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FORCED IMPREGNATION FOR THE STANDARD S10 METHOD

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INTRODUCTION

For many years, various plastics have been diffused into small pieces of tissue via the classic processing techniques for both light and electron 'microscopy (Baker, 1960; Hayat, 1970). How could plastic be made to enter the depths of larger specimens? Pondering this classic process, vacuum impregnation was conceived by Dr. Gunther von Hagens. Forced impregnation is the uniqueness of plastination. It is the process whereby the curable polymer is drawn into the cellular structure of the specimen. Recently, air pressure has been used to impregnate lungs (Henry, 1990).

Forced impregnation is the extraction of a volatile intermediary solvent (which occupies the space originally filled with tissue fluid and lipid) from a specimen and simultaneously replacing it with a curable polymer (von Hagens et al., 1987). Extraction is accomplished by using a vacuum. The intermediary solvent must have a saturated vapor pressure considerably higher than the polymer, such that the intermediary solvent may be vaporized and extracted from the specimen leaving a tissue void so that the polymer is drawn into the specimen. Both methylene chloride (dichloromethane) and acetone have been used successfully as intermediary solvents. Their respective boiling points are 39.75°C and 56.5°C (CRC, 1973) while the boiling point of the polymer (S10) is 295°C (von Hagens, 1989b). The dehydrated specimen, saturated with the intermediary solvent, is submerged in the polymer mixture and vacuum is begun. As absolute pressure is slowly decreased, because of the low SVP of the polymer, only the solvent is extracted out of the specimen. It is boiled out through the surrounding polymer (von Hagens et al., 1987) and evacuated through the vacuum pump as a vapor. As the solvent leaves the tissue a void is produced in the specimen and polymer is drawn into the specimen.

Equipment: Vacuum pump (BIODUR HI 10 or Welch 1400), Plastination (vacuum) kettle (BIODUR HI 02 or large kettle fabricated from steel or stainless steel) with a tempered glass lid or a glass desiccator, Specimen basket (BIODUR HI 09), Vacuum regulator valves (BIODUR HI 14), Vacuum tubing, Vacuum gauge, Bennert-type Manometer (BIODUR HI 20), Exhaust vent or solvent-oil separator (BIODUR HI 13), Deep freezer (explosion proof of modified to decrease explosion hazard (von Hagens, 1985; Hillebrands, 1992).

Forced impregnation has three steps:

1. Preparation of the polymer reaction mixture (impregnation bath),
2. Placement of solvent saturated specimens into the impregnation bath, and
3. Application of vacuum, producing a slow decrease in absolute pressure (increase in vacuum) which results in: a_, the volatile intermedium boiling out of the specimen and jbu the polymer reaction mixture is drawn into the specimen. For proper impregnation. steps a. and b. must occur simultaneously.

PREPARATION OF THE POLYMER REACTION MIXTURE:

Polymer Mixture: Polymer - BIODUR S10, *Hardeners* - BIODUR S3 and S6. The BIODUR S10 polymer and hardening

agents, S3 and S6, are stable when stored individually for several months at room temperature. However, their shelf life is greatly increased when they are stored in a deep freezer.

After mixing the S10 and S3 together, the reaction between the polymer and the hardener commences and the silicone molecules start to elongate and form chains. To retard this reaction, the reaction mixture must be kept cold (-10°C to -20°C). The S10 polymer is usually packaged in 10 kg containers. However, it can be shipped in 50 kg tubs, while the S3 is packaged in 0.1 kg bottles and the S6 in 1 kg bottles. The S10 polymer and the first hardening agent, S3, are thoroughly mixed together at a 100:1 ratio. Once the polymer and hardener have been mixed, this reaction mixture is placed in the vacuum kettle and deaerated to remove the air which was stirred into the reaction mixture during mixing. Full vacuum is applied for several minutes, until bubbles cease to rise to the top of the polymer.

PLACEMENT OF SOLVENT SATURATED SPECIMENS INTO THE IMPREGNATION BATH:

After the reaction mixture has been deaerated, the dehydrated specimens, which are saturated with the intermediary solvent, are submerged in the impregnation bath. The volatile intermedium (methylene chloride or acetone) must have a high vapor pressure and a low boiling point while the polymer reaction mixture must have a low vapor pressure and a high boiling point. It is important to submerge the solvent saturated specimens rapidly to prevent evaporation of the solvent from the surface of the specimen. Dry spots are produced by this evaporation and these areas do not impregnate properly, leaving a whitish discolored area on the impregnated specimen. Acetone soaked specimens tend to float. A specimen basket with a mesh lid is beneficial to keep the acetone drenched specimens submerged, as well as, to remove the specimens when impregnation is complete. Specimens soaked in methylene-chloride sink due to its high specific gravity (1.3). A grid placed on the bottom of the vacuum kettle or a wire specimen basket will keep the specimen from lying on the bottom of the vacuum chamber and thus allow the polymer reaction mixture to reach more of the surface of the specimen. It is beneficial to allow the specimens to sit in the reaction mixture over night before applying the vacuum. This allows the surface solvent and the polymer reaction mixture to equilibrate.

APPLICATION OF VACUUM, PRODUCING A SLOW DECREASE IN ABSOLUTE PRESSURE (INCREASE IN VACUUM) WHICH RESULTS IN: a, THE VOLATILE INTERMEDIUM BOILING OUT OF THE SPECIMEN AND b, THE POLYMER REACTION MIXTURE IS DRAWN INTO THE SPECIMEN.

a. A slow continuous extraction of the volatile intermedium from the specimen is required. The solvent is vaporized in the specimen, it boils from the specimen, it rises through the surrounding polymer mixture, and is pumped out through the pump into the oil-solvent separator or exhaust vent. Extraction of the intermedium creates a void in -the tissue and sets up a pressure gradient between the specimen and the impregnation bath.

b. The void in the tissue and the resulting pressure difference draws the polymer reaction mixture into the tissue. Thus for ideal impregnation as the solvent is boiled out of the specimen, the polymer reaction mixture is simultaneously drawn into the specimen. If the solvent is extracted (boiled out) too fast, the polymer can not flow to all parts of the specimen. The structural framework of these parts will collapse and the specimen will shrink.

The rate of increase of vacuum (decrease of absolute pressure) is extremely important. Since the solvent is less viscous than the polymer reaction mixture, the solvent must be extracted slowly to allow enough time for the viscous polymer to move into the tissue void created by the departing solvent. Pumping speed (rate of extraction of the solvent) is dependent on the properties of the specimen that is being impregnated. A large specimen with a high

density will dictate a slower impregnation speed while a small or thin specimen with a low density will accommodate a faster impregnation speed (Nel, 1990). Often a variety of specimens are to be impregnated at the same time. Therefore, a slower rate of extraction and impregnation will assure uniform impregnation of each specimen in its entirety. ***Rate of extraction*** may be monitored by two methods and preferably both: 1. Rate of bubble formation, and 2. Monitoring and recording the rate of decrease in absolute pressure via a gauge or Hg column and Bennert Manometer. Bubbles about 1 cm in diameter are formed as the solvent is vaporized and extracted from the specimen and pulled out through the surrounding reaction mixture and finally the vapor is pumped out through the pump. An ideal extraction rate of the intermediary solvent is when a few bubbles slowly rise to the surface and gently burst. Very rapid boiling is not acceptable and indicates that the solvent is being extracted far too rapidly and will likely lead to inferior specimens.

During forced impregnation, vacuum and changes in vacuum are monitored on a vacuum gauge or Hg column and later by a manometer. Both a gauge (or column) and a manometer are beneficial as a gauge monitors the entire range of decrease in absolute pressure (1 atmosphere) while the Bennert Manometer monitors only the last one-third of the atmospheric decrease, from 20-22 cm of Hg to zero. The manometer is very accurate while most inexpensive gauges are not as precise. Since impregnation of acetone saturated specimens at -20°C commences around 25-30 cm of Hg, at pressures between 78 and 20 cm of Hg, the degree of vacuum is not known unless a gauge is attached to the system. In cold polymer, solvent extraction begins at a lower absolute pressure (more vacuum) than when impregnation is carried out at room temperature. The calculated saturated vapor pressures (SVP) of methylene chloride and acetone at -25°C are 32.5 mm and 14.8 mm while that of water is 0.476 mm. SVP increases exponentially with an increase in temperature so that at -10°C their SVP are 78.0 mm, 35.9 mm, and 2.16 mm; while at near room temperature (20°C) their SVP are 343.9 mm, 160.5 mm and 17.5 mm (Henry and Johnson, 1992). Extraction of acetone in cold polymer begins around 25-30 cm of Hg. Whereas, extraction of acetone in room temperature polymer begins around 60 cm of Hg. Similarly, methylene chloride extraction commences at a higher absolute pressure than that needed for acetone indicating the importance of a vacuum gauge to monitor the decrease in absolute pressure. An example of a vacuum-impregnation sequence in a -20°C freezer for acetone saturated specimens could be similar to the following:

Day 1 - Submerge specimens in cold polymer reaction mixture. Day 2 - Apply vacuum, stabilize at 35-40 cm Hg. Day 3 - If no bubbles, decrease absolute pressure (increase vacuum) slowly to 30-25 cm Hg. Day 4 (and thereafter) - If no bubbles, decrease pressure 1-2 cm daily. If bubbles are observed, wait 24 hours, then, if no bubbles decrease pressure.

Vacuum regulation: If a vacuum pump (even with a low pump speed) is hooked directly to the plastination kettle and turned on, it will pull a total vacuum in a few hours. Therefore, a valve-system must be installed so that absolute pressure may be decreased and stabilized in a controlled manner. Two needle valves (HI 14) are ideal, as they afford fine adjustment and assure < 1 cm Hg increments of adjustment. Most valves available commercially have a course adjustment and are not as suitable. The valve may be installed as a ***by-pass*** valve or as an ***in-line*** valve. As the by-pass valve is closed. absolute pressure decreases. However, as the in-line valve is opened, pressure decreases.

Vacuum pumps should have a relatively low pump speed or capacity and be an oil driven vane type. A pumping capacity of 1.5m^3/hr [.9cfm (25Lpm)] (BIODUR HI 10 or Welch 1400) is adequate for most sizes of plastination kettles (small or large up to 125 cm x 75 cm x 50 cm). If you already have a pump with a greater capacity, it

possibly can be adapted for use. However, do not purchase a large capacity pump as they cost more and you are likely to experience difficulty with its operation since it is being over worked. Most high speed pumps are not designed to pump high volumes of air for prolonged periods of time. If the original pump breaks down, having a second pump available to serve as a back up is beneficial. The oil level of the pump should be monitored daily. To minimize damage to the pump from the solvents, the oil must be changed after each load of specimens has been impregnated.

Evacuation is carried out faster and needs to be faster if impregnation is done at room temperature. The polymer reaction mixture is less viscous at room temperature and hence can flow into all regions of the specimen easier and faster. The reaction mixture also has a short pot-life at room temperature. Impregnation time is cut more than one half when carried out at room temperature. Cold temperature extends the pot-life of the reaction mixture indefinitely. Therefore, when not being used at room temperature for impregnation, store the polymer reaction mixture in the deep freezer. Remember, the vacuum is always increased gradually. If in DOUBT, LOWER the PUMPING SPEED.

Impregnation is complete when the absolute pressure has been stabilized around 2-10 mm Hg for a few days and/or plastination bubbles (1-1.5 cm) have given way to large, 4-5 cm bubbles which are water vapor. It is beneficial and desirable to pump off any water vapor (large bubbles at the end of the impregnation cycle) for a few days. When impregnation is completed, the vacuum is released and the plastination kettle atmosphere is restored to atmospheric pressure. It is important to allow the impregnated specimens to remain in the impregnation bath at atmospheric pressure for a day before they are removed from the impregnation bath. This allows the polymer in the specimen to equilibrate with the impregnation bath.

SPECIAL CONSIDERATIONS:

Specimens covered with skin or capsule present special problems. These dense layers are penetrated by the polymer reaction mixture at a very slow rate. Therefore, this type specimen tends to shrink during routine forced impregnation because the solvent (low viscosity) is extracted before the polymer (high viscosity) can penetrate this barrier. To prevent shrinkage and to assure an ample supply of polymer is beneath the capsule, such specimens should be infiltrated with the polymer reaction mixture just before impregnation and possibly during impregnation. A large gauge needle and a small volume syringe may be used. However, due to the viscosity of the reaction mixture, a hydraulic pumping device or an automatic pistol grip injection syringe is beneficial (Tiedemann, 1987).

INCOMPLETE IMPREGNATION:

Diluting the polymer with xylene can enhance flexibility and softness to certain tissue. The percentage of xylene added may vary from 1-2% up to 25%. Gastrointestinal organs are sometimes processed by this procedure. Xylene mixes with and dilutes the polymer reaction mixture so that as impregnation proceeds both xylene and polymer (at the mixed ratio) are drawn into the specimen and hence, proportionately less polymer is drawn into the specimen. After impregnation is completed, and the specimen is brought to room temperature, the xylene vaporizes out of the specimen. This results in a specimen with a little less polymer in each cell and tissue compartment and hence a lighter and more pliable specimen. As the xylene evaporates from the higher percentage xylene-impregnated specimes, the surface polymer is drawn rapidly into the specimen, leaving the surface dry with little or no polymer. The dry surface can be remedied by wiping polymer reaction mixture onto the surface of the specimen.

SUMMARY

Forced impregnation is a reliable method to deposit silicone into the cellular structure of a specimen. It may be done at room temperature or in a deep freezer. At room temperature, impregnation is faster. However, the pot-life of the reaction mixture is relatively short. Impregnation in the cold (around -20°C) is slower, but the pot-life of the reaction mixture is extended indefinitely. Therefore, less polymer is wasted and cost per specimen is reduced. The cost of the S10 polymer is similar to other types of silicone products available commercially but not suited for plastination. Routinely, the process must not be hurried. Vacuum must be increased slowly and should be monitored via a gauge or Hg column and a manometer. Impregnation is complete when the vacuum has been decreased nearly one atmosphere to a reading of 2-10 mm of absolute pressure and plastination bubbles have ceased.

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