The effect of osmium staining on lamellar spacing in thin polystyrene-polyisoprene diblock copolymer films

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Abstract. Thin films of lamellar-structured polystyrene-block-polyisoprene were vapour stained with osmium tetroxide and compared using transmission imaging in an environmental scanning electron microscope to unstained films of the same polymer. Staining was found to swell the affected phase by a factor of 1.15 in-plane, compressing the unstained phase to a factor of 0.71 of its original size as it did so. Additionally, images exhibiting contrast between phases in the unstained sample were successfully taken with conventional transmission electron microscopy, showing that staining is not necessary for imaging such samples.

1. Introduction

Electron microscopy is a routine technique for directly examining the small-scale structure of polymers, but is hampered by the fact that many polymers are chemically similar and electrically insulating. To get around such difficulties, polymer samples are typically prepared by coating with conductive material and by staining one phase of a multi-component material to increase the material contrast - a review of such procedures can be found in Sawyer and Grubb [1].

The development of the environmental scanning electron microscope (ESEM) [2] enabled the imaging of insulating or wet samples in their native state, without any artefacts from sample preparation and without fear of sample charging or other such image distortions. Samples imaged “naked” in the ESEM can look significantly different to those which have undergone preparation for traditional SEM [3] and so it is reasonable to assume that staining, being another preparation method, may cause its own share of artefacts.

This work will examine a polystyrene-polyisoprene diblock copolymer film (block copolymers such as the triblock ABS are common structural materials and other chemistries hold great promise as photonic crystals, for instance) with a lamellar structure and the microscale effects caused by using the common stain osmium tetroxide. No other preparation (eg. sample coating) has been carried out. Imaging of both the untreated and the stained samples was carried out using a new transmission
detector for the ESEM [4], using the low acceleration voltage and hence stronger beam-sample interaction of (E)SEM to provide strong contrast.

2. Experimental Methods

The block copolymer used in this work was supplied by Dr. Patrick Fairclough and his group from the Department of Chemistry, University of Sheffield. It consisted of equal volume fractions polystyrene (PS, C₈H₈) and polyisoprene (PI, C₅H₈), which should result in a lamellar microstructure when annealed [5]. It has Mₘ = 578,000 g mol⁻¹, Mₙ = 426,000 g mol⁻¹ and a polydispersity of 1.38.

90nm thick films of block copolymer were prepared by spin-coating a solution in toluene on to glass slides, which were then vapour annealed [6] for 16.5h to bring out the lamellar structure. Annealed films were then attached to copper TEM grids, some of which were stained by exposure to osmium tetroxide vapour for one hour.

The film-coated grids were then examined in an XL30 field-emission environmental scanning electron microscope (ESEM), produced by FEI Company, Hillsboro, Oregon, US, using a new accessory enabling transmission imaging to be conducted in the ESEM on samples which can be temperature-controlled by a Peltier element. 1 Torr (133 Pa) of water vapour was required to eliminate sample charging, but temperature control was not necessary. Such apparatus has been used by Bogner et al previously [4] on hydrated samples. This procedure will be referred to as ESEM-STEM.

Additionally, films were examined in a Tecnai 20 TEM (FEI Company, Hillsboro, Oregon, US) to compare the image quality of stained and unstained samples using conventional TEM. The accelerating voltage used was 120kV. Charging of the sample had only a minimal effect when imaging with the TEM.

3. Results and Analysis

![Figure 1. Dark-field ESEM-STEM images of both unstained (left) and stained (right) annealed PS-block-PI. The lines drawn at the top of each image are examples of where traces are taken for analysis. A clear difference in morphology can be seen between the two images.](image)

Multiple images, each of a different part of the sample, were taken with ESEM-STEM in dark-field mode of stained and unstained samples, examples of which are shown in figure 1. Strong contrast can be seen from the unstained sample and as expected, the thicknesses of the two types of lamellae are essentially equal as judged by eye. To make the analysis more quantitative, line traces perpendicular to stacks of lamellae for each image were taken with the ImageJ software [7]

A custom-written C++ program using the FFTW library [8] was used to perform a discrete cosine transform on each line trace and select out the positions of either the largest peak in the power spectrum for the unstained sample (for which there was only one periodicity) or the two largest peaks
in the case of the stained sample, rejecting any wavelengths over 300nm to reduce errors due to uneven film thickness causing intensity fluctuations. The most significant components in the power spectrum should correspond to the overall envelope of the lamellar shape and so a sample with two separate lamellar sizes should exhibit two large peaks - one each for the characteristic dimension of the larger and smaller spacings.

![Figure 2. Examples of line traces from annealed PS-block-PI images (left), along with their discrete cosine transforms (DCT, right). Data from unstained images is shown by a red continuous line, while data from stained samples is shown by a blue dashed line. A third harmonic Examples of such line traces, along with the power spectrum of their discrete cosine transforms, are shown in figure 2. The unstained sample has only one main peak in its power spectrum, while the stained sample has multiple peaks. The overall results are in table 1, along with an attempt to extract the same data directly from line traces.](image)

**Table 1.** Lamellar spacings obtained from the discrete cosine transform of line trace data for unstained and stained samples (single distance for unstained, lower and upper distances for stained) as well as results obtained by attempting to obtain the peak and trough widths from direct analysis of the line traces (peak and trough widths). Due to the uneven shape of line traces, attempting to extract information directly from them will not be particularly accurate – this data is included for completeness only and is more to indicate which phase has swollen.

| Sample     | Single Distance | Lower Distance | Upper Distance | Trough Width | Peak Width  |
|------------|-----------------|----------------|----------------|--------------|-------------|
| Unstained  | (113±6) nm      | (110±23) nm    | (114±27) nm    |              |             |
| Stained    | (81±26) nm      | (130±19) nm    | (98±21) nm     | (131±26) nm  |             |

Staining the PS-block-PI film with OsO₄ causes the stained phase to swell by a factor of approximately 1.15, compressing the other phase to a scale of approximately 0.71 of its previous size as it does so. A visual inspection of the images (figure 1) suggests a similar conclusion – the stained phase (which would be the brighter component when viewed with dark-field STEM) clearly appears larger than the other, whereas the two were comparable before staining.
Having demonstrated that staining runs the risk of altering samples such as these, we return to examine its necessity in standard TEM. Such work has been previously done by Handlin and Thomas [9], who defocussed the electron beam to enhance phase contrast within the sample. As figure 3 shows, phase contrast TEM is a viable technique for indicating the morphology of unstained polystyrene-block-polyisoprene samples. This should open up new studies via (S)TEM - rather than the more recent trend towards AFM - without the dangers of swelling artefacts highlighted here.

4. Conclusion
We have investigated the effect that vapour staining of osmium tetroxide has on the characteristic dimensions of lamellae in thin polystyrene-block-polyisoprene films. Incorporation of a heavy metal stain into the polyisoprene component of the polymer causes it to swell. Hence staining is not recommended if precise measurements are required of polymer samples on the microscale or below. Also, we demonstrate that staining is not required for imaging samples even as closely related as polystyrene ([C₈H₈]ₙ) and polyisoprene([C₅H₈]ₙ) when imaged at low energies (STEM at 20kV in the ESEM) or with a slight loss of contrast at high energies (TEM at 120kV).

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