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This Week's Citation Classic

Spurr A R. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**:31-43, 1969.
[Dept. Vegetable Crops, Univ. California, Davis, CA]

The author reports a new epoxy-resin system for embedding tissue to be sectioned for observation by electron microscopy. Based on the low-viscosity epoxy resin, vinyl cyclohexene dioxide, and three other components selected mainly for low viscosity and long pot life, the medium is easily prepared and rapidly infiltrated into specimens. It is compatible with a wide range of dehydrating agents, and has been successfully used with diverse materials. [The *SCI*® indicates that this paper has been cited over 2,725 times since 1969.]

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"Advances in electron microscopy have been made through the innovations of numerous investigators. My contribution came at a time when many of my colleagues in ultrastructural research were receptive to a new epoxy-resin system of embedding materials. The primary advance made by the method is the outstandingly low viscosity achieved in the epoxy-resin mixture. Many have inquired about how the method was devised, and this 'Citation Classic' provides an opportunity to give a personal account about its development.

"My entry into ultrastructural research in the late 50s came when most procedures were geared to generally more pliable animal tissues, rather than to the heavily cutinized and hard, thick-walled, lignified elements so common in plants. Methods based on methacrylate or on the epoxy resins Araldite, Epon, and Maraglas, sometimes did not work well, or at all, with plant materials. So I started probing into the technical and industrial literature on epoxy resins. Had I been presented at the outset with the four components, the medium would have been comparatively easy to formulate, but the materials were found piecemeal. As the work progressed, trying to evaluate systems lacking in one or more of the final components was admittedly frustrating at times.

"Expectations reached a high point when it became apparent that the epoxy resin, vinyl cyclohexene dioxide, could be the basis for a system. Thereafter, the selection of additional components, all in keeping with a low-viscosity method, was based on it.

"Work was continued with numerous possible components, but primary attention was centered on the selection of an anhydride. Both solid and liquid forms were tested. Eventually, a liquid type from the Humphry Chemical Company, nonenyl succinic anhydride, yielded excellent castings. However, they were deeply colored, almost black. Through fractional distillations, a series of cuts was finally obtained that provided clear castings.

"At this point a reactive flexibilizer was needed, and many were evaluated. During this period of development the castings varied in the extreme, but finally D.E.R. 736 was found to give the best results Now only the accelerator remained to be determined. Among many tested dimethylaminoethanol proved to be significantly better than other compounds because it conveyed a long pot life in the laboratory and yet a rapid cure with elevated temperature.

"To provide some insight into the physical properties of the medium and castings, the Dow Chemical Company cooperated in tests on viscosities and deflection temperatures. I later undertook further testing at Davis. Final evaluation was largely based on the quality of the thin sections when viewed in the electron microscope.

"Apart from the utility of the low-viscosity system with plant tissues, it has been successfully used with such diverse materials as human skin and bone, and even fish scales. The long developmental effort in searching out and testing new materials evidently proved to be worthwhile to many researchers in electron microscopy. The low-viscosity system became especially advantageous to hospital EM laboratories where time is of importance in diagnosis.

"I should comment that although the background literature on epoxy resins was helpful, it provided no specifics on applications in electron microscopy. The project accordingly involved empirical approaches, yet an element of judgment was essential in the evaluation of hundreds of formulations. A high quotient of persistence kept the project on track and to its ultimate fruition. Through the auspices of the University of California, the invention became the basis for a United States patent issued in 1976."