FIB Processing for Natural Rubber with Nanomatrix Structure

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Abstracts:

Focused ion beam (FIB) processing was performed to prepare an ultra-thin section of graft-copolymer of deproteinized natural rubber and polystyrene (DPNR-*graft*-PS), in order to obtain a three-dimensional (3D) image of the nanomatrix structure through transmission electron micro-tomography (TEM-T) observation. The DPNR-*graft*-PS was immersed into OsO₄ solution for more than 7 days for the sake of enhancing a contrast of the TEM-T image during the FIB processing. *In-situ* observation with a field emission-scanning electron microscope was made during the FIB processing. The ultra-thin section prepared by FIB processing was compared with that prepared with a cryo-microtome in the TEM-T observation. A distinctly clear TEM-T image of the nanomatrix structure, comprising the natural rubber micro-particles and the nanomatrix of PS nano-particles / natural rubber, was obtained with the ultra-thin section prepared by FIB processing, but not with that prepared with the cryo-microtome. The FIB processing was proved to be indispensable for the TEM-T observation of the nano-structure of the soft-materials.

Keywords: Natural rubber, Focused ion beam, Transmission electron micro-tomography

1. INTRODUCTION

Hard, soft rubber, which results in increase in both dynamic storage modulus and dynamic loss modulus with increasing frequency in rubbery plateau region, attracts much attention as a novel viscoelastic material. This may be distinguished from ordinary rubbers as a soft material and ordinary plastics as a hard material. Recently, we prepared the hard, soft rubber with inordinate dynamic viscoelastic properties by means of a modulation of nano-phase separated structure; that is, we found that both dynamic storage modulus and dynamic loss modulus of natural rubber with the nanomatrix structure increased in the rubbery plateau region as displacement frequency rose. It is, thus, hypothesized that the inordinate dynamic viscoelastic properties are attributed to the nanomatrix structure. In order to elucidate the relationship between the nanomatrix structure and the dynamic viscoelastic properties, we must develop a new technique to observe the morphology of natural rubber with the nanomatrix structure on the basis of a novel concept.

Focused ion beam (FIB) processing is a powerful technique to form a smooth cross sectional area of materials having nano-structure, because it is performed by irradiating Ga ion laser beam into the materials¹⁾. The FIB processing is, thus, distinguished from the conventional ultra-fine processing with a microtome²⁾⁻⁵⁾, which causes shear deformation of the nano-structure of the materials due to a force loaded on them to prepare an ultra-thin section with glass or diamond knives. We may, thus, prevent a physical contact of the materials with the knives, in order to maintain a spatial position of the nano-structure of the materials, as long as we adapt the FIB processing⁶⁾. The FIB processing is, thus, expected to be indispensable for the observation of the morphology through transmission electron micro-tomography (TEM-T) method^{7), 8)}, which provides 3-dimensional (3D) image of the nano-structure.

The requirement for the FIB processing is *in-situ* observation of the cross

sectional area of the materials in order to obtain the fine ultra-thin section. A combination of field emission scanning electron microscope (FE-SEM) and FIB microscope^{9), 10)} may meet our need; for instance, the surface obtained during the FIB processing is observed with FE-SEM. Furthermore, the FIB-SEM has been used for obtaining 3D SEM images after reconstructing 2-dimensional (2D) images obtained by observing the surfaces of the materials¹¹⁾⁻¹⁴⁾, which are etched with the FIB at each interval of less than 100 nm.

In the last decade, the FIB processing has been extensively carried out for inorganic materials such as ceramics, metals and so forth^{15), 16)}. The ultra-thin sections of the inorganic materials were well prepared by the FIB processing and they were subjected to transmission electron microscope (TEM) observation, in order to obtain sophisticated images of crystalline structures¹⁷⁾. In contrast, for the organic materials, few works were reported on the FIB processing to prepare the ultra-thin sections. Morphology of the solar cells¹⁸⁾, polymer light emitting diodes¹⁹⁾ and block copolymer thin films²⁰⁾ was observed by TEM, in which the ultra-thin sections were prepared by the FIB processing. However, recently, damages of the materials during the FIB processing have been found to be a serious issue for the preparation of the ultra-thin sections through the FIB processing²¹⁾. For instance, in the case of the inorganic materials, two types of processing damages were reported: that is, the formation of amorphous layers that contain implanted Ga atoms and the precipitation of particles due to deterioration, i.e. melting, of surface with heat during the FIB processing. These damages were cured by micro-sampling method and cool ion milling method. On the other hand, more serious damages were reported for the organic materials. Sometimes, the etched surfaces were scorched during the FIB processing due to over-heating with the FIB at local portion of the area. The FIB processing has been performed after staining the organic materials with osmium tetroxide (OsO₄) at lower temperature, in

order to prevent charge-up with the FIB^{4), 21)}.

In the previous work, we prepared a graft-copolymer of deproteinized natural rubber and polystyrene (DPNR-*graft*-PS) with the nanomatrix structure, which consists of deproteinized natural rubber particles of about 1 μm in average diameter and polystyrene nanomatrix of about 15 nm in thickness²²⁾. We obtained 3D images of DPNR-*graft*-PS with the nanomatrix structure through FIB-SEM and TEMT observations²³⁾. It was found that the polystyrene nanomatrix was not continuous; hence, the value of the Young's modulus of the DPNR-*graft*-PS was smaller than expected one. In addition, the images obtained by the TEMT method were unclear, because the ultra-thin sections were prepared by using cryo-microtome with the glass knife. The relationship between the structure and properties of DPNR-*graft*-PS with the nanomatrix structure still remains ambiguous.

In the present work, an attempt to prepare the ultra-thin sections of DPNR-*graft*-PS was made by the FIB processing with the FIB-SEM in order to elucidate the relationship the nanomatrix structure and the dynamic viscoelastic properties. First, natural rubber with the nanomatrix structure was prepared to confirm the finding that both dynamic storage modulus and dynamic loss modulus of natural rubber with the nanomatrix structure increased in the rubbery plateau region as displacement frequency rose. Second, the 3D image was obtained by reconstructing 2D images of the FIB-SEM. Conditions to prepare the ultra-thin sections were investigated for the FIB processing. The observations of the nanomatrix structure were performed by TEM and TEMT method for DPNR-*graft*-PS, where the nanomatrix structure is defined to consist of natural rubber micro-particles and polystyrene nanomatrix.

2. Experimental procedure

2.1 Sample preparation

Natural rubber latex used in the present study was commercial high-ammonia natural rubber latex. The incubation of the latex was made with 0.1 w/w% urea in the presence of 1 w/w% sodium dodecyl sulfate (SDS) at 303 K followed by centrifugation at 10,000 rpm. Cream fraction was re-dispersed in 1 w/w% SDS to make 30 w/w% dry rubber content (DRC) latex and it was washed twice by centrifugation to prepare deproteinized natural rubber (DPNR) latex²⁴. The DPNR latex was diluted with distilled water to adjust 10 w/w% DRC and concentration of SDS was adjusted to 0.1 w/w%.

Graft-copolymerization of styrene onto DPNR was carried out in latex stage with *tert*-butyl hydroperoxide / tetraethylene pentamine as an initiator. The DPNR latex was charged with N₂ gas for 1 hour at 303 K. The initiator of 3.3×10^{-2} mol/kg-rubber and styrene of 1.5 mol/kg-rubber were added to the latex, respectively. The reaction was performed by stirring the latex at about 400 rpm for 2 hours at 303 K. The unreacted styrene was removed by using a rotary evaporator under reduced pressure. A film specimen of the grafted natural rubber was prepared by dipping the glass tube into the reacted latex and it was dried under reduced pressure at ambient temperature for more than a week.

2.2 Observation

Observation of morphology of the grafted natural rubber was made with a TEM, JEOL JEM-2100 at accelerating voltage of 200 kV and FIB-SEM, SII SMI-3050SE at accelerating voltage of 3 kV. The FIB-SEM was also used to make a ultra-thin section of grafted natural rubber according to the literature⁴. The ultra-thin section of the graft-copolymer was prepared by a Sovall Instruments MT6000 Ultra-microtome at -80°C. The thin sections were stained with OsO₄, after annealing the section at 353 K for 30 min.

3D images were obtained by reconstructing more than 100 2D images, i.e.

TEM images. The sections with *ca.* 100 nm thick were prepared and stained with Ruthenium tetroxide (RuO_4) and OsO_4 in the similar way as described above. A special specimen holder that enables us to rotate the section up to -70° - $+70^\circ$ was used to take a high resolution 3D image. Tilt angular range in the particular experiment was -59° to $+63^\circ$ with an increment of 1° , which was aligned and reconstructed according to the protocol described in ref.²⁵⁾

3. Results and Discussion

Figure 1 shows a plot of storage modulus *versus* displacement frequency in the rubbery plateau region for natural rubber and natural rubber with the nanomatrix structure (DPNR-*graft*-PS). The value of the storage modulus of natural rubber and DPNR-*graft*-PS increased as the frequency increased. The increase in the value of the storage modulus may be explained to be due to the viscoelastic properties of the rubbers in the rubbery plateau region; the higher the frequency, the higher the storage modulus. The rubbers are more elastic in the high frequency region. Figure 2 shows a plot of loss modulus *versus* displacement frequency in the rubbery plateau region for natural rubber and DPNR-*graft*-PS. The value of the loss modulus of natural rubber decreased as the frequency increased. In contrast, the value of the loss modulus of DPNR-*graft*-PS increased, as the frequency increased. The decrease in the value of the loss modulus in the high frequency region may correspond to increase in the value of the storage modulus, since the elasticity is inconsistent with the viscosity. However, it is quite difficult to explain the fact that the values of both loss modulus and storage modulus of DPNR-*graft*-PS increase as the frequency increases. We have to explain the reason why the elasticity and viscosity increase with increasing the frequency in the view point of the morphology. Thus, an attempt to develop a new technique to observe the nanomatrix structure in nano-meteric scale was made in the present study.

Figure 3 shows FE-SEM image of the etched surface of the DPNR-*graft*-PS, in which carbon coating was performed to prevent damage of an edge of the surface etched with the Ga ion beam (see complementary Figure 1). The rough surface of the coated carbon may be explained to be due to non-uniform deposition of the carbon on the DPNR-*graft*-PS. The image was taken in an oblique direction by FE-SEM, because the film specimen of the DPNR-*graft*-PS, placed on a flat plate in a chamber, was perpendicularly etched with the FIB and it was observed in the oblique direction. The bright domains represent polystyrene (PS) and dark domains represent deproteinized natural rubber (DPNR)²⁴, since it was stained with OsO₄. The contrast of the domains was sufficient to take the FE-SEM image, since the DPNR-*graft*-PS was stained with OsO₄ for more than 7 days, which prevented the damage of the DPNR-*graft*-PS during FIB processing. In general, it is known that insufficient staining (short time) may result in a significant damage of the DPNR-*graft*-PS due to local heating by charging up a specimen during FIB processing, whereas over-staining (long time) may lead to less contrast because OsO₄ goes into not only DPNR phase but also PS phase (over-staining). We, thus, determined a suitable time, i.e. 7 days to stain the DPNR-*graft*-PS with OsO₄. In the image, the sample surface was rather bright and rough, which was assigned to polystyrene. This assignment was proved by the image of etched surface; for instance, the bright domains existed on the sample surface and the matrix, because the film specimen was prepared by coagulation of DPNR-*graft*-PS latex, in which natural rubber particles of about 1 μm in average diameter, covered with PS layer, were dispersed in water.

Expanded image of the etched surface is shown in Figure 4 (see complementary Figure 2). The edge of the etched surface shown at top in Figure 4 was situated rather front compared with a bottom bed; hence, the magnification of the top edge is a little bit higher than that of the bottom bed. This may be explained to be due

to the oblique angle between an electron-gun and sample surface. The image was, thus, modified by correction of voxel dimensions for oblique-angle imaging (55°) and overlay correction for x-y drift. The absolute dimensions in the x-direction are obtained from a calibrated FIB magnification scale. Because of oblique FE-SEM imaging at an angle of 55° , distances in the y-direction have to be corrected in terms of projection effects using $y' = y \sin(55^\circ)$.

The corrected 2D FE-SEM image of the DPNR-*graft*-PS is shown in Figure 5, in which a contrast of the image is extensively enhanced to distinguish the bright PS domains from the gloomy DPNR domains. The bright PS nanomatrix was quite distinct near the top of the image, whereas this turned into ambiguous near the bottom. This is attributed to a difference between distances of secondary electron detector from top and bottom of the etched surface, due to the oblique angle; the lower the height from the top of the etched surface along to the Y-axis, the worse was the focus. Thus, the scanning region was limited in Y-direction, which resulted in the rectangular image in Figure 5. The image was almost similar to that obtained by TEM observation (shown below in Figure 6), suggesting that the structure of the DPNR-*graft*-PS was not damaged by FIB processing.

Figure 6 shows TEM image for the ultra-thin section of the DPNR-*graft*-PS prepared by FIB processing, in which the dark domains represent PS and the bright domains represent DPNR, because the ultra-thin section was stained with RuO_4 . The image of the ultra-thin section was taken after putting it on the grid substrate. The dimension of the ultra-thin section, thus obtained, was about $6\ \mu\text{m} \times 9\ \mu\text{m} \times 54\ \text{nm}$. The thickness of 54 nm may be a remarkable virtue of the FIB processing, because the thickness of the ultra-thin section prepared with the cryo-microtome is more than 100 nm in an ordinary circumstance. Furthermore, in Figure 6, several damages of the ultra-thin section were found in the image: for instance, curled edge of the ultra-thin

section and white spot as a void due to over-heating with the FIB at local portion of the area during the FIB processing. This may be explained to be due to charge-up caused by less staining with RuO_4 ; hence, we could not see the nanomatrix structure near the curled edge and white spot.

TEM image for the ultra-thin section of the DPNR-*graft*-PS prepared with the cryo-microtome equipped with the glass knife is shown in Figure 7. The bright domains represent PS and the dark domains represent DPNR, because the ultra-thin section was stained with OsO_4 . The dimension of the ultra-thin section obtained with the cryo-microtome was significantly larger, when it was compared with that obtained by FIB processing. The thickness and width of the ultra-thin section were about 130 nm and at least 20 μm , respectively. In the TEM image, some voids were found in the ultra-thin section as a brilliant domain due to charge-up; more exactly, they existed on the right hand side of the ultra-thin section. The voids were considered to be formed by cutting with the knife, because they existed with several non-periodic waves in the ultra-thin section. Perhaps, the film specimen may be deformed, when the force was applied to prepare the ultra-thin section. The cavitation may occur in the nanomatrix to form the voids due to weak attractive force between the PS nano-particles and natural rubber. The formation of the voids may be one of the disadvantages of the cryo-microtome.

Disadvantage was also recognized for the FIB processing. Figure 8 shows TEM image for the ultra-thin section of the DPNR-*graft*-PS prepared by the FIB processing, which is an expanded image of a part of the ultra-thin section shown in Figure 6. In the TEM image was found a sharp crack (marked as white arrow), whose width and length were about 10 nm and 6.6 μm , respectively. The crack may be formed by rapid volume contraction due to quick cooling, after heating the ultra-thin section during the FIB processing. The crack propagation may occur to release local strain

energy of the ultra-thin section due to quick cooling and it remains appeared due to the staining with OsO_4 as a chemical pinning agent. This implies a conflict between the advantage and disadvantage of the FIB processing; that is, the advantage to prepare thin film becomes the disadvantage to form sharp crack due to quick thermal transport. This fracture behavior is completely distinguished from the mechanical fracture behavior that forms a large crack. Consequently, it is found that the slow cooling is a necessarily requirement for the preparation of the ultra-thin section with the FIB.

Figure 9 shows expanded TEM images of the DPNR-*graft*-PS, in which (A) represents the TEM image for the ultra-thin section prepared by FIB processing and (B) represents the TEM image for the ultra-thin section prepared with the cryo-microtome. Two staining agents were used to distinguish the images from each other: that is, RuO_4 for (A) and OsO_4 for (B), which were known to stain PS phase and natural rubber phase, respectively. In Figure 9 (A) and (B), various sizes of the natural rubber particles were distributed in the PS nanomatrix, reflecting the wide distribution of particle size of natural rubber latex. In contrast, we could not determine the thickness of the nanomatrix, because the 2D-image was made with transmitted electron, which may be different from a real image. For instance, the thickness of the nanomatrix shown in 2D-image may become apparently larger than that in the real image, when the nanomatrix possesses curvature (Figure 10). Thus, it is necessary to perform 3D-observation of the ultra-thin section, in order to obtain quantitative results.

3D TEMT image for the ultra-thin section prepared by the FIB processing is shown in Figure 11 (see complementary Figure 3 (A) and (B)), in conjunction with that prepared with the cryo-microtome, where the magnification is similar to that of Figure 9. Bright domains in the images represent PS and dark domains represent natural rubber. The natural rubber particles of about 1 μm in average diameter were shown to be well dispersed in the PS nanomatrix. Curvatures of the natural rubber particles were

slightly shown in the image, because average size of the natural rubber particles was absolutely larger than the thickness of the ultra-thin section. The 3D image for the ultra-thin section prepared by the FIB processing appeared to be the same as that prepared with the cryo-microtome. Furthermore, the 3D images were quite similar to the 2D images shown in Figure 9, since the size of rubber particles was quite large as compared with the thickness of the ultra-thin section. The 3D observation was, thus, not effective for the nanomatrix structure at this low magnification, i.e. micro-metric scale. This demonstrates that the 3D image is more effective to observe nano-structure at high magnification, i.e. nano-metric scale.

Figure 12 shows 3D TEMT image for the ultra-thin section prepared by the FIB processing in conjunction with that prepared with the cryo-microtome at extremely high magnification, i.e. nano-metric scale (see complementary Figure 4 (A) and (B)). In the images, the nanomatrix of about 15 nm in thickness was observed in detail. The nanomatrix was discontinuous. Especially, in the 3D TEMT image for the ultra-thin section prepared by FIB processing (Figure 12 (A)), the PS nano-particles of about 5 nm in diameter were found to be closely dispersed in the nanomatrix. In contrast, partially disconnected nanomatrix was shown in the 3D TEMT image for the ultra-thin section prepared with the cryo-microtome (Figure 12 (B)). The difference in the image between Figure 12 (A) and (B) may be explained to be due to the preparation method of the ultra-thin section. In an ordinary circumstance, the spatial position of the nano-phase separated structure is maintained by the FIB processing due to no physical contact, whereas it is distorted with the cryo-microtome due to the physical contact with the glass knife. Based upon this empirical rule, the spatial position of the PS nano-particles may be maintained in the nanomatrix in the case of the FIB processing. However, the position may be distorted in the case of the cryo-microtome processing. This demonstrates that the FIB processing is indispensable for the

observation of the nanomatrix structure.

Furthermore, by adapting the FIB processing for the preparation of the ultra-thin section, the distance between the PS nano-particles was found to be about 5 nm. Perhaps, in the nanomatrix, natural rubber may be placed in between the PS nano-particles; so that, the nanomatrix may consist of the PS nano-particles and natural rubber. Consequently, it was proved by TEMT observation of the ultra-thin section prepared by the FIB processing that the nanomatrix structure was complexly hieratical structure comprising of the natural rubber micro-particles and the nanomatrix of the PS nano-particles / natural rubber. The complexly hieratical structure may provide various strain fields into natural rubber. Thus, the observed morphology may be closely related to the inordinate viscoelastic properties of natural rubber with the nanomatrix structure (DPNR-*graft*-PS); that is, the elasticity and viscosity increased with increasing the frequency. More detailed study on the relationship between the nanomatrix structure and the viscoelastic properties is now being conducted and it will be reported in our subsequent paper.

4. Conclusion

TEM and TEMT observations of the ultra-thin sections prepared by FIB processing were made for the DPNR-*graft*-PS with the nanomatrix structure. The dimension of the ultra-thin section prepared by FIB processing was about 6 μm X 9 μm X 54 nm, which was extremely smaller than that prepared with the cryo-microtome. The ultra-thin section was completely stained with OsO_4 for more than 7 days to enhance the contrast of the TEMT image during the FIB processing. It was demonstrated by TEM and TEMT observations that the FIB processing was superior in non-destructive nature to prepare the ultra-thin section; that is, the spatial position of the nanomatrix structure was maintained by the FIB-processing due to no physical

contact, whereas it was distorted with the cryo-microtome due to the physical contact with the glass knife. The nanomatrix structure was found to be the complexly hierarchical structure comprising of the natural rubber micro-particles and the nanomatrix of PS nano-particles / natural rubber. In the nanomatrix, the PS nano-particles of about 5 nm in diameter were closely dispersed, whose distance was about 5 nm. The FIB processing was proved to be indispensable for the TEMT observation of the nano-structure, i.e. the nanomatrix structure of DPNR-*graft*-PS.

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Figure captions

Figure 1 Logarithmic plot of storage modulus *versus* displacement frequency in the rubbery plateau region for natural rubber and natural rubber with the nanomatrix structure (DPNR-*graft*-PS). Open circles represent natural rubber and closed circles represent DPNR-*graft*-PS.

Figure 2 Logarithmic plot of loss modulus *versus* displacement frequency in the rubbery plateau region for natural rubber and natural rubber with the nanomatrix structure (DPNR-*graft*-PS). Open circles represent natural rubber and closed circles represent DPNR-*graft*-PS.

Figure 3 FE-SEM image of the etched surface of the DPNR-*graft*-PS, in which carbon coating was performed to prevent damage of an edge of the surface etched with the Ga ion beam. Positively charged Ga ion at about 50 pA was used for etching process. FE-SEM image was obtained at accelerating voltage of 3 kV. Field emission SEM images of the DPNR-*graft*-PS were taken after etching its surface at each 140 nm intervals with Ga⁺ ion.

Figure 4 Expanded 2D FE-SEM image of the etched surface. The edge of the etched surface was situated rather front compared with a bottom bed. The magnification of the front edge was, thus, a little bit higher than that of the bottom bed.

Figure 5 Corrected 2D FE-SEM image of the DPNR-*graft*-PS. The absolute dimensions in the x-direction are obtained from a calibrated FIB magnification scale. Because of oblique FE-SEM imaging at an angle of 55°, distances in the y-direction have to be

corrected in terms of projection effects using $y' = y \sin(55^\circ)$. The bright PS nanomatrix was quite distinct near the top of the image, whereas this turned into ambiguous near the bottom.

Figure 6 TEM image for the ultra-thin section of the DPNR-*graft*-PS prepared by FIB processing, in which the dark domains represent PS and the bright domains represent DPNR, because the ultra-thin section was stained with RuO_4 . The dimension of the ultra-thin section, thus obtained, was about $6\ \mu\text{m} : 9\ \mu\text{m} : 54\ \text{nm}$.

Figure 7 TEM image for the ultra-thin section of the DPNR-*graft*-PS prepared with the cryo-microtome equipped with the glass knife. The dimension of the ultra-thin section obtained with the cryo-microtome was extensively larger, when it was compared with the ultra-thin section obtained by FIB processing. The thickness and width of the ultra-thin section were about 130 nm and at least $20\ \mu\text{m}$, respectively.

Figure 8 TEM image for the ultra-thin section of the DPNR-*graft*-PS prepared by the FIB processing, which is an expanded image of Figure 7. The white arrow represents a crack propagating in the ultra-thin section.

Figure 9 Expanded TEM images for the DPNR-*graft*-PS, in which (A) represents the TEM image for the ultra-thin section prepared by FIB processing and (B) represents the TEM image for the ultra-thin section prepared with the cryo-microtome. Two staining agents were used to distinguish the images from each other: that is, RuO_4 for (A) and OsO_4 for (B).

Figure 10 Schematic illustration of nanomatrix structure in the ultra-thin section. The

relationship between 2D-image and 3D image are well represented in the schematic illustration. The interface in the 2D image is thicker than that in the 3D image.

Figure 11 3D TEMT images for (A) the ultra-thin section prepared by the FIB processing and (B) the ultra-thin section prepared with the cryo-microtome. Bright domains in the images represent PS and dark domains represent natural rubber. The natural rubber particles of about 1 μm in average diameter were shown to be well dispersed in the PS nanomatrix.

Figure 12 3D TEMT images for (A) the ultra-thin section prepared by the FIB processing and (B) the ultra-thin section prepared by cryo-microtome processing at extremely high magnification.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

(A)

(B)

Figure 9

Figure 10

(A)

(B)

Figure 11

(A)

(B)

Figure 12