Improved Sectioning of Polymers Using an Oscillating Diamond Knife for Transmission Electron Microscopy

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Polymers are viscoelastic materials that can often deform during microtome sectioning. Similar to plastic embedded biological materials, many methods have been developed over the years to not only improve the image contrast of these materials but also to harden the material for improved sectioning during microtomy[1]. Even with these improvements, a common artifact, compression, during the sectioning of this class of materials remains problematic.

Compression is caused by several factors: hardness of the sample, embedding media, wedge angle of the knife, interaction between the diamond and sample surface, section thickness and cutting speed. It has been found that reducing the knife angle from 45° to 35° leads to a reduction in compression[2]. Recent efforts to further reduce the compression of ultra-thin sections have led to the invention of an oscillating diamond knife[3].

The newly developed ultra sonic™ oscillating diamond knife from DiATOME Ltd. was used to prepare ultra-thin sections at room temperature for transmission electron microscopy (TEM). The knife has a piezo actuator that when set to the desired frequency and amplitude, produces an oscillation of the knife parallel with the cutting edge. Three classes of multiphase polymers were chosen for this report to demonstrate the advantages of the 35° oscillating diamond knife. High-impact polystyrene (HIPS), a rigid thermoplastic with soft rubber domains, (2) a multi phase soft thermoplastic polyolefin (TPO) and (3) a styrene/acrylate (S/A) latex emulsion polymer,

High impact polystyrene (HIPS) is commonly used in packaging, housewares, toys, appliances and recreational products. Samples were prepared for sectioning by first cutting a small block from a molded sample then trimming that block to shape to form a trapezoid. The trapezoid face is then microtomed polished (*sections are not collected*) to provide a smooth face for staining. The specimen is stained by immersion in 2% aqueous osmium tetroxide overnight at room temperature. The osmium stain will crosslink the polybutadiene rubber particles and make them harder. After staining, the specimens are rinsed in distilled water and thin-sectioned using a DiATOME ultra sonic oscillating diamond knife (turned on/off) with a Leica UC6 microtome. Sections are collected onto 400 mesh copper grids and examined with a JEOL JEM-1230 TEM operating at an accelerating voltage of 120kV and spot size 3. Digital images were acquired with a Gatan Multiscan CCD camera and processed with Adobe Photoshop.

Even though the stain has increased the hardness of the rubber particles, the large denser particles tend not to section well, with the denser particles compressing during sectioning. In some instances, deformation of the rubber particles can occur as well as crazing of the material. Because these particles are added as stress concentrators to increase impact strength, it is natural that most of the compression and crazing occur around them during sectioning. Thin-sections of the HIPS material were cut using a standard knife and the oscillating knife. Significant changes in the length of the sections were observed, with the sections cut with the oscillating knife measuring the size of the sample block face. Figure 1 shows images of the HIPS cut with a standard knife

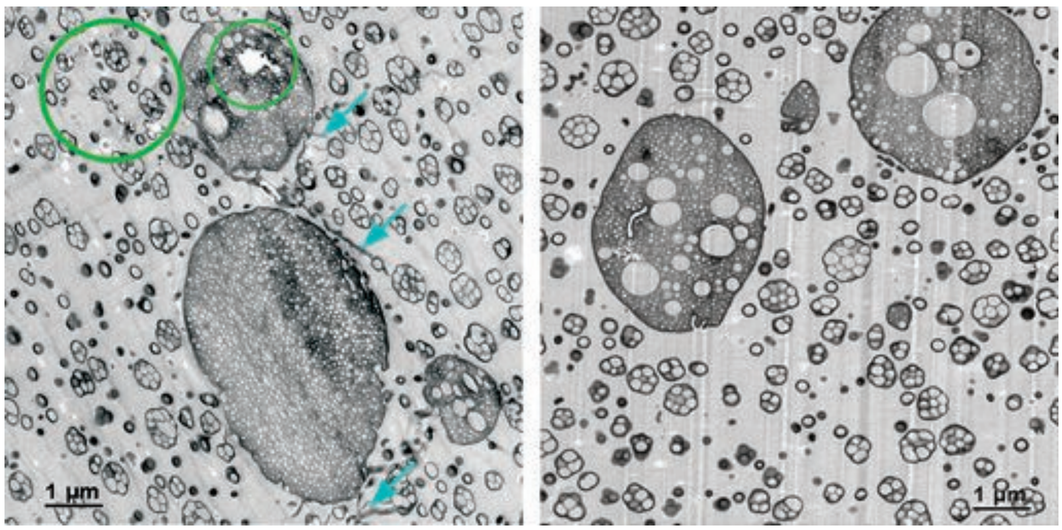

Figure 1. TEM image of HIPS sample cut with a standard diamond knife (left) showing deformation of the rubber particles (circled in green), crazing (blue arrows) and compression. The morphology is better preserved when using the oscillating knife (right).

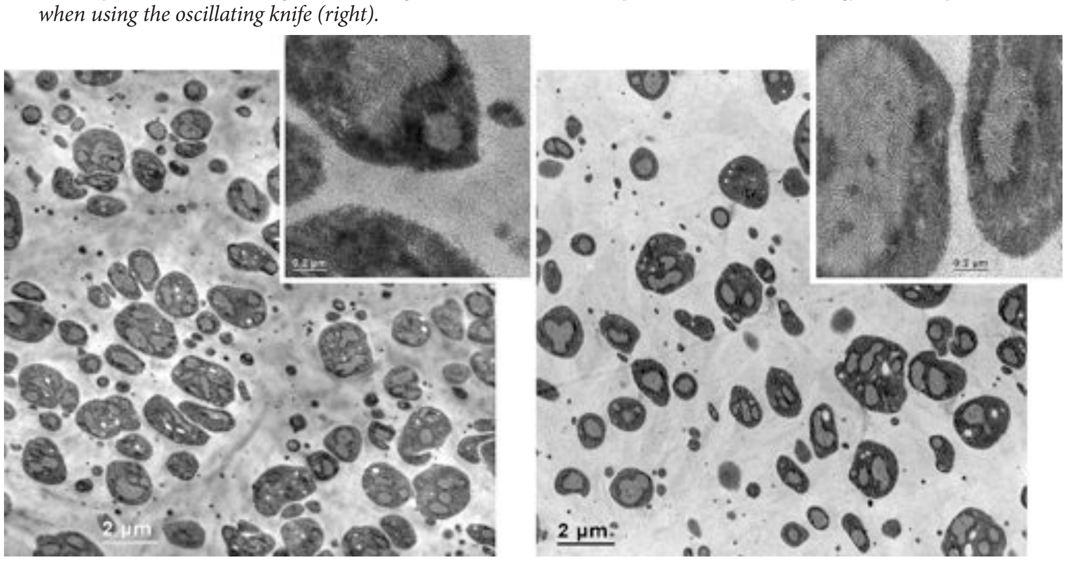

Figure 2. The TPO prepared by polishing the material with the oscillating knife (right) was found to have a similar morphology as the sample prepared by the conventional method (left).

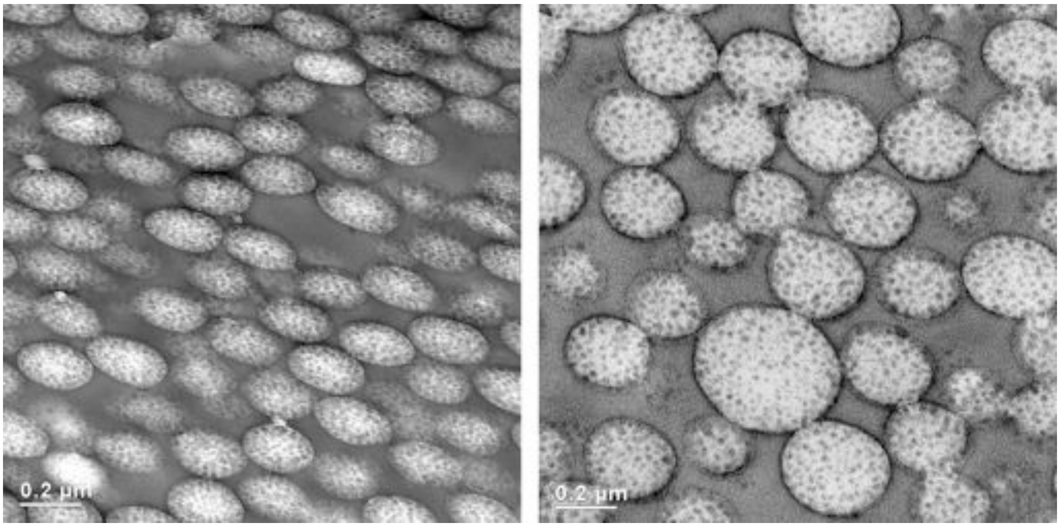

Figure 3. The S/A latex sample on the left was cut with a standard knife and resulted in highly compressed sections. The same block face was then cut with an oscillating knife which resulted in compression free sections and truer round particles.

having compression and deformation at the large particles. When the sections cut with the oscillating knife were examined by TEM, no compression was observed. In fact the particle morphology was much better preserved as the rubber particles were round in shape, as compared to looking ovular in the sections cut with the standard diamond knife.

Thermoplastic olefin materials are usually first polished with a cryo-microtome due to the softness of the material and then vapor stained over ruthenium tetroxide before being thin-sectioned at room temperature[4]. For this analysis the TPO was instead polished at room temperature using the oscillating diamond knife, then stained and sectioned. Figure 2 shows that the oscillating knife was able to provide a smooth polish at room temperature and, upon examination by TEM, shown to preserve the particle morphology better than the TPO prepared by the conventional method. This alternate sample preparation method results in saved set-up time over cryo-microtomy as one doesn't have to wait for an instrument to cool down or multiple samples to get to an equal temperature before polishing. While we were successful in preserving particle morphology with this particular material, it is not known whether all thermoplastic olefins could be prepared this way due to varying percent rubber affecting the hardness of the material.

Emulsion polymers such as a styrene/acrylate latex present their own challenges for microtomy. First off, the dried latex must be embedded into an epoxy that will not react with it. Previous tests conducted found Pacer Industries two part Z-Poxy™ resin to be the best choice for this material[5]. The only drawback to using this epoxy is that it cures softer than most tradition epoxies used in electron microscopy. This was found to be especially true when cast into BEEM capsules. To resolve this, the embedded latex was cured as a thinner film on a sheet of parafilm. Room temperature sectioning, if possible at all, resulted in highly compressed sections (Figure 3). If the sections compress too much then one must cryo-section this embedded material, which also leads to compression and elliptical shaped latex particles. Through scanning electron microscopy (SEM) imaging we know that these S/A particles are round in structure. Again we used the oscillating knife to resolve this problem and were able to get thin-sections with greatly reduced compression. This resulted in us being able to observe the true particle morphology by TEM, as the particles appeared spherical rather than elliptical. This is a very important result to help us better understand the internal structure inside the particle, which is not possible when whole particles are examined in the SEM.

The use of the new DiATOME ultra sonic™ oscillating diamond knife has been found to further reduce and almost eliminate section compression. Thin-sections made with the oscillating knife better preserved the polymer morphology. Not only were particles round, but no deformation or crazing was observed in the material. Other advantages of the knife were the ability to obtain thinner and more uniformly thick sections. In several instances we have also eliminated the need for cryo-microtomy which has saved time in both sample preparation and analysis time. ■

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