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A Simplified Method for Formulation of Epoxy Resin Embedding Media

E. Ann Ellis
Texas A&M University, College Station, TX
eann.ellis@worldnet.att.net

In a recent paper on the revised formulation of Spurr low viscosity embedding medium with ERL 4221[1] the importance of maintaining an appropriate anhydride:epoxide (A:E) ratio was discussed. By understanding a few simple concepts about epoxy resin formulations and setting up a formulation table it is possible to create new resin mixtures with good sectioning properties and other desirable properties such as decreased viscosity and increased beam stability.

Before starting a formulation you need to know the molecular weight of the anhydride and the WPE (weight per epoxide equivalent)[2] of the epoxy resin component. The molecular weights and WPEs are usually printed on the bottle or can be obtained from the vendor. An A:E ratio of 0.7:1.0 -1.0:1.0 is used for most biological specimens. Increasing the A:E ratio results in a harder block; decreasing the A:E ratio results in a softer block. Table 1 shows a basic formulation spreadsheet where the molecular weights of the anhydrides and the WPEs of the epoxy resin components can be entered. The A:E ratio is entered under the anhydride for the molar ratio and the molar ratios of the epoxy components are entered under the epoxy components. The calculations are done as shown in each column and row.

TABLE 1: Formulation Table for Epoxy Resin Mixtures

COMPONENT	DDSA	Araldite 502	Quetol 651
Mol. Wt. or WPE[A]	266	225[A]	87[A]
A:E Ratio[B] or Molar Ratio[C]	1.0[B]	0.5[C]	0.5[C]
Proportion by Wt.	266×1.0 = 266	225×0.5 = 112.5	87×0.5 = 43.5
Total Wt.	266 + 112.5 + 43.5 = 422		
% by Wt.	266/422 = 63.0%	112.5/422 =26.7%	43.5/422 = 10.3%
Formulation in Grams	6.3 g	2.67 g	1.03 g

The molar ratio is not the same as the A:E ratio. The molar ratio involves the relation of two components in the same class, either anhydrides or epoxides. For example, the total ratio of A:E is 1.0:1.0; since there are two epoxy components involved to give 1.0 for the epoxide component of the ratio, the molar ratios for Araldite and Quetol can be any combination that gives a total of 1.0 (0.4 for Araldite and 0.6 for Quetol or 0.5 and 0.5 for each). When using two anhydrides, the same principle applies as explained for the epoxide components. The formulation method is based on percentage by weight so that any amount of resin mixture can be made by multiplying the percentage by weight for each component by the total weight of resin mixture.

Formulations of epoxy resin mixtures were necessary when Luft's Epon[3] was introduced since the WPE of Epon 812 varied greatly from batch to batch. Now that the WPE's of most epoxides are fairly consistent, it is not necessary to calculate new formulations that often. I use the formulation method to vary the viscosity or hardness of a mixture for a particular type of specimen. In the formulation example given above, I used Quetol 651, a low viscosity, straight chain epoxy resin, to lower the viscosity of an Epon-Araldite mixture and maintain the good sectioning properties of a firm to hard block.[4] The table below gives the viscosities of the standard formulations and those of new formulations with Quetol 651 to reduce the viscosity of the working formulation. I normally use benzyldimethylamine (BDMA) as the accelerator for all epoxy resin mixtures at the rate of 0.20 ml/10 grams of resin mixture.

TABLE 2: Viscosity of Different Resin Formulations

RESIN FORMULATION	VISCOSITY IN CENTIPOISE
EPON 812-ARALDITE 502	2500
QUETOL 651-ARALDITE 502	800
LUFT'S EPON 812	550
LX-112 LUFT'S FORMULA	340
QUETOL 651-LX-112	250
ERL 4221 SPURR	180
QUETOL 651-ERL 4221 SPURR	100
VCD SPURR	60

The formulations in Table 2 were made on the basis of an A:E ratio of 1.0:1.0 and a molar ratio for each epoxides of 0.5 for the Araldite containing formulations. The Luft's Epon[3] formulation used an A:E ratio of 0.7:1.0 with the molar ratio of nadic methyl anhydride equal to 0.35 and the molar ratio of dodecenyl succinic anhydride equal to 0.35. The combined molar ratios of the two anhydrides equal 0.7 [0.35 +0.35 = 0.70]. The Spurr resin[5] based formulations used an A:E ratio of 0.88:1.0 as given in the original Spurr publication.

The significance of maintaining an appropriate A:E ratio for a given embedding formulation[2] has not been appreciated in recent years. The formulation originally proposed by Luft using dodecenyl succinic anhydride, nadic methyl anhydride and Epon 812 had an A:E ratio of 0.7:1.0; the Spurr low viscosity formulation had a ratio of 0.88:1.0. Experience over the past thirty years in my lab has shown that an A:E ratio of 0.9-1.0:1.0 gives a good quality block when using Epon-Araldite combinations. In cases where I use an Epon 812 substitute in Luft's formulation, I have found that raising the A:E ratio to 0.9 :1.0 generally results in a block with better sectioning quality.

Audrey Glauert has reported in her publications over the years and I have observed likewise that the benzene ring structure of Araldite based formulations and the cyclic structures of ERL 4206 and ERL 4221 give better beam stability than those of the straight chain Epon substitutes. I often use formulation methods and combinations of cyclic and/or benzene ring based epoxides with the straight chain Quetol 651 to produce resin mixtures with lower viscosity and better beam stability. Generally, I use only one anhydride, either DDSA or NSA, for a given mixture since anhydrides absorb water more readily than the other resin components. A single source of anhydride makes it easier to identify a wet component if such should happen. As in the case of all resin mixtures, I prepare a small batch of resin and polymerize it to check the overall properties before I commit to infiltrating and embedding specimens. ■

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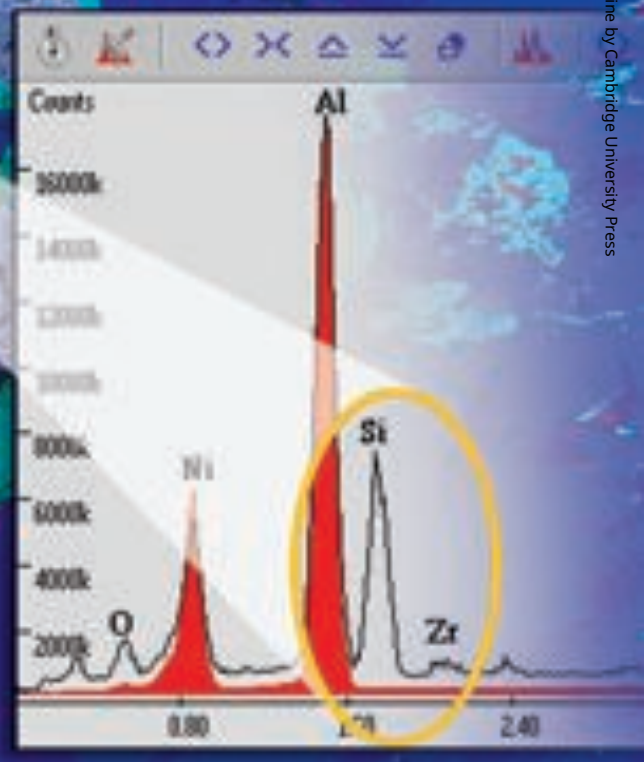

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