Ruthenium Tetroxide Staining of Polymers

Introduction

Transmission electron microscopy can yield excellent structural detail of polymers in ultrathin sections, but only if the polymer is stained with a heavy electron dense metal. Ruthenium tetroxide is a widely used stain for this purpose. Compared to osmium tetroxide also commonly used for polymer staining, RuO_4 is a more powerful oxidizing agent and stains more polymer types. RuO_4 is also less volatile and toxic than OsO_4. Small samples may be either stained ***en bloc*** with a liquid ruthenium tetroxide solution, usually 1 %, or ultrathin sections may be exposed to vapors of ruthenium tetroxide. This documents shows the results of staining polymers such as polyethylene (PE), ultra-high molecular weight polyethylene (UHMWPE), and polyamide blends, either stained ***en bloc*** with a solution of RuO_4 or by exposing to vapors of RuO_4. In the examples here, ultrathin sections were cut on an RMC PowerTome PCZ ultramicrotome equipped with an LN Ultra cryosectioning attachment.

Figure 1.
The RMC Boeckeler PowerTome equipped with the LN Ultra cryosectioning attachment.

Instrumentation

The RMC Boeckeler PowerTome PCZ (PT PCZ) was used to section a specimen embedded in a hard epoxy resin. This PT PCZ ultramicrotome is equipped with a touchscreen PC and a trinocular microscope with high resolution camera. Furthermore, this PowerTome PCZ is equipped with an LN Ultra cryosectioning attachment. The PowerTome as well as the LN Ultra can be controlled and programmed by the included touchscreen PC and/or the ultramicrotome controller. The camera allows several people to visualize the process of sample sectioning. The camera can also capture images and HD video to be used in reporting, documentation, and training. Furthermore, the built-in measuring tool enables measurement of blockface and sample dimensions by using the image displayed on the touchscreen.

Procedure

The following procedures are routinely used at the Fraunhofer Institute for Microstructure of Materials and Systems IMWS/IWM, Halle, Germany. They were kindly provided by Dr. Sven Henning.

Preparation and handling of RuO_4 solution must be performed by qualified personnel only! Use a fume hood suitable for RuO_4/OsO_4 handling! These chemicals are toxic and corrosive. RuO_4 must not come into contact with grease, paper, alcohol, or other organics, as it will explosively oxidize them!

Vapor Staining of ultrathin sections with Ruthenium Tetroxide (RuO_4)

Chemicals: Ruthenium(III) chloride, sodium hypochlorite (5 % solution in water), $NaHSO_3$ (sodium bisulfite)

Lab glassware: Desiccator, sample vials (e.g. 10 mL) with PE snap cap, Pasteur pipettes, small beakers, double-sided adhesive tape, glass slides or stiff polymer foil.

Preparation of staining agent: Add a small amount (spatula tip) of ruthenium(III) chloride to approx. 2 mL of the sodium hypochlorite (5 % solution in water).
Use a sample vial with PE snap cap.

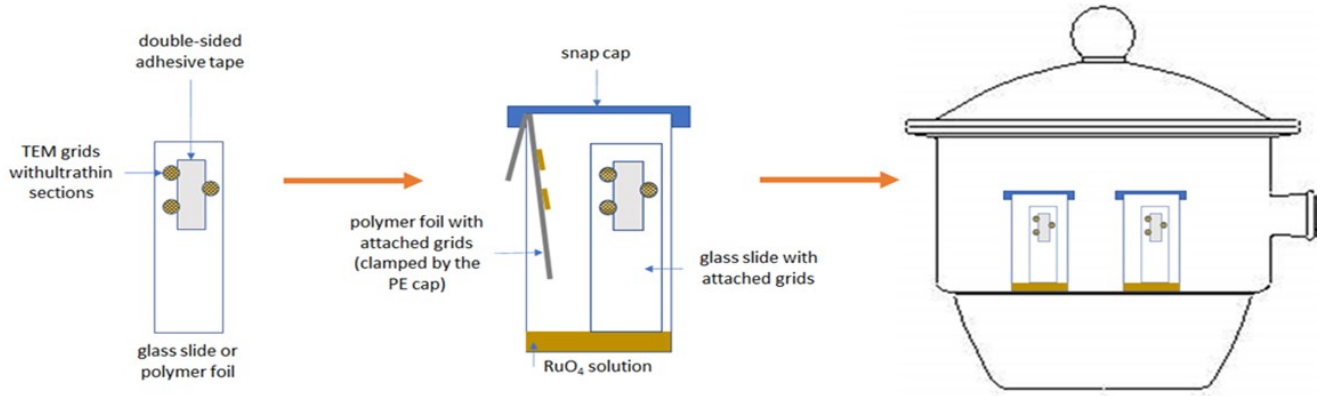

Figure 2. Setup for vapor staining of grids.

Time: 10 min. to 1 hour, depending on sample (determined empirically).
Application: ASA (acrylonitrile styrene acrylate), polyhydroxyalkanoates (PHB, PHV etc.), polyolefins, a variety of polymer blends and block copolymers.
Comments: Use carbon coated or uncoated TEM grids (formvar-coated grids will be stained).
Longer exposure times may lead to corrosion of grids. Gold grids may be necessary.
Deposition of staining agent and/or condensation on grids/sections can be avoided by reducing the amount of staining agent (lowering the liquid level in the vial).
Use the plastic film sheets where conductive carbon-pads for SEM are attached.
Use the adhesive tape as an indicator: when it turns black, staining is completed.

En Bloc Staining by Ruthenium Tetroxide

En bloc staining refers to staining the specimen block which has already been trimmed, but not yet sectioned. The ruthenium tetroxide penetrates several micrometers into the polymer specimen. This outer layer is then sectioned with a cryoultramicrotome.

Chemicals: Ruthenium(III) chloride, sodium hypochlorite (5 % solution in water).

Lab glassware: Desiccator, sample vials (e.g. 10 mL) with PE snap cap, Pasteur pipettes, small beakers.

Preparation of staining agent: Add a small amount (spatula tip) of ruthenium(III) chloride to 2 mL of the sodium hypochlorite (5 % solution in water).
Use a sample vial with PE snap cap.

Neutralization: Collect leftover and/or used ruthenium tetroxide solution in a sealable container; disposal by authorized companies only.
Immerse used glassware and tools in neutralization agent overnight before regular cleaning.

Preparation and handling of RuO_4 solution must be performed by qualified personnel only!
Use a fume hood suitable for RuO_4/OsO_4 handling! These chemicals are toxic and corrosive.

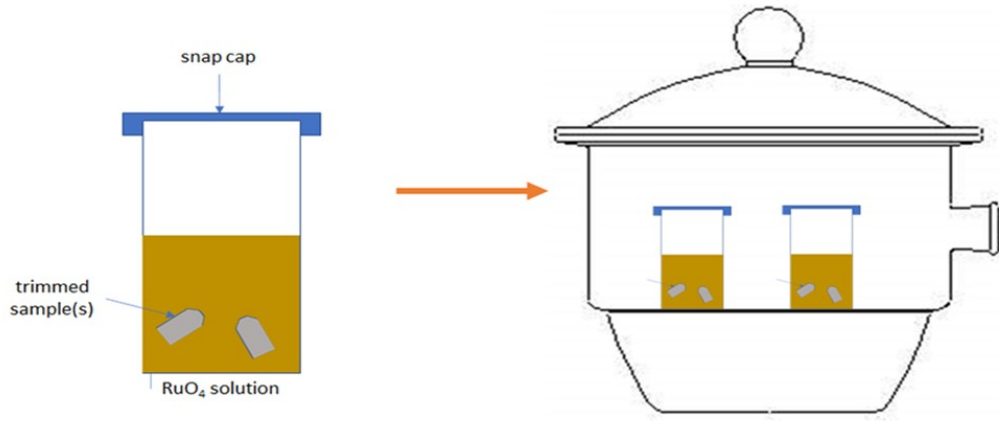

Figure 3. Setup for *En Bloc* Staining of Sample Blocks.

Time: 20 min. to 2 hours, depending on sample (determined empirically).
Application: Polypropylene, polyethylene, PTFE/ETFE, polyolefin films, heterophasic polyolefins, a variety of polymer blends.
Comments: Longer immersion times may lead to sample degradation.
The color of the staining agent should be clear red/brown. If it turns dark brown to black, it is spent.
Use fresh RuO_4 solution if longer staining times are needed.

Results

The micrographs displayed below were kindly provided by Dr. Sven Henning of the Fraunhofer Institute for Microstructure of Materials and Systems IMWS/IWM, Halle, Germany.

Vapor Staining

Vapor staining is easier to control and gives cleaner results than *en bloc* staining. It is used primarily for sections already on grids. But the grids should be carbon coated or gold grids should be used, as the RuO_4 will oxidize copper grids.

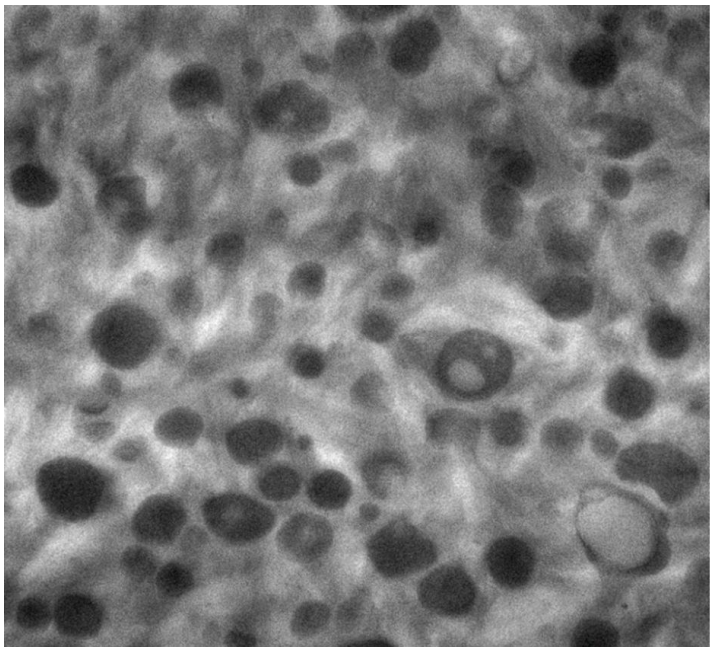

Figure 4. A Polyamide Blend
Polyamide + polyolefin modifier Specimen was cryoultramicrotomed at –80 ° C Then grids were post-stained with RuO_4 vapor.

ASA modifier particles are clearly visible in the polyamide matrix. Image contrast is improved by the application of a DCE (differential contrast enhancement) routine.

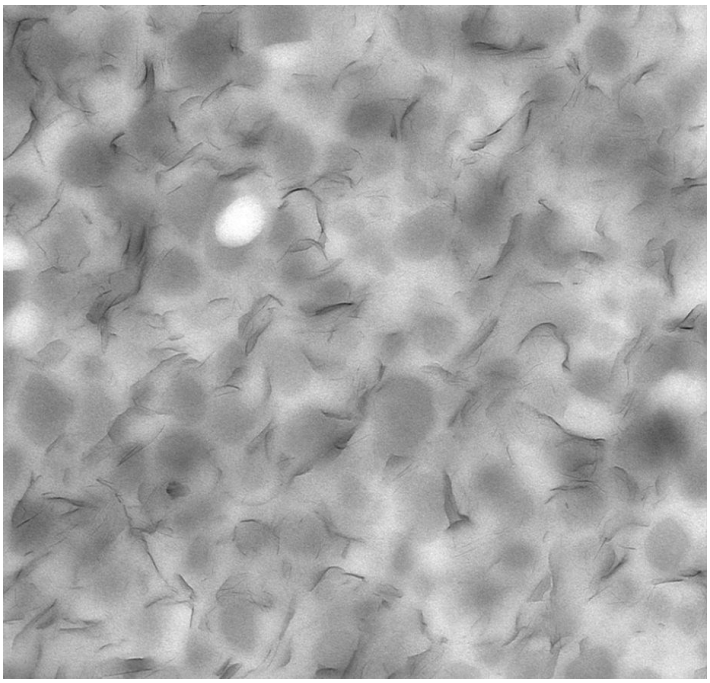

Figure 5. A Polyamide/ASA blend nanocomposite Polyamide + polyolefin modifier + layered silicate Specimen was cryoultramicrotomed at –80 ° C Ultrathin sections were post-stained in RuO_4 vapor. TEM: LEO 912, 120 kV.

ASA modifier particles are visible in the polyamide matrix. Exfoliate layered silicate nanofiller is readily identified.

En Bloc Staining

The *en bloc* staining technique stains stronger than vapor staining, but may degrade the specimen. It has the advantage that the polymers become cross-linked and may section better. Figures 6 and 7 below display excellent results with this technique.

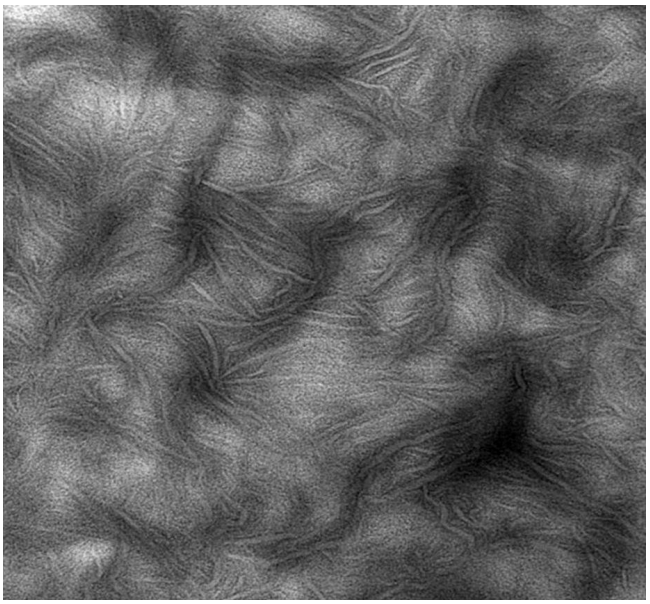

Figure 6. Ultrahigh Molecular Weight Polyethylene (UHMWPE)
Material: GUR 4120 (TICONA) ***en bloc*** staining with RuO_4 for 1 hour.
Specimen was then cryoultramicrotomed at –120 ° C.
TEM: LEO 912 120 kV.

Important properties of UHMWPE are controlled by the semi-crystalline morphology (arrangement of lamellae and the thickness of the amorphous and crystalline domains).

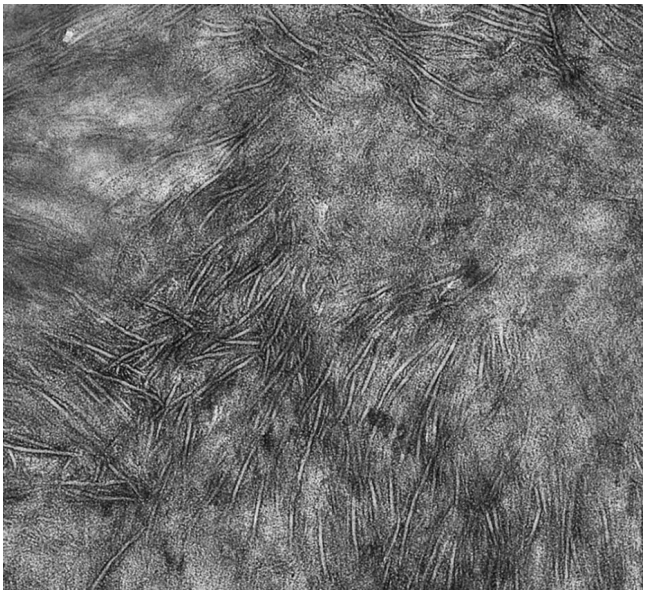

Figure 7. Polyethylene (PE)
Stained ***en bloc*** with a RuO_4 solution for 2 hours.
Specimen was then cryoultramicrotomed at –120 ° C.
TEM: LEO 912, 120kV.

Important properties of PE are controlled by the semi-crystalline morphology (arrangement of lamellae, thickness of the amorphous and crystalline domains).

Because ruthenium tetroxide can stain a greater number of polymer functional groups than osmium tetroxide, it should be considered as an alternative stain. It can often yield excellent results and reveal additional structural information.

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