Solutions to the Problem of Substitution of ERL 4221 for Vinyl Cyclohexene Dioxide in Spurr Low Viscosity Embedding Formulations

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Spurr low viscosity embedding medium[1] was introduced in 1969 and used vinyl cyclohexene dioxide (VCD or ERL 4206) as the low viscosity epoxy resin together with nonenyl succinic anhydride (NSA), the flexibilizer DER 736, and the accelerator dimethylaminoethanol (DMAE or S-1). This low viscosity formulation has been used extensively for embedding plant materials, microrganisms and other difficult specimens. Over the years numerous concerns about the toxicity and carcinogenicity of VCD have been raised. VCD (Fig. 1)[2] was finally removed from the market in 2005 and replaced with ERL 4221 (Fig. 2)[2], a cyclic epoxide with a higher molecular weight and higher viscosity. Vendors instructed that ERL 4221 be substituted for VCD at the same rates as used in the original Spurr formulation. This direct substitution resulted in a more viscous resin and blocks were extremely hard and brittle and lacked the sectioning qualities of the original formulation. Vendors then suggested modifications to the dehydration schedule and reductions in the amount of DMAE to improve the quality of the blocks.

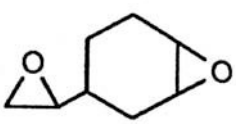

Fig. 1 Structure of VCD

However, these suggested modifications do not address or solve the problem with the replacement epoxide. VCD, a cycloaliphatic diepoxide, has an average WPE (weight per epoxide equivalent)[3] of 76; ERL 4221, a cyclic diepoxide, has a WPE of 137. The source of the problem is raising the anhydride:epoxide ratio from 0.88:1.0 in the original formulation to 1.6:1.0 by directly substituting ERL 4221. Increasing the anhydride:epoxide molar ratio always results in a harder block and when the ratios are higher than 1.0:1.0 there is increased hardness and brittleness along with problems in trimming and sectioning the block. The significance of maintaining an appropriate anhydride:epoxide ratio for a given embedding formulation[3] has not been appreciated in recent years; however, substituting ERL 4221 for VCD reiterates this importance. The corrected formulation presented here maintains the anhydride:epoxide ratio at 0.88:1.01 and preserves the good sectioning qualities and beam stability of the original Spurr formulation.

Table 1 shows the corrected standard formulation in grams of Spurr low viscosity embedding media based on batches of 10 grams without the flexibilizer, DER 736, included in the formulation calculations. This corrected formulation of ERL 4221 and NSA maintains the anhydride:epoxide ratio of 0.88:1.0 as done in the original formulation and block hardness is controlled by varying the amount of DER 736 with the same proportions as recommended by Spurr[1]. Modifications of the formulation for harder or softer blocks are given in Table 2. The accelerator, DMAE, is used at the same rate as given in the original publication. It is suggested that individuals prepare small batches of resin and vary the amount of DER 736 to determine the optimal amount of flexibilizer for their particular specimens.

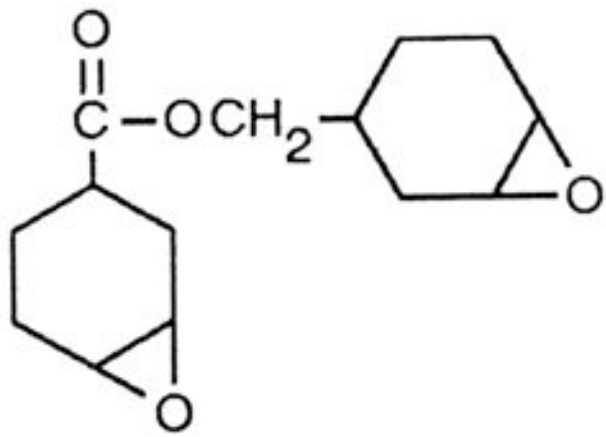

Fig. 2 Structure of ERL 4221

Viscosity of the original, standard formulation was 60 centipoise (cP). With the increased WPE of the replacement epoxide there should be an increase in viscosity of the corrected formulation. Viscosity of the new, corrected formulation was determined by comparing flow rates to those of the standard Spurr formulation with VCD. There was a three fold increase in flow rate, which gives an approximate viscosity of 180 cP for the corrected formulation using ERL 4221. Due to the increased viscosity of ERL 4221, care should be taken to mix the resin components thoroughly. This increased viscosity is still much lower than the viscosity of Epon-Araldite[4] formulations and offers the good cutting qualities and beam stability that many people prefer as compared to formulations based on Luft's Epon[5].

Quetol 651, a low viscosity aliphatic resin introduced by Kushida[6] in 1974 has been used in my lab for over twenty years to lower the viscosity of various formulations including those based on Luft's formulation and as a replacement for the Epon component in Mollenhauer's Epon-Araldite[7]. We routinely use the formulation given in Table 3 which uses ERL 4221 and Quetol 651 in a molar ratio of 1.0:1.0 and maintains the anhydride:epoxide ratio at 0.88:1.0. DER 736 is used at the same rate as given in the original Spurr formulation. The accelerator, benzyldimethyl amine (BDMA), is used at the rate of 0.20 ml/10 grams of the ERL 4221, Quetol 651, NSA mixture. The viscosity of this embedding formulation is 90-100 cP, approximately half that of the standard formulation without Quetol 651. As with the other formulations given here, it is recommended that the amount of DER 736 be varied to determine the optimum for a particular type of specimen. Increasing the amount of DER 736 results in a softer block; decreasing the amount of DER 736 yields a harder block.

TABLE 1: Standard Formulation in Grams

GRAMS	ERL 4221	NSA	DER 736	DMAE
10	4.10	5.90	1.43	0.1

TABLE 2: Hard and Soft Formulations in Grams

FORMULATION	ERL 4221	NSA	DER 736	DMAE
Hard	4.10	5.90	0.95	0.1
Soft	4.10	5.90	1.90	0.1

TABLE 3: QUETOL 651:ERL 4221 Formulation in Grams

GRAMS	Quetol 651	ERL 4221	NSA	DER 736	BDMA
10	1.40	2.22	6.38	1.43	0.20 ml

Although ERL 4221 has a higher molecular weight and viscosity and should not be as toxic as VCD, it has the same parent structure as VCD. The same safety precautions should be exercised in handling ERL 4221 and Quetol 651 and formulations using these epoxides as have been recommended in the past for VCD and other low viscosity formulations[8]. In addition, precautions should be taken to keep resin components and mixtures dry by use of oven dried containers, utensils and embedding molds. ■

References

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9. Appreciation is expressed to John Arnott, Ladd Research, Williston, VT, for providing information on the WPE of ERL 4221 before such information was available to the general public.

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