

PRINCIPLES OF PLASTINATION - DEHYDRATION OF SPECIMENS

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Specimens to be plastinated are often moist which necessitates the removal of tissue fluid (**dehydration**) before forced impregnation or plastination can be carried, out. Dehydration removes the specimen fluid (water), as well as, some fat. The tissue fluid is replaced with an organic solvent. To be a dehydrating agent, the solvent must be miscible with water and may consist of a variety of chemical structures (ketones or alcohols). Either alcohol or cold acetone maybe used as a dehydrant for plastination. Methylene chloride (chlorinated hydrocarbons) is not a dehydrating agent. Shrinkage accompanies dehydration and may be minimized by: 1) using cold acetone (known as freeze substitution) or 2) starting dehydration in a lower % of ethanol. With freeze substitution, the ice in the specimen is replaced by the dehydrating liquid (acetone). It is essential to use an adequate volume of dehydrating liquid (either cold acetone or ethanol). The recommended ratio is: 10 volumes of dehydrating fluid to 1 volume of tissue. It is necessary to monitor the concentration of the dehydration fluid at weekly intervals. Once the fluid content has remained similar for a few days, the specimen is moved to a fresh dehydrating solution. **Cold ACETONE (-15° to -25°C):** usually has been considered the best method of dehydration. However, dehydration with acetone must be carried out in the cold and not at room temperature; warm acetone will cause excessive shrinkage and complete dehydration may not occur.

Disadvantages: must be done in a deep freezer and acetone is a hazardous material.

Advantages: Minimal shrinkage; Acetone serves as the intermediary solvent; Superior specimens are produced; Dehydration time is shorter and previously used acetone (70% - 90%) maybe used to commence dehydration. **ETHANOL:** Specimens are started in a low % of room temperature ethanol (50%), allowed to equilibrate and later placed in

ascending concentrations of ethanol, ie: 60%, 70%, 80%, 90%, 100%. Carried out at room temperature; therefore, less deep freezer space is necessary. Specimens can be stored in 70% ethanol. Specimens from embalmed tissues, containing standard embalming fluids, are cleansed of the polyvalent alcohols (glycerin or ethylene glycol) or phenols. Specimens are defatted.

Disadvantages: excess shrinkage and the dehydrated specimens must be saturated with intermediary solvent [acetone or methylene chloride (dichloromethane)]. Why? The saturated vapor pressure (boiling point) of ethanol is too low to be slowly extracted at -15°C and allow concurrent influx of the silicone polymer. As for the choice of intermediary solvents, methylene chloride may be more cost and time efficient, but it is more hazardous. An inherent problem with using acetone is that the specific gravity of ethanol and acetone are similar (0.79) making it difficult to determine when the ethanol has been totally replaced with acetone. When specimens are totally dehydrated they are ready for impregnation with the silicone polymer mixture.

RECLAMATION of ACETONE by FREEZE VACUUM DISTILLATION

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SUMMARY

Reclamation of large volumes of acetone by freeze-vacuum distillation was practical, simple, economical to perform, and environmentally wise. The apparatus, constructed primarily from items found within a plastination laboratory, proved to be effective for the distillation of the various percentages (45 - 94%) of acetone used for conducting this study. Three liter aliquots, of known acetone content, were distilled over a six hour period and resulted in reclamation of 94 to 98 percent acetone. Further distillation, of the remaining lower percentage acetone (2 - 20%), provided residual solutions to as low as 1 percent acetone. Freeze-vacuum distillation has served to

reduce operating costs both by reclamation of large volumes of quality acetone and significantly reducing the volume of hazardous waste which must be disposed.

INTRODUCTION

An integral part of the plastination process is dehydration of specimens, a process which exchanges tissue fluid (water) and excess fats with an organic solvent. Cold acetone (-25°C) is usually the best solvent for dehydration (von Hagens, 1986; Henry, 1992). This process, however, leads to an accumulation of waste acetone (contaminated by water and fats) which necessitates not only the purchase of new acetone, but the disposal of the old acetone as a hazardous waste. Although previous work has characterized methods for effectively distilling acetone (Roark, 1992; Grodin and Berube, 1992), the practicality of freeze-vacuum distillation for both large volume applications and significant reduction of hazardous waste has not been addressed.

MATERIALS and METHODS

- (1) Nalgene 114 L (30 gal.) tank(60x60x30cm.)
Nalgemfg. * 141000021
- (1) Large Pyrex dessicator I.D. flange 250mm with
a 55/38 sleeve Corning mfg. #3120250
- (2) 5 meter Rolls of 6mm O.D. copper tubing
- (1) Needle valve (HI14, Biodur)
- (1) Nalgene polyethylene vacuum tubing
- (1) vacuum gauge reading inches of Hg
- (2) Bi-vented 2 liter cylinders fabricated
from 18 gauge stainless steel
- (1) vacuum pump (10)
- medium size rocks (1)
- Sub zero freezer

Prior to distillation, fat was removed from waste acetone by freeze separation (Grodin and Berube, 1992). A three liter aliquot of the waste acetone (45 to 94 percent) was placed into a dessicator along with 10 medium size rocks. The rocks served as a bumping agent to catalyze the vaporization of the acetone. The dessicator and acetone were warmed in a 40°C water bath (Nalgene tank). Vacuum was applied, increased until the acetone boiled (19 to 25 inches of mercury), and then

stabilized by adjusting the needle valve. As vacuum increased, the liquid acetone vaporized and flowed, via polyethylene tubing, to a copper condensing coil in -15°C freezer. The combination of warming the liquid waste acetone and applying vacuum facilitated the vaporization of the acetone in the dessicator. With the subsequent chilling of the vapor in the condensation coil, much of the acetone returned to the liquid state and collected in the first canister. Any remaining acetone vapor passed through the second copper condensing coil and most of any remaining acetone was collected in the second canister. The system concluded with the vacuum line coursing from the second canister, out of the freezer, to an elevated vacuum pump. A needle valve, for vacuum regulation, was placed in this line. Each 3L aliquot of waste acetone was distilled for a six hour period. Reclaimed acetone was collected at 2 hour intervals. Subsequent to each collection, the vacuum was re-applied to a level sufficient to promote further boiling.

RESULTS

Reclaimed acetone (over 6 hour periods) was collected with purities reaching 98 percent from the higher distillates to 94 percent from the lower distillates (Table 1). Maximum volume loss for this operation was maintained at or below 4 percent indicating a fairly efficient system. Residual waste products ranged from 20 to 2 percent in a corresponding fashion. Further distillation of small quantities of low percentage acetone was not efficient. However, distillation of cumulative solutions (3 liters of 2 to 20% acetone) did prove effective for reducing the percentage of acetone to near 1 percent. Depending on local restrictions, this concentration (1%) of acetone may be easily disposed. Even if disposal as a hazardous waste is required, the overall quantities become considerably reduced (Table 1). Therefore, this procedure reduces the cost of waste disposal and reclaims large volumes of acetone for re-use in the dehydration process.

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Table 1
3 Liter Distillations - Varying % Acetones

% Acetone	Total Volume Collected	Total Volume Remaining	Total Volume Lost	Volume lost % of total
94%	2580 ml 98% Acetone	300ml 20% Acetone	120ml	4%
87%	2425 ml 97% Acetone	460ml 14% Acetone	115ml	4%
70%	2000ml 97% Acetone	900ml 10% Acetone	100 ml	3%
58%	1370ml 95% Acetone	1370ml 5% Acetone	80ml	3%
45%	1220ml 94% Acetone	1760ml 2% Acetone	20ml	1%

RECLAMATION APPARATUS

