EMBEDDING GUIDE FOR EPON® RESINS

EMBEDDING MEDIA

Ideal qualities of embedding medium:

1. Easily available
2. Uniformity from one batch to another, no lot to lot variation
3. Solubility in dehydrating agents
4. Low viscosity as monomer for penetration
5. Uniform polymerization
6. Little volume change during polymerization
7. Good preservation of fine structure
8. Good sectioning quality that includes homogeneity, hardness, plasticity and elasticity
9. Resistance to heat generated by sectioning
10. Adequate specimen stainability
11. Stability in electron beam
12. Electron lucent

Epoxy resins, polyester resins and methacrylates are in general use. For general electron microscopy epoxy resins have most properties required.

EMBEDDING MIXTURE CONTAINS

1. Epoxy resin
2. Plasticizer and/or flexibilizer
3. Hardener
4. Accelerator (catalyst)

Plasticizer does not become part of cross-linked structure. It is necessary in some mixtures to prevent block from becoming too brittle and to improve sectioning properties.

Flexibilizer reacts with epoxy resins and becomes part of cross-linked structure. They are less likely to be lost under electron beam than plasticizers.

Hardener: Nature influences hardness of block.

Plasticizers, flexibilizers and hardeners influence hardness of blocks. Proportions may be varied to alter hardness. Accelerator also influences hardness but variation in concentration may cause blocks to become brittle and difficult to section. Sectioning properties depend on hardness and extent and nature of cross-links formed during polymerization.

ARALDITE RESINS

CHARACTERISTICS

Glycerol based aromatic resin that has very little volume shrinkage on polymerization.

High viscosity:
- Araldite 502 = 3000 cps at 25°C
- Araldite CY 212 = 1300 to 1650 cps at 25°C

Rather low softening temperature.

Not reactive with alcohols. Transitional solvent (acetone or propylene oxide) necessary between dehydration alcohols and embedding.

Sections have excellent stability under electron beam and stain well.
EXAMPLE OF EMBEDDING MIXTURE (LUFT, 1961)

**Araldite 502** 27 ml
**DDSA (hardener)** 23 ml
**DMP-30 (accelerator)** 0.75 to 1 ml

Mix thoroughly!

Araldite 502 contains plasticizer as supplied by the manufacturer. Final hardness may be varied by changing the resin/hardener ratio.

Polymerize at 45° C for 36 hr or 60-70° C overnight.

**EPON**

Epon 812 has been the most widely used embedding resin. The manufacturer (Shell) has discontinued production. Substitutes, such as Poly-Bed 812 have been developed by Polysciences, Inc.

**CHARACTERISTICS**

Glycerol based aliphatic epoxy resin.

Relatively low viscosity: Epon 812 = 150-220 cps at 25° C.

Easily hardened at low temperatures.

Not reactive with alcohol. Requires use of transition solvent (acetone or propylene oxide).

Cutting quality influenced by:

1. hardener/epoxy ratio
2. final block hardness
3. temperature and duration of polymerization

Sections show greater contrast in electron microscope than araldite sections. Epon has inherent granularity which may limit high magnification and high resolution studies.

EXAMPLE OF EMBEDDING MIXTURE (LUFT, 1961)

**Mixture A**
- **Epon 812** 62 ml
- **DDSA (hardener)** 100 ml

**Mixture B**
- **Epon 812** 100 ml
- **NMA (hardener)** 89 ml

Combine mixture A with mixture B. Add accelerator (BDMA or DMP-30) in proportion of 1.5-2%.

<table>
<thead>
<tr>
<th></th>
<th>ml</th>
<th>ml</th>
<th>ml</th>
<th>ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture A</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Mixture B</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>BDMA or DMP-30</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Mix components thoroughly!

Polymerize at 45° C for 12 hr and 60° C for 24 hr at 60-70° C overnight.

Mixtures A and B can be stored in refrigerator for several months. Epon is hydroscopic so storage bottles should be well sealed.

NMA reacts with permanganates. Mixtures with NMA cannot be used in conjunction with peramanganates.

**EPON-ARALDITE COMBINATIONS (MOLLENHAUER, 1964)**

<table>
<thead>
<tr>
<th>Mixture #1</th>
<th>#2</th>
<th>#3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epon 812</strong></td>
<td>25 ml</td>
<td>62 ml</td>
</tr>
<tr>
<td>Araldite 502 or 6005</td>
<td>15 ml</td>
<td>-</td>
</tr>
<tr>
<td>(or CY 212 in Europe)</td>
<td>-</td>
<td>60 ml</td>
</tr>
<tr>
<td><strong>Araldite 506</strong></td>
<td>-</td>
<td>81 ml</td>
</tr>
<tr>
<td><strong>DDSA (hardener)</strong></td>
<td>55 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td><strong>Cardolite NC-513</strong></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(flexibilizer)</td>
<td>-</td>
<td>25 ml</td>
</tr>
<tr>
<td><strong>DBP (plasticizer)</strong></td>
<td>2-4 ml</td>
<td>4-7 ml</td>
</tr>
<tr>
<td><strong>DMP-30</strong></td>
<td>1.5%</td>
<td>1.5%</td>
</tr>
<tr>
<td>or <strong>BDMA</strong></td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td><strong>Relative hardness</strong></td>
<td>medium</td>
<td>soft-medium</td>
</tr>
<tr>
<td><strong>Image contrast</strong></td>
<td>high</td>
<td>medium</td>
</tr>
<tr>
<td><strong>Tissue preservation</strong></td>
<td>good</td>
<td>excellent</td>
</tr>
</tbody>
</table>

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NOTES

Developed for plant tissues but applicable for wide range of specimens with little modification.

Mixture #1 is easier to section than media containing epon or araldite alone.

Mixture #2 is easier to section than mixture #1.

Mixture #3 is slightly more difficult to section than mixtures 1 & 2 and image contrast is lower. Preservation of specimen is excellent and mixture is particularly useful for specimens (ex. pollen grains) which tend to be pulled out of the block during sectioning.

BDMA should replace DMP-30 in mixture #3 since DMP-30 causes precipitate formation during infiltration.

ERL (VINYL CYCLOHEXENE DIOXIDE) AKA SPURR’S RESIN

CHARACTERISTICS

Cycloaliphatic diepoxide.

Lowest viscosity of resins: 7.8 cps at 25˚ C. Facilitates rapid penetration into specimen.

Miscible with ethanol. Transition solvent can be eliminated

Resistant to electron beam.

Resistant to oxidation by permanganates.

EXAMPLE OF EMBEDDING MIXTURE

Spurr’s low-viscosity embedding resin (Spurr, 1969)

All components have low viscosity.

ERL 4206 7.8 cps at 25˚ C

DER 736 30-60 cps at 25˚ C

NSA 117 cps at 25˚ C

Viscosity of final medium is 60 cps at 25˚ C.

SPURR RESIN MIXTURES

<table>
<thead>
<tr>
<th>Mixture</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERL 4206</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>DER 736 (flexibilizer)</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>NSA (hardener)</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>DMAE (S1, accelerator)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Hardness</td>
<td>Firm</td>
<td>F/Hard</td>
<td>Hard</td>
<td>Soft</td>
<td>Firm</td>
<td>Firm</td>
</tr>
<tr>
<td>Pot life (days)</td>
<td>3-4</td>
<td>3-4</td>
<td>3-4</td>
<td>3-4</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Polymerization time (hrs)</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>16</td>
</tr>
</tbody>
</table>

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TECHNICAL DATA SHEET 884

NOTES

Mixture A is the standard mixture. Mixtures B-F are modifications.

Mixture E has more accelerator. Time required for polymerization is reduced by storage (pot life) time is shortened.

Mixture F has reduced accelerator. Time required for polymerization is increased, but mixture can be stored in refrigerator for over 2 weeks.

Note: 0.4 gm accelerator = 0.5 ml measured in a plastic disposable syringe.

ORDERING INFORMATION:

<table>
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<tr>
<th>CAT #</th>
<th>DESCRIPTION</th>
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<tbody>
<tr>
<td>02334</td>
<td>Epon® Resin 828</td>
</tr>
<tr>
<td>08792</td>
<td>Poly/Bed® 812 (Luft formulations) Embedding Kit / DMP-30</td>
</tr>
<tr>
<td>21960</td>
<td>Poly/Bed® Araldite 502 Mini Kit</td>
</tr>
<tr>
<td>21959</td>
<td>Poly/Bed® 812 Mini Kit/BDMA (Glauert Version)</td>
</tr>
<tr>
<td>21844</td>
<td>Poly/Bed® 812 Embedding Kit/BDMA (Glauert Version)</td>
</tr>
<tr>
<td>08791</td>
<td>Poly/Bed® 812 Embedding Media</td>
</tr>
</tbody>
</table>

TO ORDER

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