Preparation Methods for EM Studies of Polymers

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Introduction

Polymers are organic materials, and most of the preparation methods for transmission electron microscopy (TEM) are much the same as for biological specimens. Bulk samples are cut in the ultramicrotome, and latex particles are dropped onto a Formvar-coated grid. However, the staining possibilities of polymers are limited. Polymers mainly consist of saturated hydrocarbon, and unlike biomaterial there are few reactive groups for staining. This has two consequences for the microscopist: it is difficult to obtain contrast, and the sample is very beam-sensitive. Biological samples are also organic material and sensitive to the beam, but the staining process converts them into materials that are usually stable to the beam [1].

Polymers are easy to prepare for scanning electron microscopy (SEM) largely because they do not contain water. Because they are not conducting, they are usually sputter-coated with Au/Pd.

Staining Methods

Staining methods for TEM studies of polymers are limited, but there are some possibilities. The most used are the following:

- Osmium tetroxide, which stains double bonds and may be used for polymers that contain a double-bond (most rubbers).
- Ruthenium tetroxide is a more powerful oxidization agent and also stains aromatic double-bonds, as in polystyrene (PS).
- Negative staining with uranyl acetate may be used for latex particle sizing.
- Beam sensitivity effects on polymers that allow differentiation of one phase from another.

Size Distributions of Latex Particles

Latex is a stable dispersion of polymer microparticles in water prepared by emulsion polymerization. Latexes are mainly used as binders for coating applications, paints, and adhesives. The particle size and particle size distribution are important parameters that are possible to study in TEM.

To obtain a size distribution of latex particles, one drop of the latex should be dripped onto a Formvar-coated grid, dried, and examined in TEM. The latex is diluted to around 1% at a particle size of 1 μm. Smaller particles demand higher dilution than larger particles to form a monolayer on the Formvar surface. If the latex is too concentrated, several layers are formed and the particles cannot be separated. If the latex is too diluted, too few particles are present to perform a reliable size distribution determination.

If the latex particles have a low glass transition temperature (T_g) and are film-forming, the particles must be stabilized in some way. If the latex does not contain any reactive groups, negative staining may be used, where the background is stained instead of the particles. Adding an inorganic salt to the latex before drying will demarcate the particles during drying, as the salt is accumulated in a ring around the particles. The same method is used for particles that are destroyed by the electron beam. A black ring of the same diameter appears when the particle is evaporated. Examples of latex particles are shown in Figure 1: (a) PS latex particles are seen as uniform round particles without any staining because of the relatively good radiation stability of PS, (b) unstained soft acrylic latex particles without staining that float out during drying and connect with other particles if they are close enough, and (c) negatively stained acrylic latex particles.

Heterogeneous Latex Particles

To study the relation between polymerization condition and morphology of heterogeneous latex particles in TEM, a system of PS/acrylicates was adopted as a model system [2–3]. Polystyrene is stable, whereas acrylics are sensitive and decompose in the electron beam. A blend of these polymers gives a contrast in TEM that is enough to distinguish between the phases.

An example of two-phase latex particles is shown in Figure 2, where the PS shell is very stable in the electron beam and the polymethyl-methacrylate (PMMA) core is very beam-sensitive. The latex was dried and crushed by a spatula. A

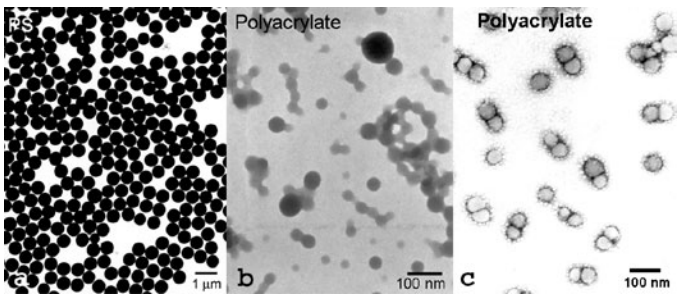

Figure 1: TEM of latex particles. (a) PS latex particles are seen as uniform round particles without any staining because of the relatively good radiation stability of PS. (b) Unstained soft acrylic latex particles without staining float out during drying and connect with other particles if they are close enough. (c) Negatively stained acrylic latex particles.

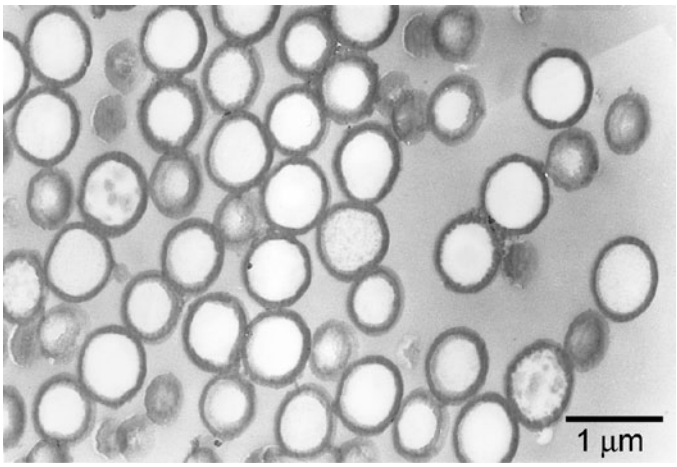

Figure 2: Two-phase latex particles PS/PMMA embedded in epoxy resin and sectioned.

small amount was placed in the tip of a BEEM® capsule that was filled with epoxy resin. Sections were cut in an ultramicrotome. The section thickness was set to 60 nm. Commercial latex particles usually contain several different acrylic polymers that are difficult to distinguish. In addition, the particle size is smaller, and the distribution is much broader than the particles in the present model system.

Polymer Blends

Multiphase polymer blends are difficult to cut into smooth sections because the sample is not even. To obtain the best results when sectioning, the hardness of the sample should be optimized for the knife type and the cutting speed in the microtome. This situation is not possible with multiphase polymers because different polymer phases have different hardness. The fracture passes most easily through the softest polymer. The section thickness varies between sections and also within the same section. This means that not all sections are useful, but some are. The best way is to cut sections and choose the ones that look good in TEM. It is difficult to estimate the condition of the sections in light microscopy (LM).

Sections may be picked up on grids with small openings—600–700 mesh without a support film. Because of the very faint contrast, each small defect in the Formvar film may be visible, and this distorts the image. The preparation steps are illustrated in Figure 3. Images 1 and 2 are from a rubber blend, and images 3–6 are from a latex film resulting from a mix of two latex particle types with different T_g. The rubber blend was stained with OsO_4. The latex film contains one phase that is partly PS, and hence it was possible to stain this phase with RuO_4. Both specimens were cryo-sectioned at a knife temperature of −40°C and a sample temperature of −70°C [1].

An attempt was made to study polymer blend structures in SEM because it is an easier and more available microscope [4]. A common method for studying polymer blends in SEM is to dissolve one of the polymers in a solvent and observe the remaining phase. A solvent must be found that will dissolve one of the phases but not the other. An ethylene-propylene-diene (EPDM)/nitrile rubber (NBR) blend studied in TEM was prepared for SEM. Because both EPDM and NBR are cross-linked rubber, the solution method would not be useful for observing the morphology. Instead the samples were stained

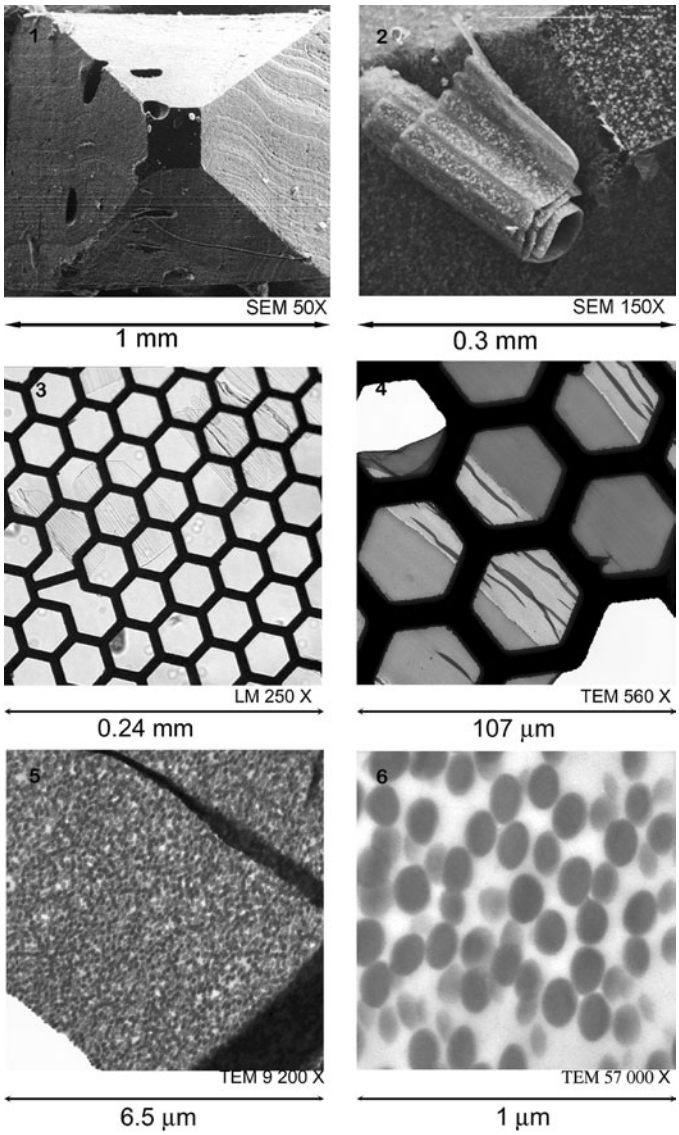

Figure 3: Preparation steps for a polymer sample. Image 1: The sample is trimmed to a pyramidal shape with a razor blade. The tip is further trimmed into a small rectangle with a glass knife in the ultramicrotome. If the sample is stiff it must be trimmed to a very small size (of the order of 0.1 mm) to obtain thin sections in the microtome. Image 2: SEM micrograph of a curled microtomed section on the edge of the cut surface. Image 3: Light microscopy photograph of the grid. The sections are visible, but the contrast is low, and the sections are very faint. Because the sections are small, hexagonal 600-mesh grid was used. Normally no support film was used. Image 4: TEM image at low magnification. The contrast is much higher, and the sections are clearly visible. The thicknesses of the sections vary. The wrinkling occurs because of the elasticity of the sample. Image 5: TEM image at higher magnification. The structure of the sections starts to be resolvable at this magnification. Image 6: TEM image at high magnification. This magnification is needed to make a good assessment of the morphology.

with OsO_4 and examined in backscattered electron (BSE) mode in SEM [3]. To avoid topographic contrast, the surface must be flat. To obtain a smooth surface, the sample was cooled on dry ice and cut with a razor blade. The sample was then placed in OsO_4 vapors for 24 hours. If the samples were sputter-coated with a heavy metal, no contrast from the polymer would be visible in BSE mode. No conductive coating was needed for these polymers. Other polymers needed to be coated with a thin layer of carbon to reduce charging. The SEM accelerating voltage was adjusted

to optimize the image quality. The result is presented in Figure 4. TEM micrographs of unstained and OsO_4-stained sections (Figures 4a and 4b) are compared to SEM micrographs in SE mode and BSE mode (Figures 4c and 4d). All micrographs have the same magnification. The dark areas in the unstained TEM micrograph are carbon black agglomerate. Individual particles may be distinguished at higher magnifications. The NBR phase contains more double bonds than the EPDM phase and therefore becomes dark when stained. When the images are compared, it is obvious that the carbon black is present mostly in the NBR phase. The carbon black particles are visible as bright spots in the SE-mode image because they protrude above the matrix. In the BSE mode, the NBR phase appears brighter than the EPDM phase because it contains a higher amount of osmium (higher atomic number), yielding a higher number of backscattered electrons. The morphology seen in SEM is in accordance with the morphology observed in the TEM. The backscattered SEM image contrast is inverted relative to the stained TEM image. The carbon black distribution is visible in the SE-mode SEM image and in the unstained TEM image. An advantage of SEM over TEM is that larger areas may be studied, for instance a cross section of an extruded object.

Macro-Porous Particles

Figure 5 shows both SEM and TEM micrographs of macro-porous trimethylolpropane trimethacrylate (TRIM) particles that are used for chromatography [1]. The surface morphology is visible in the SEM images. To see inside the porous particle, TEM is necessary. To prepare the TEM specimen, a small number of the particles were placed in a BEEM® capsule, and epoxy resin was added. The sample was sectioned at room temperature (T_g around 100°C). This polymer does not contain any stainable groups. A faint contrast is obtained from the difference in stability to the electron beam between the polymer and the epoxy resin

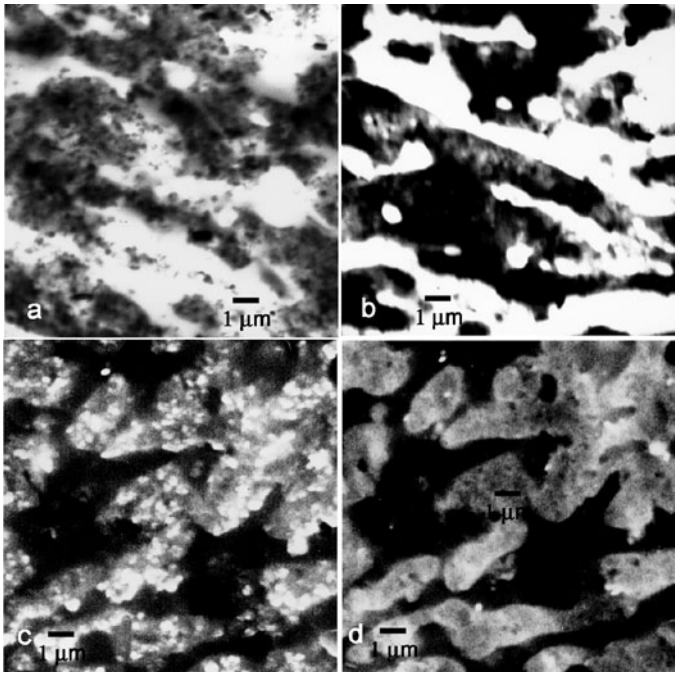

Figure 4: Carbon black filled NBR/EPDM 70/30 as it appears using different microscopy methods. (a) TEM unstained, (b) TEM OsO_4 stained, (c) SEM SE mode, and (d) SEM BSE mode.

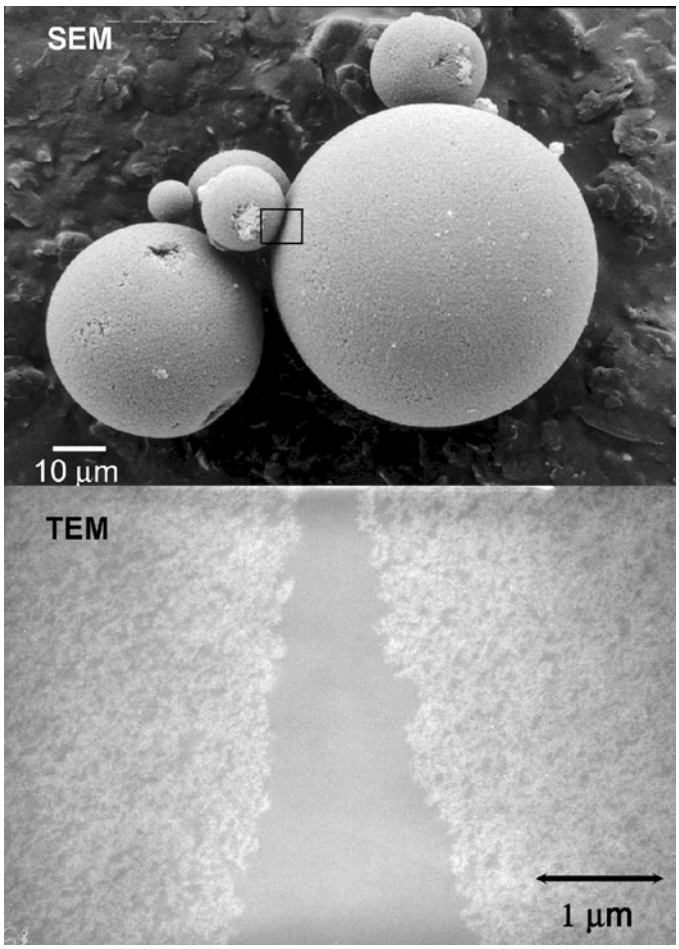

Figure 5: SEM (upper) and TEM (lower) images of macro-porous TRIM particles that are used for chromatography. The TEM micrograph is captured as indicated in the SEM micrograph. The space between the particles and the pores is filled with epoxy resin.

(Figure 5). The polymer appears lighter than the epoxy resin. It is difficult to focus before the section cracks and vanishes. A higher accelerating voltage gives less beam damage but lower contrast. For that reason the highest possible voltage, 100 kV for this instrument, was used. The objective aperture was set to a size smaller than the optimum aperture size required at higher magnifications.

Conclusion

Electron microscopy can be a valuable tool for characterizing polymer morphology. However, because of the lack of double bonds and other reactive groups in many polymers, staining opportunities are limited, and other means of contrast enhancement are needed.

References

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