SPI-Chem Araldite 6005 Kit
USE INSTRUCTIONS

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Introduction:
"Araldite", a member of a family of thermosetting epoxy resins was first introduced to the electron microscopy field by Glauert and Glauert1 (1958) who found that the resin had excellent properties as an embedding medium for electron microscopy. The epoxy resins, in general, demonstrate low shrinkage (1 or 2%) during polymerization, they are stable during exposure to the electron beam and are insensitive to oxygen and water.

Embedded specimens are therefore relatively free from polymerization damage and the plastic does not sublime or otherwise degrade in the vacuum of an electron microscope. The resin, therefore, provides continual support and preservation of fine specimen detail.

The mechanical properties of the cured resins are functions of the resin monomer and the curing agents which are components of the kit are described below:

"Araldite 6005" resin is a low molecular weight, low viscosity, unmodified epichlorohydrin-bisphenol A condensation product.
Dodecenyl Succinic Anhydride (DDSA), a cross-linking agent that is the anhydride of substituted dibasic succinic acid, and is commonly referred to as the "hardener" or "curing agent".
N-benzyl, N-N-dimethylamine (BDMA), a diamine used as a "catalyst" or "accelerator" which acts as an end-to-end linking agent.
Dibutyl phthalate (DBP), a "plasticizer" which imparts an elastic property to the cured resin.
Formulation:
The usual proportions used to form the cured solid resin are as follows:

Resin Component	Typical Medium Hardness Formulation
Araldite 6005	10.0 ml
DDSA	10.0 ml
BDMA	0.5 ml
DBP	0 to 3.0 ml

For additional formulations, the original work of Mollenhauer2 (1959) should be consulted.

Mixing of Resin Components
The preparation and mixing of the resin components must be thorough and performed only in a properly functioning laboratory fume hood. With time, some of the components will separate, so additional mixing is necessary just prior to the time the resin mixture is to be used.

The cutting properties of the final polymer depend on the components of the resin monomer mixture. The use of dibutyl phthalate (DBP) materially aids the cutting properties of the polymerized resin. Although the amount of the DBP added to each mixture should be optimized for a given specimen and/or experimental condition, DBP is generally used in the proportion of about 0.5 to 2.0% of the monomer mixture. Likewise, it is desirable that only the minimum amount of accelerator (BDMA) be used, and should be added just prior to actual use of the resin mixture. A plastic mixture to which accelerator has been added cannot be kept unrefrigerated for more than several hours before it begins to polymerize.

Sample Preparation:

DEHYDRATION:
Dehydration in a graded series of aqueous ethanol solutions (up to 100% ethanol), followed by propylene oxide or dehydration in acetone has been assumed.

Infiltration:
Infiltrate using the complete resin mixture in the following schedule:

(a). 2:1 ratio of solvent (propylene oxide or acetone) to resin mixture with accelerator, for 1 hour at room temperature (~25°C).
(b). 1:2 ratio of solvent (propylene oxide or acetone) to resin mixture with accelerator, for 1 hour at room temperature.
(c). 100% resin mixture with accelerator, for 1-2 hours at room temperature.

For some tissues it may be advantageous to apply a vacuum to the sample in the 100% resin mixture for about 5-10 minutes or until bubbles from the tissue are no longer visible. Soaking the tissue for periods longer than that outlined above, generally does not improve tissue preservation. If materials science samples are being embedded, such as porous ceramics or catalyst samples, then the vacuum impregnation is almost certainly going to be important.

EMBEDDING:
Place sample in flat embedding molds or capsules containing the fresh resin mixture and orient the sample if necessary. An identification code can also be added to the embedding medium in the mold or capsule.

CURE:
Gently place the embedments in the oven to cure overnight (8-16 hours) at 80°C.

References:
1Glauert, A.M., and Glauert, R.H., J. Biophys. Biochem. Cytol. 4, 191 (1958).

2Mollenhauer, H.H., J. Biophys. Biochem. Cytol. 6, 431 (1959).

When things go wrong:
We are talking now about a resin that won't cure "properly". Yes, this does happen and we hope that these further comments, based on our own years of experience might be helpful to someone having such problems.

The number one reason for such problems is improper dehydration. Yes, the entire specimen being embedded must be completely dehydrated and if there are still trace amounts of moisture remaining, the block will not sure. If the block tends to cure overall, but is not curing in the vicinity of the specimen, then that is almost certainly the explanation for the improper curing. Some specimens are much more difficult to dehydrate than others, therefore if yours are in that category, you should consider one of the partially water soluble resins, such as L. R. White, Unicryl, or Monostep. Or if you have a really difficult to embed system, try the SPI-Chem™ Low Acid GMA, it is completely water soluble and no dehydration is needed.

There can be real reasons why individual components are not any longer "good". In this kit formulation, the DDSA is the most moisture sensitive, in that moisture will rather quickly be absorbed by the anhydride, and this will result in a substantial increase in the free acid. The first signs of this happening are the presence of small "pores" in the cured resin and in the extreme, the resin won't cure at all. The reaction is not reversible, and unlike come components, where moisture can be driven off by the application of some heating, in this case the reaction is not reversible.

In terms of shelf-life, the BDMA is the "first to go". It releases ammonia, but in most instances, one can compensate for this by adding an excess of BDMA to their formulation. The only downside to doing this is that the block cures to a much darker color.

Another concern of many users is that when the DDSA is added to the DMP-30, the mixture can result in a "reddish" color, but upon curing, the reddish color disappears and the block does cure to its normal color and clarity. This variation in color effect at this point is due to some trace level impurities present in the DDSA. One can work hard to remove them, so that this color effect is not experienced, however, the main effect of such removal is to increase substantially the cost of the DDSA to the user. No one has ever reported a single time any negative effect of this temporary color effect. So we make comment about the color effect only so that if you see it, you don't think this is in of itself a cause for concern.

And remember that like everything else in life, nothing is "forever" and that is certainly true of organic chemicals. Be sure to follow recommended storage instructions to realize maximum rather than minimum shelf life. When in doubt, it is usually cheaper to dispose of out of date chemicals rather than to risk erroneous results from an important experiment. For that reason, we believe it is always better to order amounts that are more closely tied to short and medium term needs rather than to purchase quantities that could last a life time.