

Microscopy101

No More Epon 812: This Product Does Not Exist Today

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The Problem

Several years ago I saw an interesting poster on the ultrastructure of a semi-difficult specimen and asked the student about the specimen preparation, which was done very nicely. She explained the fixation and staining very well; but she could not tell me what embedding medium she used. After some help from her advisor, I was told that they used an Epon 812 substitute, which was the same as Luft's Epon [1]. That did not answer my question because there are products being sold as Epon 812 substitutes that are not the same chemistry as the Epon 812 recommended by Luft, and the product marketed as Epon 812 has not been manufactured for thirty years. It turns out they were using a substitute of different chemistry that resulted in a better embedding medium for their specimen. Epon is a brand name for epoxy resins produced by Shell Chemical Company, and Epon 812 was a brand name for tri-glycidyl ether of glycerol [2, 3]. Epon 812 was introduced as an epoxide for electron microscopy embedding formulations by Kushida [4] in 1959 followed by Finck [5] (1960) and Luft [1] (1961). To some people, Luft's Epon 812 became the "holy grail" of epoxy resin embedding media for biological specimens. They thought that they could only use Epon 812 as the epoxy resin for their work: to them, nothing else would give the same results (Figure 1). The resin as manufactured was an aliphatic resin mixture of di- and tri-glycidyl ethers of glycerol. The original resin had a WPE (weight per epoxide

equivalent) of 140–160 [3]. Over time there was a gradual drift upward in the WPE to 140–170, and there were wide ranges in the values for viscosity [2]. The last bottle of the Shell product in the author's lab dated to the 1980s and had a WPE of 196. These changes led to concerns about changes in anhydride:epoxide ratios and overall resin consistency from batch to batch. However, it was very easy to recalculate formulations based on the proper anhydride:epoxide ratio for each new bottle of Epon 812.

Substitute Resins

When Shell stopped producing Epon 812 in 1984, a number of electron microscopy vendors started to contract with other resin production companies. Some vendors substituted other low-viscosity resins (designated as Epon 812 replacements). Other vendors procured tri-glycidyl ether of glycerol and sold it as their brand name with the numbers 812 in the product name: Poly/Sciences Inc., Poly/Bed 812: Ladd Research Industries, LX-112; and Ted Pella Inc., Eponate 12. The resulting brand-name products listed above were, and continue to be, better quality sources of tri-glycidyl ether of glycerol and can be substituted directly in the old formulations for Luft's Epon or Mollenhauer's Epon-Araldite formulation. Labs that have used appropriate WPE and anhydride:epoxide ratios found that they had better quality embedding mixtures with lower viscosities, which improved their overall embedding and sectioning.

Designations for Substitutes

For a while, many labs would list the products they used by specific brand names in publications, and other people were then able to reproduce their work. People could go from one lab to another and reproduce their work because they had kept records of the brands of epoxide that they used for the tri-glycidyl ether of glycerol component and their calculations. All too often now, there is only a comment in the "Materials and Methods" section that the authors used an Epon 812 substitute or simply that they used Epon without a brand name and no indication of the chemistry. Thus, the component was not really a tri-glycidyl ether of glycerol. When people go to other labs or purchase supplies for a new lab, they may or may not know what to do to reproduce the previous work they did. They should not be wasting weeks or months to reproduce the previous work that they had

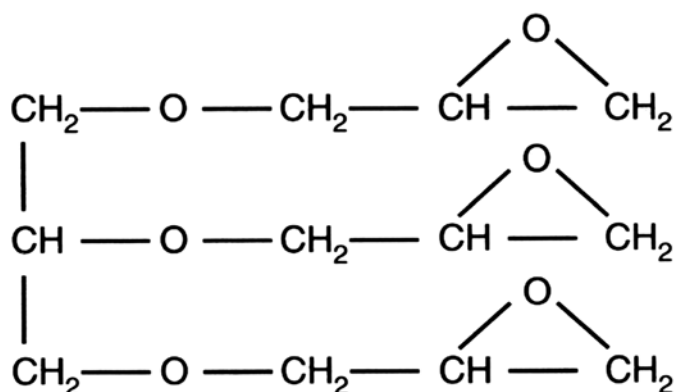


Figure 1: Tri-glycidyl ether of glycerol. From Glauert and Lewis, 1998 [2].

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done simply because they do not know the proper chemicals required for embedding.

There are plenty of substitutes or similar chemicals available from a number of vendors. However, I want consistency in my epoxy resin embedding media, and I want my students to be able to reproduce their work also. When you buy a brand name product, you need to know that you are getting the same chemical every time. You need to check the MSDS sheet, which tells you the proper chemical name (poly-glycidyl ether of glycerol (note that older publications use the designation tri-glycidyl ether of glycerol) and the CAS number, which is 90529077-4. The modern correct name is 1, 2, 3-propanetriol glycidyl ether. If the vendor cannot supply the proper chemical constituents of the resin and the appropriate CAS number, go to another supplier who has this information and product.

I do not have any Epon 812 substitutes in my lab; but I do have LX-112, Eponate 12, and Poly/Bed 812. All three of these epoxides are mixtures of tri-glycidyl and di-glycidyl ethers of glycerol, which they now list as poly-glycidyl ethers of glycerol with the CAS number #90529-77-4. We use several different epoxy resins in my lab, and my students do not tell me they use "Epon substitutes." They tell me what brand name they are using or need—there is no confusion. They also know not to substitute resin chemistry indiscriminately.

Conclusion

Now we must teach students and technologists to be accurate and consistent in communicating their protocols. Accurate communication of resin, embedding products, and their protocols is just as important as fixation and other protocols. There is no longer any Epon 812; but we still have tri-glycidyl ether of glycerol (polyglycidyl ether of glycerol) marketed under various brand names. The brand names that substitute for the Shell product are better than the original Epon 812 in that these products have lower viscosity and consistent numbers for the WPE.

Now is the time to clean up what we tell our students and technologists about epoxy resins so that we can have consistent quality in our embeddings. After all, it has been thirty years since Shell since produced any tri-glycidyl ether of glycerol (Epon 812).

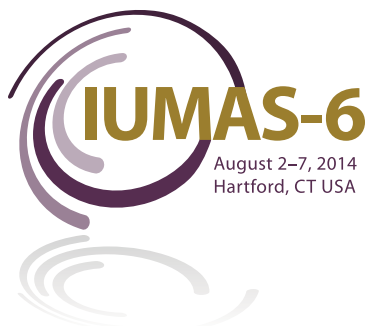
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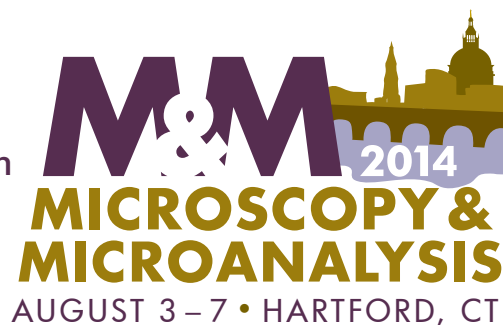
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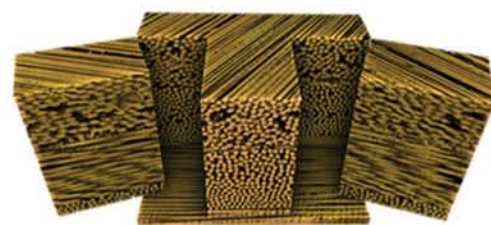


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