

THE USE OF EXPLOSION PROOF FREEZERS IN PLASTINATION: ARE THEY REALLY NECESSARY?

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SUMMARY

At first it was suspected that personnel safety might be a drawback of plastination with increased exposure to toxic chemicals for those involved with preparing and processing the specimens. The data from this study suggests that health and safety are not a major problem as long as proper precautions as described above are observed.

It is recommended, however, that all precautions be taken to minimize the risk of explosion. Although not considered optimum protection it is possible to modify domestic deep freezers, by removal of motors, compressors and lights to achieve some degree of protection.

Despite our results showing the danger of explosion to be minimal, it should be noted that optimal conditions are used throughout our laboratory. Since these optimal conditions are not always possible, it is wise to modify the freezer as suggested by Gubbins (1990).

INTRODUCTION

In the short period of its existence, plastination has proven its superiority over older methods for preservation of biological tissues (von Hagens, 1985). Despite having been adopted by many institutions around the world, the cost for the implementation of a laboratory destined to produce medium and large sized specimens is nearly prohibitive, especially when budget funds are limited.

One primary concern regarding equipment cost is the requirement for explosion proof freezers, that on the average cost \$8,000.00. Are these expensive freezers really necessary?

While plastination reduces exposure of students and instructors to formalin, the plastination process itself may add some safety and health concerns to those preparing the plastinated specimens. These concerns are the possible exposure of employees to acetone, various resins and curing agents, as well as, the danger of explosion in the dehydration and curing phases. In explosions, not only are employees at risk but also any resources that might be in the building or adjacent buildings.

For the purposes of this study, an initial hazard review was conducted to determine what hazards

might be present during the plastination process. From this prospective, the various steps of the plastination process were evaluated to: 1. determine where exposures to toxic chemicals might occur, 2. quantify possible exposures to hazardous chemicals, and 3. measure flammability of some of the chemicals.

MATERIALS AND METHODS

For the purpose of determining what chemicals would be present during the plastination process, approximately 30 mg of Biodur S10/S3 resin mixture was taken from the vacuum chamber and 10 ml of Biodur S6 (gas cure agent) from storage and both were analyzed by gas chromatography and mass spectrophotometry. The components found were: acetaldehyde or ethylene oxide, ethanol, acetone, xylene, and possible ethylbenzene and tetraethyl silicate.

Various methods were used to determine if ambient concentrations of the various chemicals exceeded levels that are considered safe. These methods included using sorbent media, flammable/toxic/oxygen (triple) gas meter, and an infrared spectrophotometer to collect average ambient concentrations of vapors during different phases of the plastination process. The triple and infrared instruments were of the direct reading type, and the sorbent media required subsequent description and outside laboratory analysis.

The recommended methods of sampling and analysis as set forth by The Occupational Safety and Health Administration and National Institute of Occupational Safety and Health are summarized in Table I.

EXPOSURE MONITORING:

Our vacuum system is setup to exhaust directly into the room air, at approximately 1.5 meters from the work bench. All samples for chemical concentration analysis of the room air were collected just above the work bench. Unless otherwise stated all measurements reflect the concentration of the chemical being manipulated plus those released through the exhaust outlet of the vacuum pump.

Chemicals:

Formaldehyde: The analysis for formalin was done when the specimens were removed from the sealed

bags of fixative and placed in running water for twenty four hours. Sampling was conducted for formaldehyde only and not methanol because the methanol is present in lower concentrations and is of a much lower toxicity.

Acetone: Sampling for acetone was conducted ambiently, at the work bench site, when the specimens were transferred to more concentrated solutions of acetone. To estimate the quantity of acetone vapors that would be released when the freezer is opened, sampling was conducted inside a deep freezer, with the door closed. The freezer contained five containers, each with 10 liters of acetone and two carboys with 20 liters each. Also, the pump exhaust was sampled at the work bench site.

Xylene: Sampling for xylene was conducted ambiently when the specimens were in the Gas Cure Phase. A cloth saturated with xylene is utilized to wipe the excess polymer from the surface of the curing specimen. This procedure aids in reducing the gloss of the specimen.

Resin components: During forced impregnation, air samples were taken above the work bench to check for the potential release of resin components into the air. During the gas cure stage, samples were taken above the curing chamber.

Equipment:

Sampling was conducted using personal air sampling pumps (SKC Hall 224-43XR) at sampling rates, times and sorbent in media filled collecting tubes as specified by the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health. These agencies also recommend methods for analysis (Table I). The sampling media used were two-section sorbent tubes with various media for collection. The first section is designed to collect the contaminants. The second section is used to determine if the first section breaks through to the second section. If so, the results were considered to represent an estimate of the minimum concentration of analyte present in the sampled air. Upon analysis using varied methods (depending on the contaminant), the amount of contaminant detected was divided by the quantity of air to yield the ambient concentration of the contaminant in air during the sampling period. The sample pumps used were calibrated before and after sampling using a primary calibration source (detergent and water solution in a volumetric burette). The burette was inverted and the soapy solution emptied into a beaker. The sample pump was connected by tygon tubing to the top end of the burette and the other open end of the burette was touched to the surface of the beaker of soap water. Bubbles from the soap film move into the burette with the flow of air created by the sample pump.

The sampling rate of the sample pump was calculated by the volume of bubbles which passed into the burette divided by the time it took to pass.

The Miran infrared spectrophotometer was used to measure the level of tetraethyl silicate during the curing process.

FLAMMABILITY MONITORING:

Because of the flammable nature of some chemical products used in plastination it seemed prudent to monitor several phases of the process to determine the likelihood of flammable vapors accumulating. A Mine Safety Appliance (MSA) Passport Personal Alarm Monitor with combustible channel calibrated to methane and a Foxboro Miran infrared spectrophotometer were used to estimate vapor concentrations during the dehydration, impregnation, and curing phases. The MSA Passport was used to estimate if potentially explosive levels might be present in the room at the vacuum pump exhaust outlet and inside the deep freezer containing the acetone for dehydration. Air sampling was performed both at room air (above the work bench, at 10 to 30 cm from the vacuum pump exhaust) and also, inside the freezer with the door ajar 1 cm and with door wide open.

RESULTS

EXPOSURE MONITORING:

The concentrations of chemicals in the air measured above the work bench are shown in Table JI. The resultant values for the reported chemicals, correlate with the various steps of the plastination procedure and the constant exhaust of the impregnation pump into the room air. Acetaldehyde was found in an average concentration of 0.04 ppm (Table II).

The room concentrations of acetone vapors, during transfer of specimens, initially demonstrated a concentration of 0.6 ppm over a 78 minute period. However, over a prolonged period of sampling (420 minutes) the acetone vapors increased up to 6.4 ppm. While acetone concentrations inside the deep freezer from two different samples were 1900 ppm and 3800 ppm. Sample break through occurred in the sampling tube.

Ethanol levels were 2 ppm. Ethyl benzene average concentration was 0.046 ppm. Ethyl silicate (found in the gas cure) average concentration was 0.035 ppm. Formaldehyde levels were 0.20 ppm when measured over a 215 minute period. Xylene levels were 0.088 ppm.

FLAMMABILITY MONITORING:

The concentrations, expressed in % lower explosive level (LED, provide by the MSA Passport Monitor are shown in Table III. LEL is the lowest fuel

to air ratio which will support combustion and propagate flame (in the presence of an ignition source). Atmospheres containing up to 10% of the LEL are recognized as safe, within an ample margin of safety.

DISCUSSION

As judged by the data in the Tables, the resultant concentration of most products monitored were very low, in fact most were below the level of analytical detection. The exposure limits given in Table II are thought to be the highest levels that a person can be exposed to on a regular basis without health risk. Generally upper limits are designed to protect more sensitive individuals, as well as, the average person. Given the present exposure limits and the data collected, there appears to be little health risk during the phases of the plastination process which were studied, as long as, the proper precautions and techniques are followed.

Proper precautions include wearing personal protective equipment when there is a possibility for physical contact with any of the chemicals, including the proper respiratory protection when vapor concentrations may near or exceed normal limits. As a good work practice, all containers should be kept tightly sealed. When working with formaldehyde, it is best to use local ventilation or wear a respirator. Ethanol was used prior to dehydration to remove any remaining glycol from the embalming fluid. The specimens were immersed into a solution of ethanol, hydrogen peroxide and water for one week.

As shown in the results, the possibility of flammable concentrations of acetone vapors accumulating during the dehydration phase exists. We must emphasize that the readings of 3,800 ppm and 1,900 ppm should be considered as estimates since sample break through occurred in the 2-chambered sorbent tube. Break through is considered to have occurred when the concentration of analyte in the down stream chamber (chamber 2) is 5% of the upstream concentration (chamber 1). With sample breakthrough the reading is not as accurate because some of the chemical has been diverted into the second chamber (portion of the sorbent tube). The break through may have been due to the high concentration of acetone and the cold temperature. The 3,800 ppm reading occurred when the lids of the plastic containers did not fit tightly. When acetone was transferred to containers with better fitting lids, the concentration in the deep freezer dropped markedly to 1,900 ppm. These concentrations, when converted to percentage by volume of air, were 0.38% and 0.19% respectively. When % LEL were calculated for these two concentrations of acetone, the resultant 0.15% and 0.07%, are below the LEL (2.6%) for acetone,

confirming the minimal flammability hazard during the impregnation phase. However, precautions should be taken.

As long as acetone vapors are maintained below zero degrees Fahrenheit, acetone vapors will not likely reach a flammable level. However, if the freezer should malfunction and the low temperature is not maintained, flammable levels of acetone can accumulate and the temperature or sparks from the compressor motor could cause a serious explosion. If vapors are allowed to accumulate in the freezer, it may even be possible for static electricity to ignite the acetone when the freezer is opened. As long as, the containers of acetone have a good seal and are airtight, acetone vapors cannot escape into the freezer and hence a household deep freezer may be considered.

Monitoring of acetone levels during the impregnation stage, suggests that with the pump used, flammable vapors probably do not accumulate around exhaust outlet of the pump. Consecutive readings of the monitor over a period of 25 minutes showed a converted % LEL of 2.3 which is far below the 10% LEL which is considered to be an adequate margin of safety. These results suggest that the flammability hazard in the vacuum pump is probably insignificant.

The vapors of tetraethyl silicate were measured during the gas cure phase. Levels found in the chamber were well below flammable levels, as would be expected, since the flash point of tetraethyl silicate is well above room temperature.

When working with chemicals that are toxic and/or have low flash points, it is prudent to limit the quantity of these chemicals present in the laboratory to only the amount needed for the present time or for a few days. For the obvious reason, larger volume equals larger spills which could result in greater personnel exposure. Greater quantities of flammable material in a given area are just more "rocket fuel" for a fire.

REFERENCES

- National Institute of Occupational Safety and Health, Manual of Analytical Methods, Third Edition, U.S. Department of Health, Education and Welfare, Publ. (NIOSH) 84-100 (1984).
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Table I - Recommended methods of sampling and analysis as set forth by OSHA and NIOSH.

Contaminant of interest	Method Used	Collection Media	Flow Rate	Sample MI/Min	Analysis Volume (L) Method
Acetaldehyde	OSHA 68	XAD-2	50	3	GC-NPD
Acetone	NIOSH 1300	Charcoal	20	2	IR
Ethanol	NIOSH 1400	Charcoal	50	1	GC-FID
Ethyl benzene	NIOSH 1501	Charcoal	50	10	GC-FID
Ethyl silicate	NIOSH 3	XAD-2	20	7	GC
Formaldehyde	OSHA 52	Xad-2	100	24	GC-NPD
Xylene	NIOSH 1501	Charcoal	50	2	GC-FID

GC: Gas chromatography.

GC-FID: Gas chromatography, Flame ionization detector. GC-

NPD: Gas chromatography, Nitrogen-Phosphorus detector. OSHA

- Occupational Safety and Health Administration NIOSH -

National Institute for Occupational Safety and Health.

Table II - Results of room air sampling conducted.

Contaminant of interest	Sample Duration (minutes)	Sample Rate (ml/min)	Results (ppm)	Recommended Exposure Limit (ppm)*
Acetaldehyde	255	101.70	0.04	100
Acetaldehyde	228	82.70	0.06	
Acetaldehyde	209	134.30	0.04	
Acetaldehyde	243	134.50	0.03	
Acetone (room)	78	25.80	0.60	750
Acetone (room)	420	18.90	6.40	
Acetone (freezer)	418	22.80	1900	
Acetone (freezer)	68	25.64	3800	
Ethanol	35	22.60	2.00	1000
Ethanol	48	15.50	2.00	
Ethyl benzene	246	43.00	0.04	
Ethyl benzene	173	50.10	0.05	
Ethyl benzene	201	50.00	0.05	
Ethyl benzene	205	49.60	0.05	
Ethyl benzene	211	55.70	0.04	
Ethyl silicate	355	20.10	0.06	10
Ethyl silicate	207	44.30	0.04	
Ethyl silicate	245	31.90	0.04	
Ethyl silicate	172	58.80	0.00	
Formaldehyde	215	100.0	0.20	1
Xylene	173	50.10	0.10	100
Xylene	201	50.00	0.09	
Xylene	211	55.70	0.08	
Xylene	246	43.00	0.08	
Xylene	205	49.60	0.09	

*Recommended exposure limits are those adopted by the American Conference of Governmental Hygienists, specifying a maximum time weighted average concentration, or Threshold Limit Value, under which it is believed that nearly all workers may be repeatedly exposed, day after day, with no adverse effects.

Table III - Percentage Lower Explosive Level (LED for acetone measured in the room air, inside freezers, and around the vacuum pump exhaust outlet.

Area Sampled	% LEL Direct Reading	Converted
Room Air	0.0	0.0
Vacuum pump exhaust outlet	2.12	2.30
Inside freezer (door ajar) Inside freezer (door wide open)	0.0	0.0
	0.0	0.0

* Time weighted average reading in 25 minutes. The measurement at the vacuum pump exhaust outlet was made between 10 to 30 cm from the outlet. During the air sampling, there were approximately 30 brain slices in the impregnation chamber freezer. Five containers with approximately 10 liters of acetone in each, plus 2 carboys of 20 liters of acetone were inside the dehydration freezer.