

perfused tissue is immersed in hot water for at least several hours to complete polymerization, the tissue is removed with alternating treatments of 5% KOH and distilled water, and the resulting cast is cleaned with 5% formic acid (15 min) and distilled water. The cast is dried by lyophilization, mounted on a stub, and sputter coated for routine SEM observation.

Example corrosion casts from various tissues including heart [6], lung [7], urinary bladder [8], and salt gland [9] are shown. Some of the types of anatomical information that can be obtained from casts include: distribution and 3-D anatomy of the microvasculature of tissues, venous valve structure, location of arterial sphincters, distribution and size of nuclear imprints, and some simple quantitative measurements, including vascular volume and vessel dimensions. Accurate, fine detailed measurements of vascular casts also are possible using 3-D morphometry techniques, scanning electron microscopy of stereo pairs, and digital image analysis [10]. Because of the natural fluorescence of the blue dye in the Mercox resin, corrosion casts also can be studied with confocal microscopy [11].

References

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Benzyldimethylamine (BDMA): Catalyst of Choice with Epoxy Embedding Media

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The catalyst 2,4,6-Tri(dimethylaminomethyl)phenol (DMP-30) has been used to catalyze and polymerize epoxy resin embedding media since the earliest years of biological electron microscopy. Although DMP-30 works well, it is a highly viscous compound and media polymerized in this fashion likewise show a high viscous character. What follows is a rapid loss in fluidity in the medium, as well as shortened pot life, two factors that are absolutely essential in order to achieve complete specimen infiltration. Opting for less viscous catalysts, such as BDMA, presumably should yield lowered viscosity and prolonged fluidity in embedding media, thus ensuring complete infiltration and strengthened tissues subsequent to microtomy and ultrastructural study.

The differences between BDMA and DMP-30 become obvious following simple tests that define basic properties such as Average Flow Time (AFT: time necessary for known volume of catalyst to exit a viscosimeter), Volume Flow Rate (VFR: amount of catalyst flowing/time) and resulting Viscosity (cP; measure of fluidity and a correlate of AFT/VFR). The tests are relatively simple and can be performed by filling a vertically-oriented viscosimeter with a known volume (9ml) of the catalyst and then recording the time necessary for the component to flow, under natural gravity, from the viscosimeter. It's possible to accurately predict (in advance) that if a chemical flows rapidly from the viscosimeter, it does so because the product is low in viscosity. On the other hand, if the product flows very slowly, it does so because the opposite is true. Applying these tests to BDMA and DMP-30 shows the following:

Table I
Characteristics of BDMA vs DMP-30

	Average Flow Time	Volume Flow Rate	Viscosity (cP)
BDMA	5.57 sec/9ml	1.97 ml/sec	0.84 cP
DMP-30	2:11:96 min/9ml	0.0682 ml/sec	25.0 cP

These results clearly show that BDMA has a very rapid AFT, increased VFR, and thus is dramatically less viscous (@ 0.84 cP) than is DMP-30 (@ 25.0 cP). But do these interesting numbers by themselves suggest that utilizing BDMA, in lieu of DMP-30, would produce lowered viscosity and the attendant advantages? An LX 112-based embedding medium often preferred and well-known to this microscopist was prepared and catalyzed differently. The AFT, VFR, and resulting viscosity were tested immediately upon mixing and 60 minutes later.

Table II

LX 112/NSA/NMA catalyzed with BDMA or DMP-30

Time from Initial mixing	Average Flow time	Volume Flow Rate	Viscosity (cP)
@ 5 min with BDMA	1:19:85 min/9ml	0.1127 ml/sec	19.1 cP
@ 5 min with DMP-30	1:46:47 min/9ml	0.0845 ml/sec	37.1 cP
@ 60 min with BDMA	4:13:83 min/9 ml	0.0355 ml/sec	60.0 cP
@ 60 min with DMP-30	7:41:09 min/9ml	0.0195 ml/sec	160.7 cP

Hardening of any embedding medium naturally is accompanied by a loss of fluidity. These characteristics seem more prevalent when DMP-30 is the catalyst of choice. Immediately after mixing (5 minutes), DMP-30 causes a two-fold increase in viscosity as compared with BDMA, and this is accelerated even more after 60 minutes. It seems reasonable to presume that the medium with the lower viscosity, because it will remain fluid longer, will infiltrate tissues more completely. The information presented here also strongly suggests that the microscopist should not devote many hours to the process of infiltration, a common technique in many embedding protocols. This is not time efficient as it is clear that, once prepared, any combination of ingredients will undergo a two- and three-fold increase in viscosity and loss of fluidity within minutes of preparation. This is exactly what occurs when epoxy resins are placed in a mix with acid anhydrides and catalyzed with tertiary amines. Useful infiltration can only occur during the early pot life of the embedding medium. DMP-30 is of historical value and continues to be widely utilized today. BDMA, on the other hand, has always been recognized as a *catalyst of choice* by pioneer electron microscopists¹.

References

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Acknowledgement:

Appreciation is extended to Dr. Gerald S. Kirby for assistance with viscosity measurements.

The Most Likely Sources of EDX Copper Peaks in Samples Run by TEM

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It is well known that TEM copper grids will give an EDX background of Cu peaks during EDX measurements. While there are a number of potential sources for these characteristic Cu peaks, the most intense source is likely the Cu grid itself. You can eliminate this source by using Be grids. Other potential sources can be addressed