compounds.

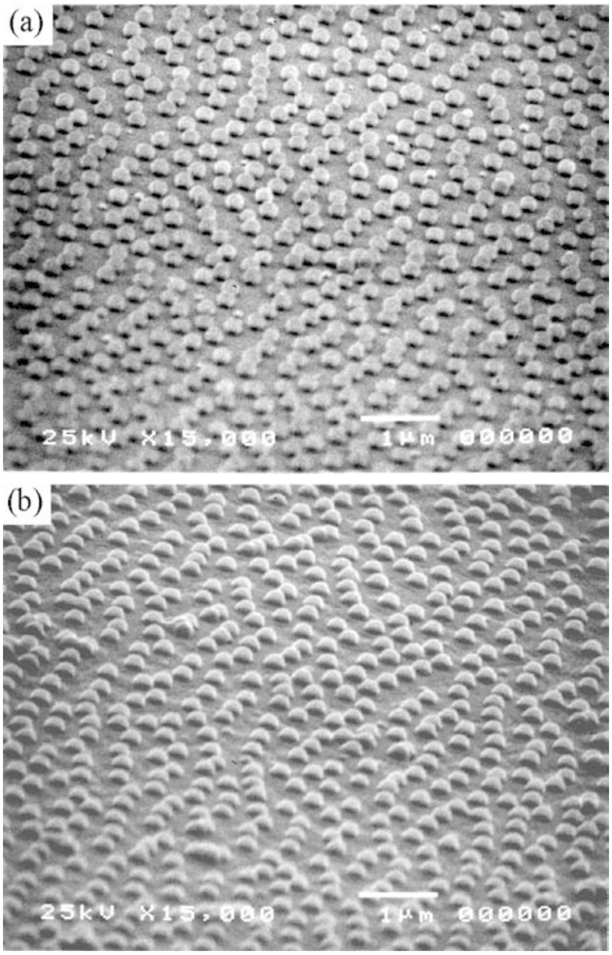

**Fig. 13a, b** SEM photographs of self-organized P(ST-*co*-MAPDS) particle monolayers on methylated glass plates **a** before heating and **b** after heating at 110 °C. Self-organization, [latex] 0.025 wt%, temperature 40 °C

## Physical stabilization of P(ST-*co*-MAPDS) particle monolayers

The cationic particles self-organized at hydrophobic surface were easily peeled off by adhesive tape, though the particle monolayers exhibit high stability towards ultrasonic irradiation. Therefore, cationic particle monolayers were heated at 110 °C above $T_g$ of the particles in order to improve physical stability. Figure 13 shows SEM photographs of cationic particle monolayers before and after heating at 110 °C.

Self-organized particles become hemispherical and strongly attached to the solid surfaces after heating.

Cationic P(ST-*co*-MAPDS) particle monolayers were effectively formed on the alkylated glass plates with silane coupling agents through hydrophobic interaction, in contrast to anionic P(ST-*co*-NaSS) particles having less hydrophobic surfaces. The morphology of the particle monolayers was controlled by adjusting the strength of the hydrophobic interaction between the particle and the plate. Remaining active ester groups on cationic particle monolayers were reactive enough to functionalize the solid surfaces. The cationic particle monolayers were physically stabilized by heat treatment above $T_g$ of the particles.

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