S-10 TECHNIQUE

PLASTINATION

General Protocol of S-10 Technique

Fixation

- Types of fixation
  - Immersion
  - Infiltration
  - Injection
  - Perfusion
  - Dilatation
- Solutions
  - Formaldehyde solution (low to 10%)
  - Others:
    - Kaiserling's solution yields a red-brown color to the specimens.
    - McCormick's, Klotz, or Jore's solutions may help preserve color
- Dilate fix hollow organs and fix specimen in anatomically correct positions.
- Intravascular injection with red/blue epoxy resins, latex or gelatin.
- **RINSE** - formalin out of specimen with cool running tap water (1-5 days).

OPTIONAL - BLEACH - if necessary to brighten stained or darkened tissue.

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Dehydration

- **DEHYDRATE:** UNTIL 99% - 100% DEHYDRATED (3-6 WEEKS) IN:
  - COLD ACETONE
  - **Ethanol**
- **INTERMEDIARY SOLVENTS:**
  - ACETONE
  - Dichloromethane (methylene chloride)

OPTIONAL - STAIN: Add Biodur AC 10 Stain in the last acetone bath.

– 1.- Pre-cool the specimen (5ºC 12h in water)

– 2.- Acetone baths (-25ºC):

- Acetone 97% 1-2 weeks
- Acetone 100% 1 week
- Acetone 100% 1 week
  - After one week in each acetone bath, the purity is checked with an acetonometer after stirring the acetone bath
  - Make acetone changes at -25ºC, it will be less evaporation and we work under inflammation poin (-19ºC)

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Impregnation

- **IMPREGNATE:** Silicone polymer (**Biodur S10**) plus catalyst and chain extender (**Biodur S3**) (100:1 to .7 ratio).
    - In a deep freezer @ -15°C is recommended.
    - Gradually decrease absolute pressure (increase vacuum) one atmosphere (3-5 week period).
    - ROOM Temperature impregnation is possible and is completed much faster (2-3 weeks).
- When impregnation is complete:
    - **DRAIN** excess polymer from impregnated specimens, while in deep freezer, back into plastination chamber.

Polymerization

- **POLYMERIZE** (Cure) (Harden)**:**
    - **SLOW Cure (Precure):**
        - Room temperature (1 - 6) months), manicure specimens daily, then use gas cure **(Biodur S6)**.
        - Room temperature (1 - 2 weeks) + gas cure.
    - **B. FAST Cure**:
        - Gas cure (3-7 days) vaporize S6. Enhance volatilization by using an aquarium pump to bubble air through the S6 or a fan to circulate the S6.
        - Wipe excess polymer daily.
        - Bag specimen for 1-3 months.

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- **PROSECT SPECIMEN:**

  - Plan prossection: Do not attempt to show every detail of the specimen.
  - Highlight and enhance desired features.
  - Dilate hollow organs.
  - Consider leaving more tissue than you want to show in the final specimen (e.g. vessels, nerves, muscles, tendons). These can be cut after plastination, which will yield clean even surfaces.

Fixation

– Types de fixation

- **Immersion**: plunge a specimen into a liquid
- **Infiltration**: an accumulation of an abnormal substance in tissue, - may use in large specimens
- **Injection**: the act of forcing a liquid into a part
- **Perfusion**: the act of pouring over or through
- **Dilatation**: the act of dilating or stretching

WHY FIX?

- Fixative - is incorporated into the cell membrane and molecular structure of the specimen, making the specimen firm and minimizing shrinkage.

- To denature any tissue enzymes which:
  - May otherwise remain active even after dehydration and plastination and cause tissue decomposition,
  - May interfere with polymerization after impregnation.
- To disinfect the specimen (destroy bacteria, etc.).

- Fixative Solutions:
  - **FORMALIN** (1-20%) - most common fixative used.
  - Formalin = formaldehyde gas saturated in water (33-38%)
    - Dilute 33% stock solution as if it were 100% to 1-20% solution.
    - To prevent a fixative coat (hardened layer) that retards diffusion of fixative, start with lower concentrations (1-5%) and later increase to a high concentration (5-20%).
    - For more flexible specimen use lower % solutions (1-3%), for a shorter period of time.
    - If necessary to store specimen rather than continue processing it, remove the specimen from the fixative solution after a few weeks and flush with water for two day and put back into the low% solution.
  - Nothing or decrease fixation time to 24 hours.

– Others:

Kaiserling's solution yields a red-brown color to the specimens.

McCormick's, Klotz, or Jore's solutions may help preserve color.

TYPES OF FIXATION:

- **COLD FIXATION**: Retards tissue autolysis,
  - May enhance color preservation,
  - Does not decrease diffusion rate of the fixative.

- **RINSE** fixative out:
  - Use running tap water entering from the bottom of the reservoir,
  - One to several days.
- **BLEACHING**: Optional
  - Gastrointestinal, Urogenital, and Joint preparations.
  - 1-5% H_2O_2 (Hydrogen Peroxide):
  - 0.5% - Serosal covered organs (intestines),
  - 2% - Muscle.
  - 5% - Heavy adipose tissue.
  - **H_2O_2 Stock solution (35-50%):**
  - 2 pts stock solution to 98 pts water = 2% solution.
  - For bleaching longer than 1 week, add 2-5% formalin to prevent putrefaction.
  - Rinse with running tap water before dehydrating.

- **COLOR PRESERVATION**:
  - Cold fixation.
  - Kaiserling's solution.
  - McCormick's, Klotz, or Jore's solution.
  - Decrease fixation time.

- **COLOR ADDITIVES:**
  - ***Biodur Stain AC10** or **VisDocta VS-CS22** (topical) - add in last acetone bath:
  - Stains surface only.
  - E-20 - intravascular injection of colored epoxy:
  - 7 parts Biodur E-20 (polymer) to 3 parts Biodur E-2 (hardener),
  - penetrates capillary bed and give red color to specimen (Hearts),
  - yields a more rigid specimen (epoxy is hard).
  - Merthiolate - use in fixation solution.

Tips!!

- **Dilate-fix** hollow organs.
- Fix specimens in an **anatomically correct position**.
- **Nervous tissue:** Fix in 10 - 20% formalin for 4 - 8 months, perfuse and immerse.
- Specimens may be stored in 1 or 2% formalin solution.

Caution: Prepackaged embalming fluids may contain **long chain alcohols** (glycerol), glycols, or phenol. These may precipitate, after curing, leaving a chalky white residue on the specimen.

REMEDY for white ppt:

- Ethanol dehydration removes much of these alcohols,
- Ethanol plus H_2O_2 bath will remove most long chain alcohols,
- Most can be leached out and rinsed out using running water for several weeks.

- **FIXATION PRINCIPLES for BETTER SPECIMENS:**
  - Use low % formaldehyde fixative solutions.
  - Use Kaiserling's. McCormick's, Klotz, or Jore's Solutions - help preserve color:
  - Kaiserling's solution (1000 ml of 2 - 5 % Formalin, 30 gm K acetate & 15 gm K nitrate yields a reddish-brown color to specimens.
  - Keep fixation time minimal.
  - Use cold fixation.

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Dehydration

I.- Concept

- 1º - Remove and replace tissue fluid with an organic solvent
- 2º - To replace the solvent with an appropriate "volatile intermediary solvent" (acetone or dichloromethane), which has the appropriate properties for extraction during the impregnation process.

II.- Types of solvents:

- The solvent must be miscible with water: monohydric alcohols (methanol, ethanol) or ketones (acetone, methyl ethyl ketone).
- Minimize shrinkage by:
  - Step wise dehydration (ethanol),
  - **Freeze substitution (acetone)**.

- ***Saturated vapor pressures in mm***

^{o}C	(P) MeCl	(P) Acetone	(P) H_2O
20	357.0	125.0	17.5
5	186.0	84.0	6.5

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–	**10**	**90.0**	**37.0**	**2.16**
–	25	40	15	0.476
–	40	12.1	5.5	0.096

Standards: **CRC Handbook of Chemistry and Physics**, Vol 53: p. D151f

56.50 °C 686.7 mm Hg - T & P of Normal Boiling point of Acetone

39.75 °C 781.6 mm Hg - Normal Boiling point of Dichloromethane

100.00 °C 760.0 mm Hg - Normal Boiling point of water

Acetone:

– Acetone's boiling point **(56.5°C)** (vapor pressure) is appropriate to be extracted from the specimen in the plastination (impregnation) process. Therefore, it does not need to be replaced as ethanol does. **ACETONE is an "Intermediary solvent."**

III.- **Principles for a good deshydration**

- Tissue/fluid ratio = 1:5 (1:10 = classic ratio).
- Fill hollow organs with cold acetone prior to submersion.
- Freeze specimens in anatomical position.
- Accelerate dehydration by stirring or agitation daily.
- Keep specimens **submerged** in the dehydrant to prevent air-drying of the specimen.

Prolonged time (several months) in acetone: May decrease flexibility & durability

IV.- **Dehydration Duration**

- Time of dehydration depend of specimens (volume and hardness)
  - Brain, heart: 3-5 weeks
  - Human head, liver,etc. : 5-6 weeks
  - Stomach, intestine, etc.: 7-10 days
- Time depends also on:
  - Ratio 1:10 (specimen volume /acentone volume). High ratio less time

V.- **Equipment**

- Freezer
- Acetone containers
- Acetometers
  - Comercial Freezer (adaptation):
    - Remove the interior light
    - Remove thermostat out of lab
    - Remove compressor out of lab
  - Plastination freezer (explosion proof):
    - Safty
    - Very expensive (4-6000 €)

Deep Freezer:

Need at least 2 large - Preferably 3

Household - $1,000.00.

May be made explosion proof by removing the light in the door and placing the compressor and motor behind a wall in an adjoining room.

Explosion proof ($8-10,000), explosion safe.

- Acetone containers:
  - Stainless steel
  - Acetone resistant Plastic
  - At least one similar size to plastination unit.

Larger vat sizes - best to have specimen baskets.

Nalgene (high density polyethylene) 15 gallon cylinders [Fisher # 14-831-310B, pg 342 @ $81.06. # 14-831-310F (10 gallon) @ $90.00 work well as do 5 gallon buckets that cleaning supplies, pickles and many other items.

Custom made stainless steel with lid is what is needed for larger specimens. Custom made to fit the size of your larger specimens and large vacuum chamber.

- **Acetonometers**
  - Calibrated for accuracy at a given temperature: +15°C, +20°C, or -10°C
  - The value 100% is the maximum density of acetone (0.79)
  - The value 0% is the water density

Fisher - Tralle & Proof ThermoHydrometer, #11-595 (larger) - $40.00. These are calibrated for 60 ^{o}F. Therefore, if you are checking **cold** acetone, you should let it warm to60

^{o}F. However, a temperature scale is included so that you may adjust your result for any variation in temperature. If less **than** the temperature calibrated at (60 ^{o}F), some % will be **added**. Likewise, if the temperature is **more** than the calibrated temperature, % will be **subtracted**. This type acetonometer or (**HD 01+20^{o}C)** is needed for acetone less than 90% pure, i.e. after you have dehydrated your initial batch of specimens. With acetone of less than 90% purity, it is not crucial to know the precise %. However, the closer acetone is used near 100%, the more precise the reading % needs to be. Therefore, a more precise (with less range) acetonometer is needed: VisDocta or Biodur.

Three of the following acetonometers are more precise but measure only acetone that is in the range of 90 - 100% purity.

VisDocta - Italy:

VS-ACM +15^{o}C: 90 -100%

VS-ACM -10^{o}C: 90 -100%

Biodur - Germany:

HD 01 +20^{o}C: 0 -100%

HD 02 +20^{o}C: 90 -100%

VI.- Protocol

Cold ACETONE is **preferred** for: All specimens.

– **Cold ACETONE**: "Freeze substitution" (-25^{o}C).

- ****Recommended DEHYDRATION method!!**
- Pre-cool specimen to 5°C.
- Three changes of cold 100% Acetone: 7-day intervals.
  - Cold acetone yields less than 10% shrinkage instead of up to 50% with other methods,
  - Dehydration time cut by 50%,
  - *Check **acetone %** weekly with acetonometer (hydrometer)
  - After the purity of acetone is known, upgrade specimens into more pure acetone (higher %).
- Recommended for **nervous tissue**:
  - If room temperature acetone is used with nervous tissue: shrinkage is great.

–**Monitor** (hydrometer/acetonometer), upgrade and record % acetone weekly:

- **Measure and record used acetone %** with an **acetonometer:** Weekly check the purity with an acetonometer after stirring the acetone bath. The acetone temperature must match the temperature calibration of the acetonometer. Most acetonometers are calibrated at +15°C, +20°C, or -10°C. Therefore acetone must be warmed or cooled to match the calibrated temperature before measuring and recording the purity reading
- Weekly, put specimens in new (higher %) acetone.

–It takes 5 - 6 days for **water and acetone to reach equilibrium**.

- **Room temperature ACETONE:**
  - Three changes of 100% Acetone: 7-day intervals.
  - Not for nervous tissue,
  - More shrinkage.

Stepwise acetone dehydration (cold or warm) (70 - 90% acetone):

This method is available if you already have used acetone.

Do **not** dilute acetone by adding water:

Commence dehydration with previously used lower % acetone,

Weekly: Upgrade to more pure acetone,

DEFATTING (Degreasing)

– Decreasing the amount of fat in specimens **enhances specimen durability**;

- Fat is not completely impregnated with silicone

Defatting Agents:

1. **METHYLENE CHLORIDE (MeCl)**: Dichloromethane - is not miscible with water and therefore, not a dehydrating fluid. Therefore, the specimen must be **totally dehydrated** before immersion into dichloromethane. Its boiling point **(40°C)** (vapor pressure) is appropriate to be extracted from the specimen in the plastination (impregnation) process

- Strong defatting agent.
- Used routinely with ethanol dehydration as the intermediary solvent.

- Yields transparent adipose tissue.

- Can store specimen in Me Cl.

1 week in MeCl will defat most specimens.

HAZARDOUS to your HEALTH!

2. ACETONE:

a. Freeze substitution (adequate defatting),

- yields: **Cream to white adipose** tissue.

b. ***Enhance Defatting in Acetone***: Bring acetone with specimens out of freezer to room temperature, when at least 95% dehydration is reached **(*not brains)**,

1'. Change acetone weekly,

2'. After acetone is changed, Congeal fat in old acetone weekly in freezer and filter,

3'. **Accelerate defatting** by STIRRING.

- Yields: **White to semitransparent adipose** tissue.

STAINING DURING DEHYDRATION:

BIODUR STAIN AC10: A liquid red stain used in last acetone bath to effect (0.1 to 1 ml liquid stain in 10 L acetone).

VISDOCTA VS-CS22: Liquid reddish colored stain used prior dehydration or in last acetone bathes (to effect).

- Agitate; Specimens should not touch each other or the container (will leave an unstained area where they touch),
- Submerge specimens totally.
- Length of staining time: 6 to 24 to 48 hours,

Sample dehydration schedule:

Day one (Week 1): Immerse specimens in cold (or room-temp) Acetone (70 to 100%) (Do not make a lower % acetone; however, you may start in lower % if you already have used acetone).

After 1 week **(Week 2): a.** Stir acetone and check % of acetone purity (it should be lower than when you started) and record % of acetone.

b. Place specimens in new 100% acetone or a higher % used acetone. You can use this used acetone to start dehydration of your next group of specimens.

Week 3: Stir acetone and check and record % of acetone (It should be in the 90's).

Place specimens in new 100% acetone.

You can use this used acetone to upgrade your second load of specimens.

Defat: Bring specimens and their acetone (if in 95% or greater acetone) out to **room temperature** and let stand at room temperature to defat for at

least one week (until fat in specimen begins to become translucent or acetone turns yellow).

Week 4: a. Stir acetone and check and record % of acetone (It should be about 100%) (This acetone is full of fat and can **not** absorb much more fat).

b. If defatting is complete*: Place specimens into the polymer mix or

b'. If more defatting is necessary - into new acetone.

b". Add Biodur AC 10 Stain to effect for pink color.

c. Place this used fat-filled acetone in the deep freezer for 1 day. Next day Filter through a Teri towel to remove the fat and use the acetone somewhere appropriate in the process.

*Check specimens to see if they are defatted enough. If so, place in the cold polymer. If not defatted enough, **a.** Place in new 100% (room temperature) for 1 more week and repeat or.

b. Place in methylene chloride for 3-5 days.

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Impregnation

Concept

- It is the main step in plastination
- Replace intermediary solvent (acetone) with silicone. Forced impregnation is the process of drawing a solvent (acetone that has replaced tissue fluid) out of the cellular structure and interstitium of the specimen and **replacing it with a curable polymer**. Facilitated by the high vapor pressure of the intermediary solvent and the low VP of the polymer reaction-mixture. As pressure is decreased, the solvent boils and its vapor is pumped off leaving a tissue void and thus the polymer-mix is drawn into the tissue (cells and interstitium).

- **Physical and chemical characteristic of solvent and polimer:**
  - **ACETONE's Vapor pressure @ -10 °C = 37 mm Hg.**
  - **Dichloromethane** [Methylene chloride (**MeCl**)] **VP @-10 °C = 90 mm Hg.**
  - Boiling point of the **Polymer-mix** = 295°C, therefore it is not reactive **@-10 °C** and a pressure of **1 or 2 mm Hg.**

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– Plastination or Impregnation of a biological specimen with a curable polymer is based on the difference in boiling points of the volatile intermediary solvent and the polymer-mix. The solvent is boiled off as the absolute pressure is decreased (vacuum is increased). This leaves a tissue void and the polymer-mix is drawn into the tissue (cells and interstitium).

Equipment

PLASTINATION UNIT - Vacuum chamber for plastination:

- **Biodur:**
  - **Small** "Heidelberg kettle": 49 cm H x 31.5 cm diameter (35 L) - **(Plastination kettle HI 02)** and Specimen basket (HI09) (20 cm H x 29 cm diameter).
  - **Larger** "Heidelberg kettle": 49 cm H x 31.5 cm diameter (100 L)- **(Plastination kettle HI 03)** and Specimen basket (HI09) (20 cm H x 29 cm diameter). No basket available (you can make your own from hardware cloth and wire)

Common laboratory equipment may be used: Labcon desiccator (1 foot cube) or glass bell jar desiccator for small specimens.

Biodur, RW Henry or VisDocta: can **make plastination kettles** to your size specifications. A specimen basket to contain the specimens is recommended. This also serves to keep the specimens submerged and allows ease of draining the excess polymer at the end of impregnation.

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–**Vacuum Pumps and oil:** Oil needs to be changed after each impregnation.

–In U.S.A., Welch 1400 is recommended. @$1,300.00 [Fisher 01-096, page 1387], 2 are preferred, one is a back up. = $2,600. See list for European pumps.

–Dry (Oil-less) pumps are being used (Durability has not been established).

–Vacuum pump oil: 5 gallon @ $6.00 per gallon = $30.00

–**Valves and vacuum tubing:**

–2 needle valves, HI14 12.5 E each

–Vacuum tubing of some variety:

–24 feet of Red rubber tubing, - 1/4" i.d. x 5/8" o.d. (3/16" wall) 14-173C, Fisher Scientific, pg 1856, $39.02 (UT$21.42)/12 ft

–**Vacuum measuring devices:**

–Benneret-type Manometer @ $276.35 (UT$148.03); Fisher Scientific, pg 793, 11-292. See Biodur price list.

–Vacuum gauge @ $57.20 (UT$59.90) Fisher Scientific, pg 792, 11-279A

Protocol

After dehydration, the "solvent-saturated specimen" is **submerged** in a **polymer-mix** in a plastination kettle (vacuum chamber), in a deep freezer [-15°C (preferred)], cold room or room temperature.

"Classic" or Biodur method (cold temperature preferred): Silicone polymer (S10) plus & Chain extender and catalyst (S3), **Ratio 100:1 - 0.7.**

1. Polymer reaction-mixture: Mix 70 - 100 ml S3 (catalyst & chain extender) with 10 kg S10 (silicone polymer):
   - **a.** Keep in deep freezer once activated (mixed).
   - **b.** Ideal temperature for: **Impregnation: -15°C.**
     - **Storage: <-25°C.**
2. Dehydrated specimens are **submerged** in the reaction-mixture (S10/S3).
3. **Impregnation** - Seal port:
   - **a.** Let sit at atmospheric pressure over night,
   - **b.** Gradually decrease pressure one atmosphere: 2 to 5 weeks,
   - **c.** Monitor via pressure measuring devices (gauge, manometer and/or Hg column) and **bubbles**,
   - **d.** Complete when bubbles cease and or pressure stabilizes @ 0-5 mm Hg.

Sample schedule for deep freezer impregnation with acetone (intermediary solvent):

Day 1: Load specimens, submerge (secure with a grid) and let acetone equilibrate with the polymer.

Day 2: Start vacuum pump (with by-pass valves closed) and decrease pressure (increase vacuum)to **20** cm **Hg** and **stabilize pressure** by opening the vacuum valves until pressure stabilizes around 8 inches Hg (when using **deep freezer** @ - 15°C). Air bubbles (small 2-5 mm or even larger bubbles) will be observed rising to the top of the polymer.

At Room Temperature: decrease and stabilize absolute pressure (increase vacuum) **(38 cm) Hg(15").**

Why?? Acetone's Saturated vapor pressure is: **160.5 mm Hg @ 20°C**. Therefore, boiling [vaporization of the solvent (acetone)] occurs at a higher pressure than at **-15°C.**

Mark needle valve to demarcate the rotation points of the valve.

How to decrease and stabilize pressure?

1. Decrease pressure by **partially closing** the in-line vacuum by-pass (needle) valve.
2. Stabilize pressure by **leaving the valve** at the partially closed position when desired pressure is achieved.

Day 3: If no bubbles continually rising to the surface, Decrease absolute pressure to 9 cm (3.5") Hg by closing one of the needle valves, **1/2** turn. More air bubbles may rise. In the **freezer**, around **25"** (63 cm) **Hg, acetone** will commence to vaporize continuously, forming bubbles (1 cm in diameter) which actively rise to the surface of the polymer reaction-mix. **Impregnation has begun!** Around **20" Hg, dichloromethane** will commence to vaporize. The exact pressure for vaporization of the solvent

is temperature and pressure dependent. i.e. At **-5°C**, vaporization will commence at a higher absolute pressure than at -15°C, etc.

At Room Temperature: acetone vaporization commences @15" of Hg. Adjust (decrease) pressure accordingly.

"PLASTINATION PRINCIPAL" - ***If BUBBLES*** are forming and actively rising to the polymer surface and bursting, **DO NOT DECREASE Pressure (do not increase vacuum)!!**

Continue to monitor bubbles and pressure daily!!!

Day 4: If no bubbles continually rising to the surface, Decrease Pressure ½ - 1" Hg to around 13 cm (5") Hg by closing one of the needle valves, **1/8** or **1/4** turn. Continue the same over the next few days until bubbles continually rise to the surface.

Day 5: If no bubbles continually rising to the surface, Decrease Pressure to 10 cm (4") Hg by closing one of the needle valves, maybe **1/8** or **1/4** turn. However, by this low of pressure (high of vacuum) there will likely be bubbles*.

***Any day** you see **bubbles rising** to the surface of the polymer and bursting **do not** decrease pressure!!!

Day 6: If no bubbles continually rising to the surface, Decrease Pressure to 7 cm (3") Hg by closing one of the needle valves, maybe **1/8** or **1/4** turn.

Day 7: There will likely be many bigger bubbles (depending on the number and/or volume of specimens), **DO NOT DECREASE PRESSURE**!!!

Day 8 and forward: If no bubbles, Decrease Pressure **1 cm (1/2"**) Hg. However if bubbles are actively forming, rising and bursting, DO **NOT** DECREASE PRESSURE!!!

Around 1.5 cm (0.5") Hg pressure (1.5 cm Hg from total vacuum), the continuously rising bubbles will likely become larger. These are larger concentrations of acetone vapors. It is advisable to pump for a few days after the larger bubbles commence to assure that all of the acetone has been vaporized and hence pumped off. It may not necessary to pump until all of the bubbles disappear.

If a large load of specimens, it will likely take 4 to 6 weeks to get cessation of bubbles or a pressure of 0-5 mm of Hg (near total vacuum).

A small load will likely be done in 3 to 4 weeks, or even sooner.

****Go too slow rather than too fast!!**

Specimens tend to float in the polymer. Keep specimens submerged with a grid or in a basket.

Decrease absolute pressure (increase vacuum) **slowly** over a 3-5 week period in the *freezer*,

Or 7-14 days at *room temperature*.

Monitor vacuum via: Vacuum gauge, Manometer and **Bubbles** (Bubbles should actively rise, but slowly to the polymer surface).

Once room temperature **impregnation is complete**, the specimens are **DRAINED of EXCESS POLYMER**

A'. Post-Impregnation:

Shut off freezer for 24 hours (warmer polymer drains off specimens better).

Open vacuum chamber and raise basket to drain specimens.

Remember to: Turn freezer back on.

A". Drain excess polymer from specimens**:**

First into vacuum chamber:

Raise basket of specimens above polymer level,

Turn specimens 2 - 3 times.

Other products and reaction-mixtures:

VisDocta (Italy) method (cold temperature)**:** Polymer-Catalyst/Chain extender ration - Similar to the Classic von Hagen's method.

Dow/Corcoran method (room temperature)**:** Polymer (CT10) plus Cross-linker (CT30), **Ratio: 100:6-10.**

Neat method (North Carolina) (Room temperature)**:** Polymer (any) with not additives.

Neat method (North Carolina) (Cold temperature)**:** Polymer-Catalyst/Chain extender ration -Same as Classic von Hagen's method.

ROOM TEMPERATURE PROCESS:

- **IMPREGNATION** may be carried out at **room temperature** with the **Dow method, Neat method (U.S. generic polymers)** or even the **Biodur method**. At room temperature the polymer-mix is less viscous and hence will penetrate the tissue easier. Therefore, impregnation can take place at a faster rate (8-12 days). Larger bubbles and more rapid bubbling will commence around 27"Hg

vacuum (7 cm from total vacuum) indicating impregnation is nearing completion.

- However with the **Biodur method**, at room temperature, the polymer-mix will begin to harden and after several weeks and eventually (after many months) become too thick for impregnation. Once impregnation is complete, place the polymer reaction-mixture into the deep freezer to stop linkage of molecules.

Storage @ **-25°C** preserves the polymer reaction-mixture for a long time.

Once **impregnation is complete**, the specimens are **DRAINED of EXCESS POLYMER**:

A. Drain Excess polymer at -15°C (or turn freezer off for 1-2 days and allow specimen to warm up while draining):

- Turn specimens after a few hours.
- Allow polymer to drain into the plastination kettle for reuse.

B. Bring the **specimens to room temperature** (out of freezer and turn freezer back on) and continue to drain **Excess polymer** at room temperature (collect in a pan).

- More polymer will drain from the specimen at room temperature.
- Turn specimens.
- Blow excess polymer out of hollow organs with laboratory air.
- Dilate hollow organs with laboratory air.
- Return the drained polymer to the plastination kettle **daily** for reuse.

Specimens may be stored in the deep freezer until you are ready to cure them.

C. Proceed with Slow or Fast Curing.

Silicone polymer:

The **Biodur process** is recommended for perfect results. The 2 new US processes (Dow/Corcoran & NC Neat) seem to be a close second and work fine especially for special needs. The Dow process produces acceptable specimens but of lesser quality due to a film of polymer on the surface which minimizes surface detail. Time will tell! The NC Neat process produces specimens with usually equal surface quality as the Biodur process, but they may not cure throughout. Neat works especially well with hairy specimens. **Time will tell!**

For the Dow process, I would only purchase **or at least only mix** enough reaction mixture to do one load at a time. **Why??** The reaction mixtures (Dow/Corcoran vs. Biodur) can **not** be intermixed (they will polymerize). The Dow or NC Neat processes maybe best if deep freezes or time are barriers.

Review article: von Hagens, G, K Tiedemann, W. Kriz. 1978: **The current potential of plastination.** Anatomy Embryology 175 (4):411-421, 1978.

Henry RW, Nel PPC. 1993: Forced impregnation for the standard S10 method. J Int Soc Plastination 7(1):27-31.

von Hagens G. 1985/1986: Heidelberg Plastination Folder. Anatomisches Institut I, Universität Heidelberg, D-6900 Heidelberg.

Weiglein A, Henry RW. 1993: Curing (Hardening, Polymerization) of the polymer - Biodur S10. J Int Soc Plastination 7(1):32-35.

Recomendations

Polymer:

Currently for most uses the German **process** is **recommended**. The German polymers are about 10% cheaper than local generic polymers of the U.S.A..

A. Large quantity polymer for large chamber: 200 to 400 pounds of silicone polymer will be needed to be able to submerge legs in and plastinate them. Cost is about $14.00 per pound. = $3,000 to $5,000

1. 50 to 200 kilogram of silicone polymer to be used with catalyst for impregnation: **$20.00 - $30 per kilogram.**

B. Hardeners and activators for polymer:

1. 0.5 - 2 liters S3 (Catalyst and chain extender):
2. 2 - 10 liters S6 (Cross-linker):
3. 2 - 10 liters S7 (Chain extender):
4. 0.2 liters Specimen stain (AC 10) $23/ 0.1L

UNIVERSIDAD DE MURCIA

PLASTINATION

S-10 TECHNIQUE

Polymerization (curing)

Concept

Once a specimen is impregnated with silicone, we want to keep the polymer in the specimen. This is accomplished by hardening the polymer.

Polymerization started when the S3 (catalyst & chain extender) and S10 (polymer) were mixed. However, cold (-15°C) retards this reaction. Once the specimens are warmed to room-temperature, curing continues at a slow rate from the presence of S3. **1.** The **chain extender** causes elongation of the silicone molecules into chains. The molecules line up "end to end" and attach, which hopefully **increases flexibility**. **2.** The **catalyst** prepares the silicone molecules for cross-linking when the S6 (hardener/cross-linker) is added.

After **excess** polymer has been drained, wipe surface of specimen to remove any excess polymer.

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GAS CURE (Biodur S6) - what happens:

S3 causes the silicone molecules to form elongated chains, by the silicone molecules lining up and attaching **"end to end"** and **prepares** the **molecules** for **cross-linking**.

The longer the molecule the more flexible the specimen.

S6 or Gas Cure (cross-linker) commences **"side to side" linkage** of the silicone polymer molecules, yielding a **firmer** but more brittle specimen.

S6 is a weak acid.

- Equipment

GAS CURE: in an airtight container:

1. Plexiglas container, Nalgene tub, Cardboard box, Plastic bag, Old deep freezer or oven.

- your imagination is the limit.

2. Decrease humidity - Desiccant ($Ca\ SO_4$).
   - Excess humidity may cause a white precipitate (silicates).
   - However, some moisture is needed for curing.
3. Seal top of chamber with plastic wrap and spray glue.
4. Aquarium pump or a fan may be used to increase vaporization.

- Types of polymerization/Curing

Two basic gas cure Sequences carried out at room temperature:

SLOW CURING:

1. **Precure (slow cure):** Continue draining specimens on toweling at room temperature as long as you wish (1 month to 1 year).

1. Precure (slow cure):

a. Catalyst and chain extender are very active at room-temperature. Polymer is beginning to thicken (harden). Make sure specimen is positioned anatomically correct and keep drips wiped.

1' Silicone molecules are elongating.

b. Long term precure (2-56 weeks): may yield a more flexible specimen:

- Limbs preps of muscle and tendon.

c. Room temperature.

d. Place on absorbent disposable toweling over brown paper or newspaper to protect counter.

e. Wipe excess polymer off daily or after week 1, every other day.

1' Specimen is beginning to get firm.

f. Pack hollow organs with toweling and position in anatomical position.

g. Flow air into hollow organs to redilate.

Optional: h. Heat: 40 to 50°C:

- Don't use heat before 2 weeks post-impregnation (excess polymer will ooze out):

- Until tacky (heat speeds up tackiness, chains of molecules are elongating).

- If no heat, it may take 2-3 months to become tacky.

-Heat causes more shrinkage, especially if used to soon (in the beginning, excess polymer oozes out, because heat thins the polymer).

****Heat is not essential.**

2. FAST CURING:

a. **Manicure** 2-3 times a day:

- Until specimen is no longer oozing polymer,
- Curing occurs rapidly after S6 is added.
- Blow out excess polymer from hollow organs and lungs with air and dilate.
- Place toweling in or around organs.

b. Place drained, room temperature specimens on toweling in a contained environment.

c. Add S6 (gas curing agent) (cross-linker/hardener), which will vaporize and react with the polymer in the specimens:

1'. Increase vaporization of S6 via an aquarium pump for 5 - 10 minutes, thereby, increasing the gaseous concentration and hence accelerating curing.

2'. Cross linkage occurs at a **rapid rate when S6 is applied**, especially on the surface of the specimen.

3'. Elongation of silicone molecules has already occurred in the vacuum chamber and elongation continues at an increased rate

when warmed to room-temperature, due to the S3 which was in the polymer reaction-mixture.

d. Surface polymer hardens in 24-48 hours:

- thus sealing the organ from loss of excess polymer.

e. Must manicure surface "runs" before they harden:

Suggested procedure:

1'. Inspect specimens and wipe oozing polymer twice a day.

2'. Start gas cure late afternoon and let it proceed all night. By morning, the runs need to be wiped off, and usually by late afternoon, the surface is hardened.

a'. Can place in deep freezer to retard curing.

3'. In 2 - 5 days surface will be sealed.

4'. Place toweling in depressions and inside hollow organs (if opened) to absorb excess polymer.

e. Keep specimens in closed container for 2 - 4 weeks.

f. Store specimens in plastic bag (closed container) for 2 - 4 months to assure depths of the specimen are cured:

1'. The surface is saturated with S6 (gas cure):

- S6 continues to diffuse slowly toward the center of specimen.

2'. Dilate Cure:

a. Thin wall, hollow organs and lungs.

b. Bubble air through a bottle containing a small amount of S6 into the organ to inflate and cure hollow organs from the inside out.

1'. Use a small amount (2-5 cc) of gas cure:

c. Place organ in an enclosed environment.

FAST CURE:

- Increases chance of white spots and decreases flexibility and durability,
- Decreases shrinkage, therefore good for freshly fixed tissues,
- Allows specimen to be used within 2-3 days.

Fast cure nervous tissue for 1-3 weeks at **-15°C**, using an aquarium pump to volatilize the S6. This may help decrease shrinkage.

PreCURE:

- Only real problem is: Increased shrinkage; therefore good for long time formalin-fixed specimens (they are already shrunk),
- Decreases chance for white spot formation,
- More flexible and durable specimen,
- Nearly fool proof.

Ways to decrease shrinkage or polymer loss during slow cure:

1. Place cut surface up - oozing a function of gravity.
2. Smear surface with old, thicker, sticker polymer, (saved from draining previous specimens).
3. Place slices - between plastic wrap or glass plates,
   - turn daily.
4. Wrap specimens in plastic wrap or vacuum bag specimens 3-4 weeks.

Glossy vs. non-glossy surface:

Glossy - smear with older polymer after surface is cured.

Non-glossy - wipe often and keep on absorbent toweling.

Problems: SHRINKAGE

1. **Incomplete dehydration:**

Why? - not 1:5 tissue/fluid ratio,

- not long enough period of dehydration (especially in ethanol).

2. **Incomplete impregnation**:

Why? - too rapid evacuation (boiling),

- not low enough vacuum to vaporize acetone (decrease to 5 mm Hg).
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