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].

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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 devout 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 microscopists[1].

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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

by using a top hat aperture that is very thick and by using a Be analytical specimen holder. If you want to track down what is really causing the problem you can do a hole count procedure as explained in Dr. David William's book, "Practical Analytical Electron Microscopy in Materials Science" starting on page 57. If this procedure doesn't work for you, in a separate book, "Transmission Electron Microscopy, A Textbook for Materials Science" by David B. Williams and C. Barry Carter have an additional discussion on page 579. Since not everyone has an ion mill to make a suitable holed specimen for the hole count, what else can a microscopist do? After laying in a supply of Be grids, the next thing is to order top hat condenser apertures and install them, even if you do not set up to do the hole count.

One likes to run powder and thin section samples on copper grids but that also generates the Cu peaks in your spectra. You want an easy way to find out if the copper peaks are real or just from localized scattering. An alternative way to minimize the Cu peaks, if you won't use Be grids, is to also make a Ti grid prep, if you have enough sample. If you have some copper in your sample, then you should see the Cu lines on the EDX spectrum obtained using the Ti grid prep. These peaks would then suggest copper was present in your sample. Don't forget to run a Ti grid without your sample on it (a blank). If the blank shows copper, then you need to use the hole count procedure to find out what's wrong.

It helps to keep some Ni grids handy too. Depending on the elements suspected to be present in your sample, using grids of different composition can often allow you to verify the source of x-ray lines in your sample. Ti has very few interferences at its energy location on the EDX spectra and I like it the best for this fast second procedure.

For additional verification, if you have enough sample, submit it to your x-ray lab for a qualitative or semi-quantitative analysis. Another alternative is to put some on an SEM carbon stub fitted with a conductive carbon tab and collect an SEM EDX spectrum.

In my opinion, you should also always use a Be specimen holder to do EDX and here's why. I have a Philips CM 12 and the regular specimen holder. If I don't use my Be holder and I use the regular holder for EDX, then I get a Cu peak with a slight Zn peak. This Zn is coming from the holder because it is always close to the beam position, it's made of brass and any stray electrons or radiation can hit it. Grid bars and the specimen holder's brass alloy are the problems here and they are easy to fix by swapping them out. If you don't have a Be holder, try to borrow one for testing. You can then use the comparative results to help justify getting your own. Remember to use those top hat apertures and remove your objective aperture.

In short TRUST your EDX or WDX detector spectra BUT VERIFY what you see. Lastly, this is a complicated subject and some of this is over-simplified. You now have ways to investigate the problem and a few 'quick and dirty' means to rule out certain known problems like copper grids!!!! *Note: Be salts and oxides are toxic to humans.*