High Temperature E12 Plastination to Produce Ultra-thin Sheets

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Abstract: The E12 method of plastination classically is used to create thin 3 - 5 mm transparent slices. If thinner slices, 0.5 - 1.0 mm are desired, it is necessary to use an ultra-thin slice plastination method. By using this method, the specimen is first plastinated as a block and then cut into the thinner slices. The impregnation temperature and the percent accelerator (E600) are the key elements necessary to obtain proper impregnation of the desired tissue block and contrary to all other plastination methods high temperature (30 to 60°C) is desired. The main goal of this paper is to describe the use of high temperature for processing ultra-thin (1 mm) epoxy plastinated slices. Only by using high temperature is the polymer thin enough to penetrate into the middle of a large processed specimen.

Key Words: plastination; epoxy; E12; E6; impregnation; temperature; ultra-thin; slices

Introduction

One of the critical factors of plastination is temperature. Usually, during dehydration, degreasing or E12 impregnation of slices, the temperature does not exceed room temperature values. High temperature, up to 45°C is only used during curing. The question is: “Why high temperature is not used?” High temperatures are not used during dehydration and degreasing because high temperature increases shrinkage and during impregnation the E12/E1 impregnation mixture becomes very viscous within hours, preventing complete impregnation. The exception is E12/E1 block impregnation with curing using E6 hardener which utilizes high temperature (60°C).

The usual steps in E12 plastination are: preparing thin slices (3-5mm) of the desired specimen, cold dehydration, degreasing, impregnation and finally curing (von Hagen, 1985; Weber and Henry, 1993; Cook, 1996; Cook, 1997; Fasel, 1988; Ann, 1999; Lane, 2000; Sora, 2002; Sora, 2004). However, ultra-thin slice plastination produces slices with a thickness of 1 mm or less after impregnation (Fritsch, 1991; Seibold, 1991; Sittel, 1996; Johnson, 2000; Windisch, 2001). Therefore, after the specimen has been dehydrated, it is impregnated, cured and finally sawed to obtain the ultra-thin E12 slices. The main goal of this paper is to present the utility of high temperature in processing and creating cured E12 blocks for sawing ultra-thin plastinated slices. One of the greatest problems during plastination of bigger tissue blocks is to get the E12 polymer into the middle of the block. At room temperature, the E12 polymer is a liquid but gets thicker when temperature is decreased. If temperature is increased, the E12 polymer gets thin, almost like water. However, once the E12/E1 reaction mixture is prepared, increased temperature hastens the reaction of the E1 hardener with the E12 and after a short time the polymer mixture becomes too viscous to impregnate tissue. Therefore a different hardener, E6, is used in
making E12 blocks. The Biodur E6 hardener is an anhydride-based hardener of low viscosity (von Hagens, 1985) which permits impregnation of the specimen at 30°C to 60°C with no immediate thickening of the polymer reaction mixture.

**Materials and methods**

*Material, dehydration and degreasing:*

One male unfixed human cadaver ankle was used for this study. The foot and the distal third of the tibia were removed. The foot was positioned in 90° dorsal flexion and frozen at -80°C for one week. Next, a tissue block containing the ankle was produced for plastination by removal of the tissue 4cm distal to and 5cm proximal to the tip of the lateral malleolus. The ankle block was placed into a -25°C freezer for two days and then submerged in 25 liters of cold (-25°C) technical quality 100% acetone for dehydration. This dehydration bath was changed after 4 weeks and had been diluted to a concentration of 92%. The second dehydration bath was for 3 weeks and the final concentration was 97%. The third and final dehydration bath was for two weeks and had a final reading of 99%. When dehydration was finished, the freezer was disconnected allowing the temperature to increase to room temperature (15°C). The warmed acetone was changed for room temperature methylene-chloride (MeCl) for degreasing. Degreasing was complete when the adipose tissue became transparent after 4 weeks (Table 1).

*Impregnation:*

The dehydrated/degreased specimen was removed from the methylene chloride bath and submerged in E12 impregnation mixture [E12(polymer)/E6(hardener)/E600(accelerator) (100/50/0.2)] (von Hagens, 1985) in a Heraeus VT 6130 M vacuum drying oven (Heraeus Instruments, Kendro Laboratory Products GmbH) at 30°C. No vacuum was applied until the next morning in order to allow equilibration and penetration of the E12 mixture. To commence impregnation, the next day vacuum was applied and stabilized at 40cm Hg pressure at +30°C. From this point, pressure was continuously reduced, 8cm Hg daily, over five days until 2 mm Hg was reached. Temperature was kept at 30°C the first four days and on the last day the temperature was increased to 60°C (Table 1).

*Casting and curing:*

After impregnation, the specimen (Fig. 1) was removed from the vacuum oven and placed in a mold built of Styrofoam and lined with polyethylene foil (Fig. 2). The mold was filled with the polymer reaction-mixture [E12/E6/E600 (100/ 50/ 0.2)]. The filled mold, containing the impregnated specimen, was placed in an oven at 65°C for 4 days to harden the polymer. The block was cooled to room temperature and the mold removed (Fig. 3).

*Slicing:*

Using a contact point diamond blade saw, Exact 310 CP (Exact Apparatebau GmbH, Norderstedt, Germany), the hardened E12 block was cut into 1 mm slices (Fig. 4). Tissue, the width of the saw blade (0.4mm), between the slices was lost.

**Results**

An E12 block was produced that was hard and transparent. Ultra-thin, <1 mm slices produced from this block were transparent and hard with good optical qualities. The finished E12 slices provided excellent anatomical detail down to the microscopic level.

**Discussion**

Impregnation is one of the main steps in plastination. The main goal is to impregnate the specimen thoroughly. This requires that the resin/hardener-mixture be thin enough to penetrate the specimen and also have a processing time of sufficient length to penetrate the depths of the specimen. The viscosity of epoxy resin varies markedly with temperature. At low temperature the viscosity of the resin is high, while high temperature yields a resin of low viscosity. During impregnation at low temperatures, 5°C or less, epoxy becomes viscous. However, low temperature prolongs the processing time. This prolonged processing time with the standard E12 method (E12 polymer mixed with E1 hardener) is the reason that impregnation is preferably performed at 5°C for 2 days. But by using this method, only slices of an average thickness of 3-5mm can be impregnated because of the rapidly increasing viscosity of the reaction-mixture.

The epoxy technique described in this work produces slices by sawing 1 mm (ultra-thin) plastinated plates from an E12 specimen block. Therefore, a large tissue block, with a thickness of 10cm, is impregnated rather than thin, 3-5mm slices. In order to impregnate such a tissue block, the reaction-mixture must have a low viscosity and the impregnation time must be prolonged. This is achieved by using the E6 hardener and by increasing the temperature during impregnation. The processing time of the E12/E6 reaction-mixture depends on the quantity of accelerator E600 and on the temperature. High temperature and increased quantity of E600 both lead to faster polymerization and a decrease in the processing time. Before starting impregnation, the dehydrated and degreased tissue block is submerged in the reaction-mixture for at least 8 hours, to allow the E12/E6/E600 mixture to equilibrate.
and begin penetration into the tissue block. As well, some volatile intermediate (methylene chloride) escapes from the specimen and lowers the viscosity of the reaction-mixture. The viscosity of the E6 hardener is high, and for this reason the E12/E6/E600 mixture is quite viscous at room temperature. Therefore, impregnation is started at 30°C. At this temperature, the reaction-mixture viscosity remains low for the next few days, since viscosity decreases with increase of temperature.

The first four days, impregnation is performed at 30°C and on the fifth (last) day the temperature is increased to 60°C to aid extraction of the remaining volatile intermediate from the specimen and to aid influx of the polymer reaction-mixture. Since increasing the temperature thins the E12/E6/E600 reaction-mixture, extraction of MeCl from the impregnation bath is easier. As well, the lower viscosity impregnation mixture penetrates the tissue block easier.

This kind of temperature/vacuum regulation is performed easily in a vacuum drying oven, where simultaneous adjustment of vacuum and regulation of temperature can be performed. The use of 60°C on the last day must be monitored carefully. At this temperature, the E12/E6/E600 mixture at first becomes thinner; but after several hours the polymer-mixture becomes thicker and bubbles rise slower and splash intensely marking the onset of polymerization.

Polymerization of E12 is dependent on the chosen hardener, temperature and percent of accelerator. For the impregnation of large tissue blocks, the E6 hardener was used which provides a longer impregnation time. Hence, by varying the amount of accelerator used, the length of time available for impregnation can be
significantly altered. Using 0.2% of accelerator E600 in the E12/E6 mixture was sufficient to assure proper impregnation and curing of the block. By omitting the E600 from the E12/E6 mixture, polymerization of the block will not occur, even if temperatures of 60°C are maintained for several months (von Hagen, 1985). The plastination folder is the only source which lists, in a table, the reaction time and hardening of E12/E6/E600 mixtures. All suggested percentages of E600 have been tested and are correct. The end product, using this protocol, was an E12 block that was firm and transparent. Such blocks are necessary to cut ultra-thin slices (<1 mm).

Ultra-thin slices <1 mm are essential if the histology is to be studied on plastinated slices or if 3D reconstruction is desired (Sha, 2001; Quiet, 2003). These ultra-thin slices can only be cut from a solid E12 block. Therefore, knowledge of controlling temperature and percent of accelerator in the ultra-thin sheet plastination method is essential. Histological examination can be performed up to a magnification of 40X. Greater magnification is not possible due to the thickness (>300 microns) of the specimen (Sora, 2002). Slides can be stained by the usual methods for histological specimens (Gruber, 2001). The major advantage of this method is that the structures remain intact and the decalcifying of bony tissue is not necessary. Plastination allows the topography of structures to be studied in a non-collapsed and non-dislocated state. Therefore, morphological measurements can be performed easily and accurately.

**Literature cited**


