Vapor Coating: A Simple, Economical Procedure for Preparing Difficult Specimens for Scanning Electron Microscopy

E. Ann Ellis and Michael W. Pendleton
Texas A&M University, College Station, TX
eann.ellis@wortldnet.att.net

The Microscopy and Imaging Center at Texas A&M University is a multi-user facility involved with preparation and analysis of many different biological and materials sciences projects. Vapor stabilization and coating is an important part of our specimen preparation methodology for difficult biological and materials, especially polymer, samples. The procedure for all our vapor preparation techniques is done in a simple, economical apparatus set up in a properly functioning fume hood with a flow rate of at least 100 ft/min (Fig. 1). The apparatus is made from a glass petri dish or a glass petri dish for the bottom and an appropriate size beaker for the top. Specimens, mounted on stubs, are placed inside the chamber and the fixative (osmium tetroxide, ruthenium tetroxide or acrolein) is placed in a small container (plastic bottle cap) near the specimens.

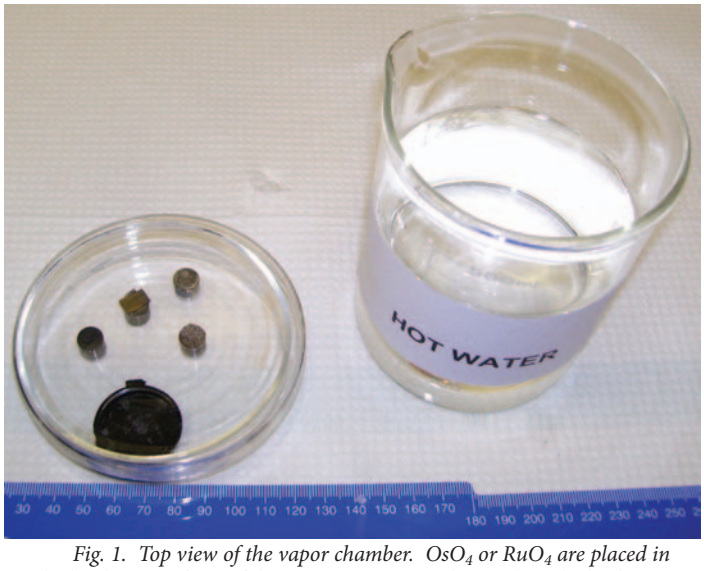

Fig. 1. Top view of the vapor chamber. OsO_4 or RuO_4 are placed in the bottle cap in front of the specimens mounted on stubs. The beaker on the right contains hot water which can be placed on top of the chamber.

Vapor fixation/coating is a forgotten methodology which still lends itself to many difficult specimens. Osmium tetroxide vapor was used in 1884[1] for fixation in studies of spermatogenesis in arthropods and in 1945 Keith Porter, Albert Claude and Ernest Fullam employed osmium vapors to fix tissue culture cells for transmission electron microscopy.[2] Since 1945 techniques for the use of osmium vapors have been passed around amoung electron microscopy laboratories by personal communication among people working on difficult specimens. A number of people realized that vapor fixation with osmium tetroxide allowed for deposition of a conductive, heavy metal in areas where it is difficult to reach with sputter coating or vacuum evaporation of conductive metals.

Our procedure for osmium tetroxide vapor treatment consists of placing specimens mounted on stubs in the vapor chamber around the periphery of a plastic bottle cap containing 1-2 ml of 4% aqueous osmium tetroxide (OsO_4). A beaker of hot water is placed on top of the vapor chamber to speed up the reaction. Some labs use OsO_4 crystals (0.25-1 g) instead of the aqueous solution. Use of anhydrous OsO_4 crystals is more expensive than the aqueous solution; however, the crystals can be used repeatedly for up to a week. Recently we exposed metal nano-particles coated with a fatty acid to 4% aqueous OsO_4 for 15 minutes and then sputter coated the specimen with 4 nm of platinum before examination in the field emission SEM. This resulted in a very stable specimen without instability because of the fatty acid coating.

Ruthenium tetroxide has been used in staining polymers for both SEM and TEM and the protocol used in our lab is based on the technique outlined by Brown and Butler.[3] Stubs with SEM specimens mounted on them are placed in the vapor chamber. Ruthenium tetroxide staining solution is prepared *in situ* by adding 1 ml of 10% (wt/vol) sodium hypochlorite to 0.02 g of ruthenium chloride ($RuCl_3 \cdot 3H_2O$) in a small plastic bottle cap in the chamber. [Both ruthenium chloride and sodium hypochlorite are purchased from Sigma-Aldrich, St.Louis, MO.] The reaction is rapid and results can be seen in less than 5 minutes. For large or compact samples, placing a beaker of hot water on top of the vapor chamber expedites the vapor coating. We often use this procedure on unstable polymers, such as elastomers, and then lightly sputter coat the specimens after the vapor treatment. Figure 2 shows a piece of packing foam that was exposed to ruthenium vapors only while Figure 3 shows a similar sample that was sputter coated only. There is greater depth of detail in the ruthenium treated specimen.

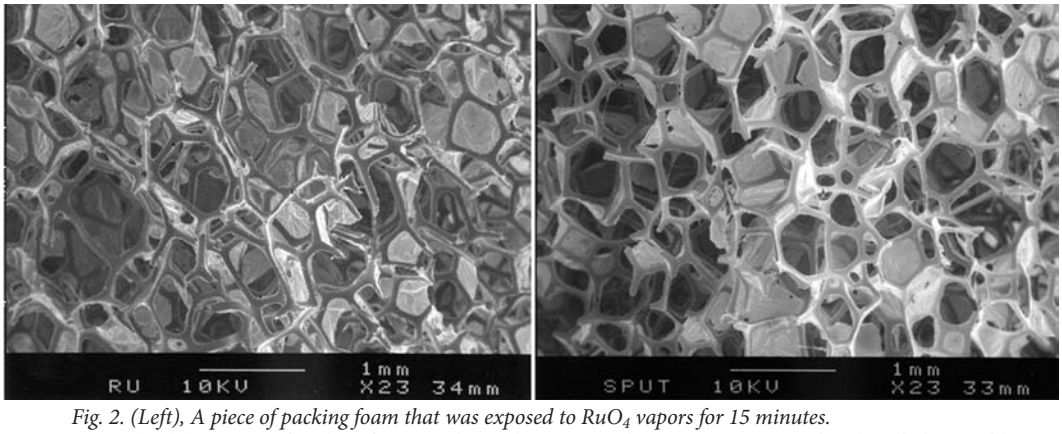

Fig. 2. (Left), A piece of packing foam that was exposed to RuO_4 vapors for 15 minutes.
Fig. 3. (Right), A piece of packing foam similar to that in Fig.2 that was sputter coated with palladium gold.

We use vapor stabilization techniques with biological as well as materials samples and find that the use of vapor stabilization followed by light sputter coating is often the only way that we can prepare difficult polymer samples for SEM and TEM. ■

References

1. G. Gilson. La Cellule 1(1884)96.
2. K. R. Porter et al. J. Exp. Med. 81(1945)233.
3. G. M. Brown and J. H. Butler. Polymer 38(1997)3937.

https://doi.org/10.1017/S1551929500055553 Published online by Cambridge University Press