Effect of the Addition of a Cross-Linker and the Water pH on the Physical Properties of Films of pH-Responsive Polymer Particles at Air/Water Interfaces

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ABSTRACT: We investigated how the stiffness and physical properties of films of pH-responsive polystyrene nanoparticles with poly(2-(N,N-dimethylamino)ethylmethacrylate) hairs (PDMA−PS particles) at air/water interfaces could be varied by using the cross-linking of 1,2-bis(2-iodoethoxy)ethane (BIEE) and by varying the water pH. The physical properties of the film were investigated by using a Langmuir trough, atomic force microscope, and monolayer particle interaction apparatus. Films without large (μm-sized) holes were prepared by reacting BIEE with the PDMA−PS particles in the ethanol spreading solution for times ≤6 h and then by spreading this solution on a pH 5.8 water surface. Longer reaction times or a pH 9.0 water surface gave films with large holes. The holes were the result of the presence of aggregates of various sizes, which could not efficiently pack in the film. The aggregates were formed by interparticle adhesions because of the BIEE presence and physical attractive forces. The pH 5.8 water gave less aggregation than the pH 9.0 water because of the stronger interparticle electrostatic repulsions that resulted from the higher particle charge in pH 5.8 water than pH 9.0 water. The holes in the films could be decreased by reacting BIEE with the PDMA−PS particles on the pH 5.8 or 9.0 water surface, while the film was compressed to give a close packing density. The stiffness of the film of PDMA−PS particles increased, when BIEE was reacted with the PDMA−PS particles in the spreading solution for 1 h and was then spread on a pH 5.8 water surface. The film stiffness, however, did not increase, when BIEE was reacted with PDMA−PS particles on the pH 5.8 or 9.0 water surface for 1 h. This stiffness difference was explained by the fact that the former method gave a thicker film than the latter method.

1. INTRODUCTION

Polymer particles adsorbed at air/liquid interfaces are used in various nanotechnical and environmental applications. For example, polymer particles have been adsorbed at air/aqueous interfaces to stabilize foams without the use of surfactants. Ordered monolayers of latex particles formed at air/water interfaces help define the effectiveness of the applications of the films. A hard (stiff) film would be less damaged than a soft film, if another object came into contact with it. A soft film, however, has the advantage of being able to deform to the desired shape, when being transferred to another material. Many industrial and technological applications require the films to show some degree of stiffness and some degree of softness. For example, organic composite-coated sheets of steel can be made by coating the steel with an organic film. That film must be strong enough to resist mechanical damage when the coated steel sheets are used in moving parts, but the film must be soft enough to allow the film to press and deform well. It is therefore important to be able to control the stiffness and physical properties of a film of particles at the air/water interface.

If the particles in a film at the air/water interface are not connected to each other, then they are capable of moving laterally within the film. As a result, the particles can move within the film when another material contacts the film from above or below. The ability of the particles to move in the film would give a film that can be easily deformed, that is, a deformable or soft film. Control over the degree to which the

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particles can move in the film would give a film whose stiffness and physical properties could be adjusted to suit the desired applications, where a reduced particle movement would give a less deformable film. The stiffness of a film of polystyrene particles with poly(2-(N,N-dimethylamino)ethyl methacrylate) hairs (PDMA−PS particles) at an air/water interface has been increased by embedding the particles in a matrix of poly(2-hydroxyethyl methacrylate) (PHEMA).9 This was achieved by forming mixed Langmuir monolayers of PDMA−PS particles and PHEMA at the air/water interface. An additional way to decrease the movement of the particles in a film is to increase the interparticle attractions within the film. Reducing the charge of the particles would reduce the interparticle electrostatic repulsions and thereby increase the magnitude of the attractive forces acting between the particles. The charge of PDMA−PS particles at air/water interfaces can be adjusted by the pH of water, where the charge of the particles has been reported to decrease as the water pH is increased from pH 2.0 to 9.0.8 The physical properties of PDMA−PS particles at air/water interfaces of different pH values have been investigated.7 The particles tended to aggregate more at the air/water interface when the pH of water was 9.0 than when it was 5.8. In spite of this, the stiffness of the film of PDMA−PS particles on the pH 9.0 water subphase did not appear to be greater than the stiffness of the film formed by the particles on the pH 5.8 subphase. Thus, the increased magnitude of the attractive forces between the particles caused by the particle charge decrease was concluded to be insufficient to increase the stiffness of the film of particles. Thus, an additional method is needed to decrease the movement of the particles in the film at the air/water interface to be able to increase the stiffness of the film and to be able to better control the physical properties of the film.

A possible way to decrease the movement of the polymer particles in a film at an air/water interface is to react the PDMA−PS particles with a cross-linker. The shells of a partially quaternized 2-(N,N-dimethylamino)ethyl methacrylate-N-(morpholinol)ethyl methacrylate diblock copolymer micelle have been cross-linked by reacting these micelles with the bifunctional quaternizing agent of 1,2-bis-(2-iodoethoxy)ethane (BIEE) in aqueous solution at 60 °C.8 BIEE was seen to cross-link the unquaternized 2-(N,N-dimethylamino)ethyl methacrylate groups in the micelles. The reaction was carried out at high dilution, that is, a solution with a low micellar volume fraction was used to cross-link the shells of the micelles and to avoid intermicellar cross-linking.9 The structure of the 2-(N,N-dimethylamino)ethyl methacrylate groups is the same as that of the poly(2-(N,N-dimethylamino)ethylmethacrylate) hairs in the PDMA−PS particles. Thus, adhesions between PDMA−PS particles may be induced, if we use BIEE to cross-link the PDMA hairs on the PDMA−PS particles. As a result, a film with different physical properties, such as different particle packing and stiffness, may be achieved.

BIEE has predominately been used to internally cross-link a micelle in water or a methanol/water mixture at high dilutions.8−12 In this study, we aimed to determine if BIEE could be used to link PDMA−PS particles in a film at an air/water interface, and whether this reaction could (1) change the packing of the particles in a film of PDMA−PS particles at the air/water interface and (2) increase the stiffness of that film. To achieve interparticle cross-linking, the cross-linking reaction was performed in the particle spreading solution and at the air/water interface when the film was compressed to give a close particle packing. As a film of PDMA−PS particles can be formed at the air/water interface by spreading PDMA−PS particles in ethanol solution at the air/water interface,6 BIEE was added to the PDMA−PS particles in the ethanol spreading solution and reacted for a specified time in the ethanol solution or at the air/water interface after the particle monolayer was formed and compressed to give a close particle packing. The effect of the PDMA−PS particle charge on the properties of the film, after the BIEE reaction, was investigated by varying the pH of the water subphase. Water subphases with a pH of 5.8 or 9.0 were used, so as to give partially charged particles or almost negligibly charged particles, respectively. Particles of different charges were used to test if the physical properties of the film were affected by the additional presence of interparticle electrostatic repulsions. We aimed to determine if the addition of BIEE and the utilized water pH could change the physical properties of a film of PDMA−PS particles at the air/water interface. We also aimed to determine which conditions should be varied to give a film with the desired physical properties.

2. RESULTS AND DISCUSSION

2.1. Physical Properties of Films of PDMA−PS Particles at Air/Water Interfaces in the Absence of BIEE. The physical properties of films of PDMA−PS particles on a pH 5.8 and a pH 9.0 water surface were first determined by measuring the surface pressure−area per PDMA−PS particle isotherms of the particle films (see Figure 1). A lower pH, such

![Figure 1. Surface pressure (Π)−area/particle isotherms of films of PDMA−PS particles on a water surface, which were prepared without BIEE and with BIEE, when BIEE was reacted with the PDMA−PS particles in the ethanol spreading solution. A volume of 220 μL of approximately 1.8 w/w particles in ethanol solution was spread on the water surfaces in each case. The concentration of BIEE in the spreading solutions was 0.541 mM. (A) pH 5.8 water subphase; (B) pH 9.0 water subphase. Black line, (1): no BIEE; red line, (2): reaction time of BIEE = 0 h; green line, (3): reaction time of BIEE = 1 h; blue line, (4): reaction time of BIEE = 6 h; pink line, (5): reaction time of BIEE = 1 day; olive line, (6): reaction time of BIEE = 6 days. The gray dashed lines show the area/particle corresponding to a value of 8.55 × 104 nm², the calculated value of the area occupied by the PDMA−PS particles with a radius of 165 nm. The gray solid line is the extrapolation of the surface pressure−area/molecule isotherm to Π = 0 and approximates the size of a particle in the close packing region of the film of PDMA−PS particles on the pH 5.8 or 9.0 water subphases.](image-url)
as pH 2.5, could not be used as the particles became too hydrophilic and sank into water after spreading. Figure 1 shows that the surface pressure increased slowly from 0 to 2 or 5 mN/m for the pH 5.8 and 9.0 water surfaces, respectively, as the area/particle was decreased from 1.90 × 10^{-1} \mu m^2 to approximately 8.86 × 10^{-2} \mu m^2. This regime is assigned to a close packing region. Decreasing the area of the film further by compressing the film caused the surface pressure to steeply increase over a small decrease in the area. This steep pressure increase resembles the surface pressure–area relationship of a close packing monolayer. Further compression of the film to areas smaller than those measured at a surface pressure of approximately 20 mN/m resulted in a plateau being observed in the surface pressure–area isotherm. This plateau is explained by the wrinkling in the monolayer. The wrinkling is thought to be formed by the ejection of the particles from the air/water interface to the air phase because of the lack of space for the particles at the air/water interface and the hydrophobicity of the particles.

The PDMA–PS particles we used in this experiment had a radius of 165 ± 85 nm. The area of these particles can be estimated from πR^2 to be 8.55 × 10^{-2} ± 2.27 × 10^{-2} \mu m^2. The gray dashed lines in Figure 1 show the area/particle corresponding to a value of 8.55 × 10^{-2} \mu m^2. The size of the PDMA–PS particles in the close packing region of the particles on the pH 5.8 and 9.0 water subphases were approximated by extrapolating the surface pressure–area/particle isotherm in the close packing region to Π = 0; see the gray solid line in Figure 1. Values of 8.04 × 10^{-2} and 8.75 × 10^{-2} \mu m^2 were obtained for the pH 5.8 and 9.0 subphases, respectively. These values are less than 5% different from the value estimated using πR^2, indicating that the isotherms were reasonable.

The surface pressure–area isotherms suggested that the PDMA–PS particles formed a closely packed film for 5 < Π < 20 mN/m and a film with wrinkling for Π > 20 mN/m, when the water subphase was pH 5.8 or 9.0. To further verify that the particles were closely packed at Π = 10 mN/m and that wrinkling was present in the films at Π > 20 mN/m, the PDMA–PS particle films prepared on a pH 5.8 water surface were transferred to mica substrates, when the surface pressure was Π = 10, 25, and 30 mN/m, that is, surface pressures that corresponded to the close packing and wrinkling regimes. Figure 2A–C shows the 5 × 5 \mu m^2 atomic force microscopy (AFM) height images of the transferred PDMA–PS particle films at Π = 10, 25, and 30 mN/m, respectively. A closely packed film is seen at Π = 10 mN/m. The film, however, was not uniformly flat. This result is explained by the non-negligible size distribution of the synthesized PDMA–PS particles. The higher surface pressures of 25 and 35 mN/m showed lines of particles above a flatter film of particles. These lines are designated as wrinkles. The number of wrinkles increased as the surface pressure increased.

The effect of the water pH on the aggregation of the PDMA–PS particles at the air/water interface can be seen by imaging films of PDMA–PS particles that were compressed to Π = 10 mN/m and then transferred to mica substrates. Figures 3A and 4A show the 5 × 5 \mu m^2 height images of the transferred films of PDMA–PS particles, which were prepared on the pH 5.8 and 9.0 water surfaces, respectively. The average sizes of the particles in the films on the pH 5.8 and 9.0 water surfaces were determined by measuring the size of each individual particle that was distinguishable in the images shown in Figures 3A and 4A by using the PhotoRuler Software (Version 1.13.0, The

Figure 2. AFM height images (5 × 5 \mu m^2) of a film of PDMA–PS particles prepared on a pH 5.8 water surface, which was transferred to a mica substrate in the absence of BIEE. (A–C) Images of the films that were compressed to Π = 10, 25, and 35 mN/m, respectively.

Genus Inocybe in Hyogo). The histograms of the sizes of the PDMA–PS particles on the pH 5.8 and 9 water surfaces are shown in Figure 5. The average size of the particles was obtained by performing a Gaussian fit to the histogram data (see Table 1). The average sizes of the PDMA–PS particles on the pH 5.8 and 9.0 water surfaces were 324 ± 112 and 308 ± 101 nm^2, respectively. These sizes are within the range of the sizes of the synthesized particles, that is, 330 ± 170 nm. The area per particle obtained from the AFM images (A_{SP, AFM}) was also calculated from the number of particles counted in the AFM image (N_{AFM}) and the size of the AFM image (2.50 × 10^3 \mu m^2) (see Table 1). The values of A_{SP, AFM} calculated from the AFM images and the size of the PDMA–PS particles in the close packing region of the particles on the pH 5.8 or 9.0 water subphases determined from the surface pressure–area/particle were approximately 24% different. This difference can be explained by the presence of tip convolution and the possibility that some of the particles were inside the film and not on the surface, causing the number of particles within the film to be underestimated.

Comparison of the particles seen in Figures 3A and 4A and the average size of the particles shown in Table 1 also show that the PDMA–PS particles appeared to aggregate more on the pH 9.0 water surface than on the pH 5.8 water surface. PDMA–PS particles are reported to be more charged in pH 5.8 water than in pH 9.0 water. A higher particle charge would cause the electrostatic repulsions between the PDMA–PS particles on the water surface to be stronger. The forces acting between the particles at the interface include the electrostatic repulsions and the attractive van der Waals, hydrophobic, and capillary forces. Thus, the interparticle forces would be more attractive for the pH 9.0 water surface than the pH 5.8 water surface, as the interparticle electrostatic repulsions would be weaker. As a
result, the PDMA−PS particles would aggregate more on the pH 9.0 water surface than on the pH 5.8 water surface.

2.2. Effect of the Presence of BIEE on the Physical Properties of a Film of PDMA−PS Particles at Air/Water Interfaces: Reaction of BIEE with the PDMA−PS Particles in the Ethanol Spreading Solution. The effects of addition of BIEE to the PDMA−PS particle film and the reaction time of BIEE with the particles on the physical properties of the particle film at the air/water interfaces were determined by measuring the surface pressure–area isotherms of the particle films and by transferring the particle films to mica substrates and observing them using AFM. The effect of water pH on the properties of the films was determined by using pH 5.8 and 9.0 water.

In this section, the films of PDMA−PS particles at the air/aqueous interfaces in the presence of BIEE were prepared by adding BIEE to the PDMA−PS particle in the ethanol spreading solution and waiting for a specified time. That particle solution was then spread at the air/aqueous interface, and 10 min was allowed for the solvent to evaporate. Next, the film was compressed, and the surface pressure–area isotherm was measured. The same volume of particles in ethanol solution was spread at the air/aqueous interface for each system,
continued to shift to lower area/particle values as the BIEE presence of BIEE appeared at lower areas than the isotherm BIEE in the Ethanol Spreading Solution isotherms of the PDMA respectively. In the case of the pH 5.8 water surface, the isotherms of the pH 5.8 water subphase and a pH 9.0 water subphase, respectively. Figures 3 and 4 were measured using the PhotoRuler Software. The histograms showing the sizes of the PDMA–PS particles on the pH 5.8 water and pH 9 water surfaces are shown in Figure 5. The average size of the particles was obtained by performing a Gaussian fit to the histogram data (see Table 1). The average size of the particles increased for both the pH 5.8 and 9.0 subphases after BIEE was added to the PDMA–PS particle spreading solution and also as the BIEE reaction time was increased. These larger particles are thought to be aggregates of PDMA–PS particles caused by the presence of BIEE. The percentage of aggregates in the film (Nagg) was approximated from the decrease in the Np_AFM values using

\[ N_{agg} = (N_{p_AFM}[no \text{ BIEE}]) - (N_{p_AFM}[\text{BIEE}]) \times \frac{100}{N_{p_AFM}[\text{BIEE}]} \]  

Here, Np_AFM[no BIEE] and Np_AFM[BIEE] are the Np_AFM values calculated for the film without BIEE and the value calculated for the film containing BIEE, respectively. The values of Nagg are given in Table 1 and can be seen to increase with the BIEE reaction time and the water pH. The \( \Delta_{NP_{AFM}} \) values were also calculated (see Table 1) and were seen to increase with an increase in the BIEE reaction time. The pH 9.0 water also gave larger \( \Delta_{NP_{AFM}} \) values than the pH 5.8 water. A decrease in the number of particles on the surface would cause the value of \( \Delta_{NP_{AFM}} \) to increase. The presence of aggregates would cause the number of particles inside the film to increase, while the

Figure 5. Histogram showing the size of each individual particle that was distinguishable in the 5 × 5 μm² AFM height images of Figures 3 and 4. A and B show the results for the pH 5.8 and 9.0 water surfaces, respectively. The PDMA–PS particles in this film were reacted with BIEE in the ethanol spreading solution. The average size of the particles was obtained by performing a Gaussian fit to the particle size histogram; see Table 1 for the average particle sizes.

Table 1. Average Size of Each Individual Particle that was Distinguishable in the Film Formed at the Air/Water Interface after the PDMA–PS Particles were Reacted with BIEE in the Ethanol Spreading Solutiona

| pH | BIEE presence | reaction time | \( N_{p_AFM} \) (PS particle) | \( (N_{p_{AFM}}) \) (PS particle/μm²) | \( A_{NP_{AFM}} \) (PS particle/μm²) |
|----|---------------|--------------|-------------------------------|-------------------------------------|-----------------------------------|
| 5.8 | no            | 0 h          | 221                           | 324 ± 112                           | 1.13 × 10⁸                         |
|     | yes           | 0 h          | 209                           | 339 ± 115                           | 1.20 × 10⁸                         |
|     |               | 1 h          | 160                           | 27.6 ± 116                          | 1.56 × 10⁸                         |
|     |               | 6 h          | 160                           | 27.6 ± 137                          | 1.57 × 10⁸                         |
|     |               | 6 days       | 137                           | 38.0 ± 125                          | 1.82 × 10⁸                         |
| 9.0 | no            | 0 h          | 241                           | 308 ± 101                           | 1.04 × 10⁸                         |
|     | yes           | 0 h          | 196                           | 18.7 ± 113                          | 1.28 × 10⁸                         |
|     |               | 1 h          | 178                           | 26.1 ± 117                          | 1.40 × 10⁸                         |
|     |               | 6 h          | 161                           | 33.2 ± 108                          | 1.55 × 10⁸                         |
|     |               | 6 days       | 100                           | 58.5 ± 174                          | 2.50 × 10⁸                         |

aThe average particle size was obtained by performing a Gaussian fit to the particle size histogram in Figure 5, which was calculated using the 5 × 5 μm² AFM height images shown in Figures 3 and 4. The number of particles counted in the AFM image (\( N_{p_{AFM}} \)), the percentage of aggregates in the film (\( N_{agg} \)), the area per particle (\( A_{NP_{AFM}} \)) calculated from \( N_{p_{AFM}} \) and the size of the AFM image (2.50 × 10⁵ nm²) are given.

allowing the effect of the BIEE reaction time to be determined from the change in the isotherms.

Figure 1A,B shows the surface pressure–area/particle isotherms of the films of PDMA–PS particles that contained BIEE, when the water subphase was pH 5.8 and 9.0, respectively. In the case of the pH 5.8 water surface, the isotherms of the PDMA–PS particle film measured in the presence of BIEE appeared at lower areas than the isotherm measured for the PDMS–PS film in the absence of BIEE, when the same nonzero surface pressures were compared. The isotherms of the PDMS–PS particle film containing BIEE continued to shift to lower area/particle values as the BIEE reaction time increased from 0 to 6 h. The isotherm with the minimum area/particle value was measured for the PDMA–PS particle film containing BIEE, whose BIEE reaction time was 6 h. An increase in the BIEE reaction time from 6 h to 1 day and 6 days caused the isotherm to shift to higher area/particle values. These area/particle values were, however, still lower than that of the PDMA–PS particle film that did not contain BIEE. In the case of the pH 9.0 water surface, the isotherms measured in the presence of BIEE appeared at higher area/particle values than the isotherm measured in the absence of BIEE, when the area/particle value corresponding to the same nonzero surface pressure was compared. Increasing the reaction time of BIEE caused the isotherm to shift to larger area/particle values.

To determine whether the apparent particle sizes were changing because of the addition of BIEE to the PDMA–PS particle spreading solution, the PDMA–PS particle films that were prepared at the air/water interface were compressed to a surface pressure of 10 mN/m, transferred to a mica substrate, and then imaged using AFM. Films of the PDMA–PS particles without and with BIEE were imaged. The effects of the subphase pH and BIEE reaction time were also determined by transferring films, which were prepared on pH 5.8 and 9.0 water subphases and reacted with BIEE for different times. Figures 3 and 4 show the 5 × 5 μm² and 15 × 15 μm² height images of the transferred particle films, which were prepared on a pH 5.8 water subphase and a pH 9.0 water subphase, respectively. Figures 3A,F and 4A,F show the films of PDMA–PS particles in the absence of BIEE. Figures 3B,E, 3G–J, 4B,E, and 4G–J show the films of the PDMA–PS particles in the presence of BIEE, when the BIEE reaction was allowed to proceed for 0 h, 1 h, 6 h, and 6 days.

Information concerning the change in the particle size and aggregation within the film upon the addition of BIEE to the film and its reaction time was obtained by comparing the 5 × 5 μm² images. The sizes of each individual particle that was distinguishable in the 5 × 5 μm² images shown in Figures 3 and 4 were measured using the PhotoRuler Software. The histograms showing the sizes of the PDMA–PS particles on the pH 5.8 water and pH 9 water surfaces are shown in Figure 5. The average size of the particles was obtained by performing a Gaussian fit to the histogram data (see Table 1). The average size of the particles increased for both the pH 5.8 and 9.0 subphases after BIEE was added to the PDMA–PS particle spreading solution and also as the BIEE reaction time was increased. These larger particles are thought to be aggregates of PDMA–PS particles caused by the presence of BIEE. The percentage of aggregates in the film (\( N_{agg} \)) was approximated from the decrease in the \( N_{p_{AFM}} \) values using

\[ N_{agg} = (N_{p_{AFM}}[no \text{ BIEE}]) - (N_{p_{AFM}}[\text{BIEE}]) \times \frac{100}{N_{p_{AFM}}[\text{BIEE}]} \]  

Here, \( N_{p_{AFM}}[no \text{ BIEE}] \) and \( N_{p_{AFM}}[\text{BIEE}] \) are the \( N_{p_{AFM}} \) values calculated for the film without BIEE and the value calculated for the film containing BIEE, respectively. The values of \( N_{agg} \) are given in Table 1 and can be seen to increase with the BIEE reaction time and the water pH. The \( \Delta_{NP_{AFM}} \) values were also calculated (see Table 1) and were seen to increase with an increase in the BIEE reaction time. The pH 9.0 water also gave larger \( \Delta_{NP_{AFM}} \) values than the pH 5.8 water. A decrease in the number of particles on the surface would cause the value of \( \Delta_{NP_{AFM}} \) to increase. The presence of aggregates would cause the number of particles inside the film to increase, while the
number of particles on the surface increased. The surface pressure—area per particle isotherms showed that in the case of pH 5.8 water, the minimum surface area/particle at which the film broke down (collapsed) decreased as the cross-linking reaction time was increased from 0 to 6 h. This decrease is explained by the aggregation of the particles in the film, causing the thickness of the film to increase with the increase in the reaction time. Comparison of the size of the aggregates seen in Figure 3B–E with that in Figure 4B–E and also the average particle sizes shown in Table 1 allows us to see that the aggregates were larger for the pH 9.0 water subphase than the pH 5.8 water subphase.

Information concerning the change in the particle packing within the film upon the addition of BIEE to the film and its reaction time was obtained by imaging the above transferred films using an image size of 15 × 15 μm². The larger size of 15 × 15 μm² allowed the particle packing to be seen more clearly. In the case of the pH 5.8 water subphase, the particles can be seen to have packed closely for BIEE reaction times ≤6 h. Longer reaction times, such as 6 days, resulted in films with worse packing and holes (see the white circle in Figure 3J). In the case of the pH 9.0 water subphase, the particle packing became worse as the BIEE reaction time was increased. The particles tended to aggregate more on the pH 9 water surface than on the pH 5.8 water surface. Large detectable holes (see white circles in Figure 4I,J) were observed in the case of the pH 9.0 water subphase in the PDMA–PS film shown in Figure 4 for reaction times of 6 h and 6 days, the thickness of each hole seen in an image was approximately the same. Additionally, no particles could be seen in the hole when the hole was magnified and the height scale was changed. A variation in the depth of the holes would have suggested that the holes were a deep wrinkle in the film. Thus, the holes in the film are concluded to be a channel that went through the film.

The particle size appeared to increase with the BIEE reaction time for both the pH 5.8 and 9 subphases. However, in the absence of BIEE, the size of the particles in the film at the air/water interface did not increase, if the PDMA–PS particles were immersed in ethanol for periods of time longer than 6 days before they were spread at the air/water interface. Thus, the absence of BIEE is thought to cause the particles to aggregate, appearing to make the particle size increase with the BIEE reaction time for both the pH 5.8 and 9 subphases. Previous studies that aimed to internally cross-link the shells of micelles reported that the cross-linking reactions needed to be performed under highly diluted conditions, so as to avoid intermicellar cross-linking. The fact that we observed interparticle aggregation under our experimental conditions suggests that the weight fraction of the PDMA–PS particles in ethanol solution was concentrated enough to allow the particles to interact and aggregate.

The size of the aggregates formed by the PDMA–PS particles in the presence of BIEE was larger for the pH 9.0 water subphase than for the pH 5.8 water subphase. Although the reaction of BIEE would have caused the PDMA hairs on the particles to have become more charged because of the quaternization, the PDMA–PS particles at pH 9.0 would still have been less charged than those at pH 5.8. A higher particle charge would have resulted in a stronger interparticle electrostatic repulsion. The interparticle electrostatic repulsion for the pH 9.0 water subphase would therefore have been less than that of the pH 5.8 water subphase, resulting in the effect of the interparticle physical attractions (van der Waals, hydrophobic, and capillary forces) being more dominant for the pH 9.0 water subphase than for the pH 5.8 water subphase. As a result, the PDMA–PS particles would have aggregated more on the pH 9.0 water subphase than on the pH 5.8 water subphase, causing the size of the aggregates to have appeared larger for the pH 9.0 water subphase than for the pH 5.8 water subphase. The PDMA–PS particles therefore appear to have aggregated on water surfaces because of the BIEE presence and the interparticle attractions resulting from the van der Waals, capillary, and hydrophobic forces. The magnitude of the interparticle attraction can be controlled by the charge of the particles and therefore the pH of water.

The images of the PDMA–PS particle films in the presence of BIEE shown in Figures 3 and 4 demonstrate that the sizes of the aggregates were not homogeneous. The variation in the sizes of the aggregates is thought to be related to the difference in the magnitude of the attractive forces that act between the particles and aggregates. As the size of the particles was only 333 nm, the magnitude of the capillary forces between single particles was small and is therefore thought not to have contributed to the interactions acting between the particles at the air/water interface. However, the magnitude of the capillary force would have increased as the size of the aggregates increased, causing the magnitude of the capillary forces acting between the aggregates to increase. An increase in the size of the aggregates would also have increased the magnitude of the van der Waals and hydrophobic attractions that could act between the aggregates. The increased attractive forces would therefore have promoted the aggregates to aggregate further, causing the size of the aggregates to increase.

The effects of the BIEE reaction times and the subphase pH on the particle packing abilities are thought to be related to the size of the aggregates and the attractions acting between these aggregates. The varied sizes of the aggregates and particles would cause the aggregates and particles to pack inefficiently at the air/water interface, resulting in holes being formed in the film of PDMA–PS particles at the air/water interface. The presence of holes in the film would cause the isotherm of the film of PDMA–PS particles to appear to be more expanded, that is, the isotherm would shift to higher area/particle values. The particles tended to form larger aggregates in the PDMA–PS particle film at shorter BIEE reaction times on the pH 9 water surface than on the pH 5.8 surface. Larger and more holes were observed in the images of the PDMA–PS particle films shown in Figure 4 for reaction times of 6 h and 6 days, when the films were prepared on the pH 9.0 water surface than on the pH 5.8 water surface. The difference in the hole formation in the films is explained by the fact that the electrostatic repulsions that act between the particles and aggregates on the pH 5.8 water surface were stronger than those present when pH 9.0 water was used. The repulsions would reduce the aggregation of the particles and/or aggregates, resulting in a smaller size distribution of the particles and aggregates and a better ability of the particles to pack at the air/water interface. However, the particles would aggregate to form holes in the film, when the sizes of the aggregates became large enough that the magnitude of the attractive forces (capillary, van der Waals, and hydrophobic) acting between the particles and aggregates in the film was larger than the magnitude of these electrostatic repulsions. These holes were seen (Figure 3) to have appeared in the films formed on the pH 5.8 water surface, when the BIEE reaction time was 6 days. The fact that large holes appeared in the films formed on the pH 9.0 water surface at shorter BIEE reaction...
times than in the case of the films formed on the pH 5.8 water surface indicates that the interparticle electrostatic repulsive force helped form a film of PDMA–PS particles without large holes.

The effect of the presence of BIEE in the films of PDMA–PS particles and the subphase pH on the strength of the films was next determined by measuring the forces between a hydrophilpos probe in water and the particle films at the air/water interface. As the hydrophilpos probe was positively charged and the PDMA–PS particles were positively charged in pH 5.8 water and almost negligibly charged in pH 9.0 water, there would have been an electrostatic repulsion between the probe and the film. This repulsion would hinder the transfer of the particle film to the probe during a force–distance curve measurement, allowing the stiffness of the film to be measured. Force–distance curves showing a steeper force–distance slope in the region where the probe contacted the film indicates a stronger (stiffer) film. Figure 6 shows example force–distance curves measured for the PDMA–PS films in the absence of BIEE and PDMA–PS films in the presence of BIEE, which were prepared by allowing the particles to react with BIEE for 1 h in the spreading solution before the particles were spread at the air/water interface and later compressed to a surface pressure of 10 mN/m. The force–distance curves were obtained using a pH 9.0 water subphase. Black squares: film of PDMA–PS particles without BIEE on the water surface, which was compressed to $\Pi = 10$ mN/m; red circles: film of PDMA–PS particles with BIEE prepared by mixing BIEE with the particles in the spreading solution for 1 h, spreading the particles on the water surface, and compressing the film to $\Pi = 10$ mN/m.

![Figure 6](image)

**Figure 6.** Approach force–distance curves measured between a film of PDMA–PS particles compressed to $\Pi = 10$ mN/m on a water surface and a hydrophilpos probe in the water. (A) pH 5.8 water subphase; (B) pH 9.0 water subphase. Black squares: film of PDMA–PS particles without BIEE on the water surface, which was compressed to $\Pi = 10$ mN/m; red circles: film of PDMA–PS particles with BIEE prepared by mixing BIEE with the particles in the spreading solution for 1 h, spreading the particles on the water surface, and compressing the film to $\Pi = 10$ mN/m.

The force–distance curves show that the reaction of BIEE with the PDMA–PS particles for 1 h increased the strength of the film at the air/water interface, when the water was pH 5.8. The BIEE reaction, however, did not increase the film strength for the pH 9.0 water subphase. The AFM images showed that the addition of BIEE to the film of PDMA–PS particles caused the size of the particle aggregates to increase for both the pH 5.8 and 9.0 water subphases. The images also showed, however, that the reaction of BIEE tended to cause the particles to pack worse in the film and for more holes to form in the film when the water was adjusted to pH 9.0 than when it was pH 5.8. The tendency of the particles in the film to pack badly and for holes to form in the film is therefore thought to contribute to the weakness of the film.

The packing density of particles at the interface and the presence of holes are thought to affect the stiffness of the film of particles at the air/water interface. This reasoning agrees with the facts known about the relation between the volume density of particles in a lattice of spheres and the flow of spheres in that lattice. Spheres have been reported to be incapable of flowing in a lattice of spheres with a maximum volume density of closely packed particles of 0.74 or 0.64, that is, close packing or randomly close packing. These spheres, however, can flow in a lattice of spheres when the maximum volume density of closely packed particles is reduced to 0.55, that is, random loose packing. A bad particle packing ability and/or the presence of holes in a film of particles at the air/water interface would reduce the maximum volume density of closely packed particles, allowing the particles to laterally flow in the film. The ability of particles to flow in such a film would cause the film of particles to appear more deformable. The particles and aggregates in a film with bad particle packing ability and/or holes would be capable of moving laterally more, when the hydrophilpos probe in the water contacted the monolayer from beneath during a force–distance measurement.

![Figure 7](image)

**Figure 7.** Elastic modulus of mica and estimated elastic modulus of a film of particles at the air/water interface. Row number: 1, mica; 2 and 5, film of PDMA–PS particles spread on the water surface and compressed to $\Pi = 10$ mN/m; 3 and 6, film prepared by reacting BIEE with the PDMA–PS particles in the ethanol spreading solution for 1 h and then spread on the water surface and compressed to $\Pi = 10$ mN/m; 4 and 7, film prepared by reacting BIEE with the PDMA–PS particles on the water surface for 1 h, while the film was continually compressed to $\Pi = 10$ mN/m. The data for the pH 5.8 water surfaces are given in row numbers 2, 3, and 4. The data for the pH 9.0 water surfaces are given in row numbers 5, 6, and 7.
The film of particles whose particles could move in a lateral direction more would result in a force curve with a lower slope than a film of particles whose particles could move less. As a result, the former film would be more deformable than the latter film.

2.3. Effect of the Method Used to React BIEE with the PDMA−PS Particle Films on the Physical Properties of the PDMA−PS Films at Air/Water Interfaces: Reaction of BIEE with the PDMA−PS Particles at the Air/Water Interface. The stiffness of a Langmuir film of particles at an air/aqueous interface may be increased, if we improve the particle packing ability in the film and therefore reduce the number of holes in the film. This may be achieved, if we modify the procedure used to react the PDMA−PS particles with BIEE and to prepare the film of PDMA−PS particles on the water surface. In this section, we tested if the strength of the films at the air/water interface could be improved by allowing the BIEE reaction to occur in the film of particles at the air/water interface, while the particles were continually compressed to give a closely packed film. In this way, the aggregates and particles would be forced to adhere to each other, causing the particle packing in the film to improve and thereby help prevent the formation of holes in the film. In this section, the films of PDMA−PS particles containing BIEE were therefore prepared by adding BIEE to the PDMA−PS particles in ethanol spreading solution, after which the solution was mixed for 30 s. That solution was then spread on a pH 5.8 water surface or on a pH 9.0 water surface; 10 min was allowed for ethanol to evaporate, and the particles were compressed to a surface pressure of 10 mN/m. This compression was continued throughout the reaction of BIEE with the PDMA−PS particles, so as to maintain the surface pressure of 10 mN/m. A surface pressure of 10 mN/m was chosen as this surface pressure corresponds to a surface pressure that can give a close particle packing in the film to improve and thereby help prevent the formation of holes in the film. In this way, the aggregates and particles would be forced to adhere to each other, causing the particle packing in the film to improve and thereby help prevent the formation of holes in the film. In this way, the aggregates and particles would be forced to adhere to each other, causing the particle packing in the film to improve and thereby help prevent the formation of holes in the film.

Figures 8A,B and 7C,D show the 5 × 5 μm² image of the film of PDMA−PS particles without BIEE and the films of the PDMA−PS particles with BIEE that were prepared by reacting BIEE at the air/water interface, respectively. Figure 8A,C and 8B,D shows the films formed at the pH 5.8 water surface and the pH 9.0 water surface, respectively. The sizes of each individual particle that were distinguishable in the 5 × 5 μm² images shown in Figure 8 were also measured using the PhotoRuler Software. The histograms showing the sizes of the individual PDMA−PS particles on the pH 5.8 water and pH 9.0 water surfaces are shown in Figure 9. The average size of the particles was obtained by performing a Gaussian fit to the histogram data (see Table 2). On comparison of the average sizes of the particles seen in the absence of BIEE with the sizes of the particles seen in the presence of BIEE, we can observe that the sizes of the particles formed in the presence of BIEE were on average larger than the sizes of the particles imaged in the absence of BIEE, regardless of the water pH. The percentage of aggregates in the film after the BIEE reaction at the air/water interface can be seen from Table 2 to be approximately 27.1 and 26.1% for the pH 5.8 water and pH 9.0 water surfaces. The $A_{\text{NP,AFM}}$ values were also calculated (Table 2) and show that the values of $A_{\text{NP,AFM}}$ increased after the BIEE reaction for both the pH 5.8 water and pH 9.0 water surfaces. A decrease in the number of particles on the surface because of the formation of aggregates would cause the value of $A_{\text{NP,AFM}}$ to increase. On comparison, the size of the aggregates formed by the individual particles can be seen (Figure 8) to be larger when the film was formed in the presence of BIEE than in the absence of BIEE. Thus, the larger particle and aggregate sizes indicate that BIEE could interact with the PDMA−PS particles at the air/water interface. Additionally, the size of the aggregates formed by the individual particles can be seen (Figure 8) to be larger when the film was formed on the pH 9.0 water surface in the presence of BIEE than when it was formed on the pH 5.8 water surface in the presence of BIEE.

The fact that the sizes of the aggregates were larger on a pH 9 water surface than on a pH 5.8 water surface can be explained by the difference in the magnitude of the interparticle electrostatic repulsions. As the PDMA−PS particles are more charged in the pH 5.8 water than in the pH 9.0 water, the interparticle electrostatic repulsions would be stronger for the particles on the pH 5.8 water surface than those on the pH 9.0 water surface. These electrostatic repulsions would have decreased the aggregation of the particles. However, the sizes of the aggregates formed with BIEE were in general larger than the sizes formed without BIEE for both the pH 9.0 and 5.8 water subphases. Thus, some of the aggregation must have been due to the presence of BIEE. This result signifies that BIEE could
measuring the forces between a probe in the water and films of particles at air/water interfaces. Figure 10 shows that in the case of both the pH 5.8 and 9.0 subphases, the reaction of BIEE with the PDMA–PS particles on the water surface for 1 h at Π = 10 mN/m gave a film with approximately the same strength as a film of PDMA–PS particles prepared on a water surface in the absence of BIEE with Π = 10 mN/m. The estimated elastic moduli of these films of particles are shown in Figure 7. On comparison of the forces and estimated elastic moduli values measured using the films formed by reacting BIEE in the PDMA–PS particle spreading solution for 1 h and Π = 10 mN/m (Figure 6) with the forces and estimated elastic moduli values measured using the films formed by reacting BIEE with the PDMA–PS particles on the water surface for 1 h at Π = 10 mN/m (Figure 10), we see that the steepest force curve was obtained when BIEE was reacted in the PDMA–PS particle spreading solution for 1 h, spread on a pH 5.8 water surface, and then compressed to Π = 10 mN/m.

A stronger film was obtained when BIEE was reacted with the PDMA–PS particles in the ethanol spreading solution than when BIEE was reacted with the PDMA–PS particles on a pH 5.8 water surface, even though both films were compressed to the same surface pressure, and both films did not show holes detectable by AFM imaging. The difference in the strength of the films can be explained by the difference in the number of directions with which a particle can react with another particle via BIEE. In the case that BIEE was reacted with the particles in the ethanol spreading solution, a PDMA–PS particle may have bound to another PDMA–PS particle that was above or below it (vertical plane) or that was next to it (in-plane), that is, there were three binding dimensions. As the aggregates increased in size, they could have bound with other aggregates or particles in three possible dimensions. As a result, the aggregates would have become bigger in both width and height. However, in the case that BIEE was reacted with the PDMA–PS particles on the water surface, a PDMA–PS particle could have bound with other particles that were next to it (in-plane), that is, there were only two binding dimensions. As the aggregates increased in

### Table 2. Average Size of Each Individual Particle that was Distinguishable in the Film Formed at the Air/Water Interface after the PDMA–PS Particles were Reacted with BIEE at the Air/Water Interface while the Surface Pressure was Maintained at 10 mN/m

| pH | BIEE presence | Reaction time (h) | Nₜ, AFM | Nₚₑ, AFM (%) | ⟨Nₜ, AFM⟩ (nm) | ⟨Nₚₑ, AFM⟩ (nm) |
|----|---------------|------------------|--------|-------------|----------------|----------------|
| 5.8 | no            | 1                | 221    | 324 ± 112   | 1.13 × 10⁸     |                 |
|     | yes           | 1                | 161    | 349 ± 121   | 1.55 × 10⁸     |                 |
| 9.0 | no            | 1                | 241    | 308 ± 101   | 1.04 × 10⁸     |                 |
|     | yes           | 1                | 178    | 349 ± 109   | 1.40 × 10⁸     |                 |

"The average particle size was obtained by performing a Gaussian fit to the particle size histogram in Figure 8, which was calculated using the 5 × 5 μm² AFM height images shown in Figure 7. The number of particles counted in the AFM image (Nₜ, AFM), the percentage of aggregates in the film (Nₚₑ, AFM), the average particle size (⟨Nₜ, AFM⟩), area per particle (⟨Nₚₑ, AFM⟩) calculated from Nₜ, AFM, and the size of the AFM image (2.50 × 10⁷ nm²) are given.

react with the PDMA–PS particles on the pH 5.8 water surface or on the pH 9.0 water surface. The formation of the aggregates on the pH 9.0 water surface is therefore explained by BIEE-related adhesions and attractions because of physical forces, such as van der Waals, capillary, and hydrophobic forces. The aggregates formed on the pH 5.8 water surface, however, are thought to be largely due to the BIEE presence.

Figure 8E,F shows the 15 × 15 μm² images of the films of BIEE particles that were prepared by reacting BIEE on the pH 5.8 water surface and the pH 9.0 water surface, respectively. A closely packed film with no large holes was observed. This result suggests that this method could be used to prepare a film with closely packed particles and no large holes.

The effect of the film preparation method on the strength of the films at the air/aqueous interfaces was determined by measuring the forces between a hydrophilpos probe in the water and films of particles at air/water interfaces. Figure 10 shows that in the case of both the pH 5.8 and 9.0 water subphases, the reaction of BIEE with the PDMA–PS particles on the water surface for 1 h at Π = 10 mN/m gave a film with approximately the same strength as a film of PDMA–PS particles prepared on a water surface in the absence of BIEE with Π = 10 mN/m. The estimated elastic moduli of these films of particles are shown in Figure 7. On comparison of the forces and estimated elastic moduli values measured using the films formed by reacting BIEE in the PDMA–PS particle spreading solution for 1 h and Π = 10 mN/m (Figure 6) with the forces and estimated elastic moduli values measured using the films formed by reacting BIEE with the PDMA–PS particles on the water surface for 1 h at Π = 10 mN/m (Figure 10), we see that the steepest force curve was obtained when BIEE was reacted in the PDMA–PS particle spreading solution for 1 h, spread on a pH 5.8 water surface, and then compressed to Π = 10 mN/m. A stronger film was obtained when BIEE was reacted with the PDMA–PS particles in the ethanol spreading solution than when BIEE was reacted with the PDMA–PS particles on a pH 5.8 water surface, even though both films were compressed to the same surface pressure, and both films did not show holes detectable by AFM imaging. The difference in the strength of the films can be explained by the difference in the number of directions with which a particle can react with another particle via BIEE. In the case that BIEE was reacted with the particles in the ethanol spreading solution, a PDMA–PS particle may have bound to another PDMA–PS particle that was above or below it (vertical plane) or that was next to it (in-plane), that is, there were three binding dimensions. As the aggregates increased in size, they could have bound with other aggregates or particles in three possible dimensions. As a result, the aggregates would have become bigger in both width and height. However, in the case that BIEE was reacted with the PDMA–PS particles on the water surface, a PDMA–PS particle could have bound with other particles that were next to it (in-plane), that is, there were only two binding dimensions. As the aggregates increased in

**Figure 9.** Histogram showing the size of each individual particle that was distinguishable in the 5 × 5 μm² AFM height images of Figure 7. A and B show the results for the pH 5.8 and 9.0 water surfaces, respectively. The PDMA–PS particles in this film were reacted with BIEE at the air/water interface, while the surface pressure was maintained at 10 mN/m. The average size of the particles was obtained by performing a Gaussian fit to the particle size histogram; see Table 2 for the average particle sizes.

**Figure 10.** Approach force–distance curves measured between a film of PDMA–PS particles compressed to Π = 10 mN/m on a water surface and a hydrophilpos probe in the water. (A) pH 5.8 water subphase; (B) pH 9.0 water subphase. Black squares: film of PDMA–PS particles without BIEE on the water surface, which was compressed to Π = 10 mN/m; green triangles: film of PDMA–PS particles with BIEE prepared by reacting BIEE with the particles on the water surface for 1 h, while the film was compressed to Π = 10 mN/m.
size, they could have bound with other aggregates or particles in two possible dimensions, causing the aggregates to have become bigger in width. The film formed by the particles reacting in three possible dimensions would therefore have appeared thicker due to the larger size (height) of the aggregates. This would have caused the film to be stronger than the film with smaller particles.

The thickness of the film formed by reacting BIEE with the particles in the ethanol spreading solution or the film formed by reacting BIEE with the PDMA–PS particles on the water surface was determined by scratching a hole or line in the transferred film and then imaging that area. Figure 11A shows the 5 × 5 μm² height image of a hole in a film, which was formed by reacting BIEE with the particles in the ethanol spreading solution and then by spreading that solution on a pH 5.8 water surface. Figure 11B shows the 5 × 5 μm² height image of a line scratched in a film, which was formed by reacting BIEE with the PDMA–PS particles on a pH 5.8 water surface. The height profiles of the white lines shown in Figure 11A,B are shown in Figure 11C. The film formed by reacting BIEE with the particles in the ethanol spreading solution gave larger height difference values than the film formed by reacting BIEE with the particles at the air/water interface. Thus, the film formed by reacting BIEE with the particles in the ethanol spreading solution can be concluded to be thicker than the film formed by reacting BIEE with the particles at the air/water interface. This film thickness difference is thought to contribute to the difference in the strength of the films.

4. EXPERIMENTAL SECTION

4.1. Materials. The following materials were used in this experiment: N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (hydrophilic, 50% in methanol, ABCR, Germany), chloroform (CHCl₃, 99.0% purity, Wako Pure Chemical Industries, Japan), methanol (MeOH, HPLC grade, Fisher Scientific, Germany), ethanol (EtOH, JIS Special Grade, Wako Pure Chemical Industries, Japan), aqueous ammonia solution [NH₃(aq), 25 wt %, analytical grade, WTL Laborbedarf GmbH, Kastellaun, Germany], BIEE (96% purity, Sigma-Aldrich), and sodium hydroxide (NaOH, 97.0% purity, Wako Pure Chemical Industries, Japan). Ultrapure water (Direct-Q 3 UV, Millipore, USA) with a specific resistivity of 18.2 MΩ cm⁻¹ was used in this study. The PDMA–PS particles had 3.21 wt % poly(2-(N,N-dimethylamino)ethylmethacrylate) loading. The diameter of the PDMA–PS particles was 333 ± 128 nm. The details of the synthesis and preparation can be found elsewhere. The pH 5.8 and 9.0 aqueous solutions used as the subphases of the particle films in this study were pure water and water adjusted to pH 9.0 by the addition of NaOH. These subphases are referred to as “pH 5.8 water” and “pH 9.0 water” throughout this study.

4.2. Preparation of the PDMA–PS Particle Spreading Solution. The PDMA–PS particles were first dispersed in ethanol to give a weight percentage of 18.4. This solution was then diluted with ethanol to give an approximate weight percentage of 1.8. This dilution was performed so as to avoid the particles aggregating and becoming stuck in the syringe used to spread the PDMA–PS particle spreading solution at the air/water interface. This diluted PDMA–PS particles in ethanol solution was used to prepare all PDMA–PS films reported in this study. Ethanol acted as the solvent to spread the particles at the air/water interface. Organic solvents commonly used to spread and prepare insoluble Langmuir monolayers at air/water interfaces, such as chloroform and toluene, could not be used in this study as polystyrene dissolves in chloroform and toluene. Ethanol, however, did not dissolve the PDMA–PS particles and also allowed the PDMA–PS particles to be well-dispersed. Ethanol also has a positive spreading coefficient, which enables it to spread at the air/water interface and form monolayers of the PDMA–PS particles.

The cross-linker of BIEE was added to 1 mL of PDMA–PS particles in ethanol solution to give a BIEE/PDMA–PS
particles in ethanol solution ratio of 1:10. The concentration of BIEE in the PDMA–PS particles in ethanol solution was calculated to be 0.541 mM. As the same volume of PDMA–PS particles in ethanol solution (1.0 mL) and BIEE in ethanol solution (1.0 μL) was used to prepare each PDMA–PS particles in ethanol solution containing BIEE, the weight fraction of the PDMA–PS particles in the ethanol spreading solution was the same for each of the BIEE-containing systems. Assuming the weight percentage of the PDMA–PS particles in ethanol was 1.8, the weight fraction of the PDMA–PS particles in the ethanol solution containing BIEE can be calculated to have been reduced by less than 0.2%.

The BIEE reaction with the PDMA–PS particles was performed in two ways. The first method was to allow BIEE to react with the particles in the ethanol solution for a specified period of time, before the particle solution containing BIEE was spread at the air/water interface to form the particle film. The second method was to add BIEE to the particle solution and mix that solution for 30 s. The particle solution containing BIEE was then spread at the air/water interface, and BIEE was allowed to react for a specified period of time. The BIEE reaction was not quenched after its addition to the PDMA–PS particle solution. Thus, the cross-linking reaction could occur in the spreading solution containing the PDMA–PS particles and ethanol and also at the air/water interface after the particles were spread.

4.3. Preparation of the Colloidal Probe Used in the Monolayer Particle Interaction Apparatus (MPIA) Force Studies. A light microscope (BX51, Olympus) and a micromanipulator (model MRM-202D, Narishige) were used to attach a silica particle (nominal diameter = 6.84 μm, Bangs Laboratory, Fishers, USA) to a gold-plated Si3N4 cantilever (V-shaped, nominal spring constant k = 0.15 N/m, OTR8–PS-W, Olympus) with an epoxy resin (Araldite Rapid). The silica probe was next modified with hydrophilic using the method reported elsewhere.5

4.4. Measurement of the Surface Pressure–Area Langmuir Isotherms. The particle films were prepared using a Langmuir–Blodgett trough (large microscopy Langmuir trough, Nima Technology Ltd, Coventry, UK), which was made from poly(tetrafluoroethylene) (PTFE) and equipped with two PTFE barriers that compressed around the center of the trough. The maximum surface area of the trough was 290 cm². The surface pressure was measured using a Wilhelmy plate of wet filter paper (8) (no. 2 240 mm, Toyo, Japan), which was suspended from a strain gauge (Nima PS4 surface pressure sensor, Nima Technology Ltd, Coventry, UK).

Before the surface pressure–area isotherms were measured, the Langmuir trough was cleaned with chloroform and then with ethanol. To eliminate any remaining solvent, water was then added to the trough and then removed. The subphase solution was next added to the trough, the barriers were compressed to a maximum, and the subphase surface was cleaned by suctioning the water surface between the two barriers. The barriers were subsequently fully expanded. The temperature of the subphase was maintained at 20 °C. Next, 220 μL of the PDMA–PS particles in ethanol solution or the PDMA–PS particles in ethanol solution that contained BIEE was spread drop-wise onto the subphase by using a 100 μL syringe (Hamilton, Switzerland). The same volume was spread for each system, allowing the isotherms of the different systems to be directly compared. Approximately 10 min was allowed for the solvent to evaporate. The particle films were then compressed with a speed of 80 cm² min⁻¹, during which time the surface pressure–area isotherm was recorded. Each isotherm was measured for a minimum of three times to ensure that the results were reproducible.

The surface pressure (Π)–area isotherms were converted to Π–area per PDMA–PS particle (AΠPDMA–PS) isotherms by calculating the number of PDMA–PS particles spread at the air/water interface (NΠPDMA–PS), using the weight fraction of the PDMA–PS particles in the ethanol spreading solution (0.018), the volume spread at the air/water interface (220 μL), the density of ethanol (0.789 g/mL), and the weight of one particle (WΠPDMA–PS). WΠPDMA–PS was calculated using the density of polystyrene (1.05 g/cm³) and the radius of one particle.

4.5. Langmuir–Blodgett Deposition. The particle films were transferred to a 1 × 2 cm² mica substrate (Grade V-5 Muscovite 01804-CA, Structure Probe, Inc, USA) via a Langmuir–Blodgett deposition. Briefly, the Langmuir trough was cleaned in the manner described above. The liquid to be used as the subphase was then added to the trough. The mica substrate was next cleaned by using a sticky tape, which removed the outer layers of the mica. The surface of the subphase was then suctioned and cleaned. The mica substrate was next attached to a minidipper (DC-20 small, Nima Technology Ltd, UK) and immersed into the subphase so that only a few millimeters of the substrate remained in the air. The particle film was then spread, and a waiting time of 10 min was allowed for the spreading solvent to evaporate. The monolayer was subsequently compressed to the desired surface pressure. Once the surface pressure stabilized, the wafer was moved out of the subphase using the dipper at a speed of 1 mm/min (the slowest speed available on the dipper).

4.6. AFM Imaging. The particle films that were transferred to mica substrates were imaged with an atomic force microscope (Multimode 3, Veeco, USA) in the tapping mode by using a silicon nitride cantilever coated with aluminum (Si-DP20, SII, Japan, nominal spring constant = 16 N m⁻¹). The substrate was attached to the piezo using a double-sided tape (NW-15SF, Nichiban, Japan) and was then imaged with a scan size of 5 × 5 μm² and 15 × 15 μm². The number of lines and the resolution of data points per line were both 512. The AFM images were unmodified (i.e., nonfiltered), except for a first-order flattening along the scan lines.

4.7. MPIA Force–Distance Curves. The forces between a particle film at an air/water interface and a colloidal probe in the water were measured using the MPIA. The MPIA combines a Langmuir trough (Riegler & Kirstein GmbH, Potsdam, Germany) and a force measurement unit. Detailed information about the MPIA can be found elsewhere.22–24

The Langmuir trough was cleaned using CHCl₃ and EtOH. Water was next added and subsequently removed, so as to remove any remaining solvent. The colloidal probe was then attached to the cantilever holder. Water was next added to the trough, and its surface was suctioned and cleaned. The temperature of the subphase was maintained at 20.0 ± 0.1 °C by using a circulation system (C25P, ThermoHaake, Karlsruhe, Germany) that ran thermostated water through the base of the trough. The calibration factor (CFmisc) that was required to convert the raw force curves (force [V] vs piezo position [nm] curves) to calibrated force curves (force [nN] vs piezo position [nm] curves) was obtained by first measuring the forces between the probe and a clean mica substrate that was placed across the edges of the Langmuir trough filled with water. The water in the trough was removed after calibration,
new water was added, and its surface was cleaned. The particle film was next spread at the air/water interface, and 10 min was allowed for ethanol to evaporate. The films were then compressed to a surface pressure of 10 mN/m, and the forces were measured between that film and the same probe that was used to measure \( \sigma_{\text{cmc}} \). At least 50 force curves were measured at each surface pressure. \( \sigma_{\text{cmc}} \) was used to convert the raw force curves (force [V] vs. position [mm]) curves measured between the particle film and the probe to calibrated force curves (force [nN] vs. position [nm]) curves. Zero force was defined at large cantilever-film separations, where no surface forces acted on the cantilever. Zero distance was defined at the onset of the linear contact region; the contact region was defined as the area where the probe was in contact with the mica substrate or with the particle film at the air/aqueous interface.

The effective stiffness (\( S_N \)) of the film of particles at the air/aqueous interface was determined from a linear fit of the repulsion seen in the contact region of the force curves. The slope of the linear contact region recorded for the repulsion seen in the contact region of the force curves. The \( S_N \) at each surface pressure. CFmica was used to convert the raw measured between the particle film and the probe to calibrated force curves (force [nN] vs. position [nm]) curves. Zero force was defined at large cantilever-film separations, where no surface forces acted on the cantilever. Zero distance was defined at the onset of the linear contact region; the contact region was defined as the area where the probe was in contact with the mica substrate or with the particle film at the air/aqueous interface.

The effective stiffness (\( S_N \)) of the film of particles at the air/aqueous interface was determined from a linear fit of the repulsion seen in the contact region of the force curves. The slope of the linear contact region recorded for the film at the air/water interface (\( S_N \)) was divided by the slope measured with the mica substrate (\( S_M \)), giving \( S_{M-N} \). If \( S_{M-N} = 1 \), then the film had a stiffness equivalent of mica. As mica has an elastic modulus of \( \pm 62 \pm 2 \) GPa, the elastic modulus of the film was estimated by \( S_{M-N} \times 62 \pm 2 \) GPa.

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**Notes**

The authors declare no competing financial interest.

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