

## A SIMPLE AND INEXPENSIVE METHOD FOR RECYCLING USED ACETONE IN PLASTINATION LABORATORIES

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### SUMMARY

This paper describes a technique that is cheap, efficient and safe for recycling acetone. We have developed a method that includes three steps: Freeze-separation, vacuum distillation and physical water extraction. It permitted us to re-use acetone with minimal loss by using equipment that we already had in our plastination laboratory with very few additions or modifications. We were able to bring our used acetone up to 99% purity, so we never had to pay for discarding used acetone and we have purchased only a minimal volume of new acetone.

### INTRODUCTION

In most plastination laboratories, acetone is used for dehydration by the freeze-substitution technique and for defatting the specimens, as recommended in the plastination process (von Hagens, 1985). The cost of both purchasing and disposing of acetone represents an important part of the operating budget.

To be able to re-use acetone, plastinators have used their imagination and sometimes, when available, money. Some will leave the used acetone in a freezer until the water and fat congeal and can be separated from the acetone (Henry, 1991). Wealthier laboratories will simply push the button of their sophisticated distillation equipment controlled by a computer. We wanted to find a compromise between these two extremes.

### MATERIALS AND METHODS

**Procedure 1:** which was called "freeze-separation". used three contaminated solutions of acetone: 1. Acetone and water (60% acetone and 40% water); 2. Acetone and animal fat (10 - 20%); 3. Routine contaminated acetone from the dehydration of biological specimens for the plastination process. The contaminated solutions were placed in a deep freezer and at designated intervals, the samples were filtered through cheese cloth to remove the congealed fat or frozen water.

The water contaminated solutions were frozen and then filtered at 1, 2, 3, and 4 week intervals. This step required no special equipment. Space in a deep freezer at -20 °C and buckets and cheese cloth were necessary. The filtered solutions were monitored with a hydrometer (Tralle and Proof Thermo-Hydrometer).

To prepare the fat contaminated solution, animal fat was put into acetone for 1 week at 20 °C and then placed in the freezer. After freezing, the mixture was filtered every 2 days through cheese cloth, 100 g. of the filtered solution was taken, and the remainder of the filtered solution was returned to the freezer. The 100 g. aliquot of filtered acetone solution was evaporated at 30°C on a rotary evaporator (Buchi Rotavapor, model RE 111) and the residual fat was weighed.

**Procedure 2:** was called "vacuum distillation" and was slightly more sophisticated but not complicated. It required a deep freezer, a vacuum pump, valves, and tubing, available in any plastination lab. Two - 4 liter filtering Erlenmeyer flasks, 2 meters of Tygon tubing, two #13 rubber stoppers, 1 meter of glass tubing, marbles, and a styrofoam box big enough to contain one of the Erlenmeyer flasks were purchased.

Three liters of a contaminated acetone solution were poured into the first Erlenmeyer flask with the marbles acting as anti-bumping granules. This flask was heated in hot water to 45° - 50 °C and transferred into the styrofoam box containing hot water at 50°C. The box and hot water acted as a heating mantle and prevented rapid cooling of the mixture to be distilled due to the extraction of energy generated by the distillation process.

This first flask was connected to the second flask which was placed in the freezer at -20°C. Flask 2 acted as the condenser. It was connected to the vacuum line and enough vacuum was applied to obtain boiling in the first flask. Boiling stopped by itself when the temperature reached 35 °C - 40°C. The cost of the supplementary equipment for this step was less than \$300.00.

*Procedure 3:* called "physical water extraction" was conducted at room temperature by adding granules of molecular sieves (4-8 mesh, beads effective pore size 0.3nm) to the distilled acetone. These granules are utilized to remove residual water from the acetone and completed the purification process. Molecular sieves were bought from Fisher Scientific for \$30.00/500 grams.

One liter samples of distilled acetone were put at room temperature. 200 grams of molecular sieves were added and mixed into the 'distilled acetone solution. This mixture was left undisturbed for 1 week. After 1 week, the acetone was filtered using filter paper and purity was monitored with the hydrometer. The molecular sieves were left in a hood to permit evaporation of acetone vapors. The sieves were dried by heating to 120°C for 24 hours and may be reused for many years.

## RESULTS

### FREEZE SEPARATION:

This procedure was the cheapest way to purify acetone. The efficiency of leaving the samples in the freezer, undisturbed for prolonged periods to aid removal of the two major contaminants found in the dehydration process, water and fat, was much better for fat removal than for water.

1. Acetone and Water: Even after 4 weeks at -20°C and filtering through cheese cloth, the purity of the acetone did not improve appreciably.

2. Acetone and fat: Table 1 shows the very high efficiency of freeze-separation for removal of fat. Note, the almost complete separation of fat from acetone within 2 days of freezing at -20 °C. Table 2 shows monitoring of the same samples with a hydrometer. It confirms the total inaccuracy of hydrometer monitoring when fat content is high.

3. Used Acetone From Plastination Lab: The mixtures of used acetone were kept at -20 °C, filtered through cheese cloth and monitored once a week with a hydrometer for up to 5 weeks. Like the water-contaminated specimens, we did not observe changes in the density of the mixtures even if we had residues left on the filters after each filtration. The only exception happened with one jar that was "forgotten" in the freezer for 4 months. This acetone had been used for freeze-fixation and was 60% pure (hydrometer monitoring). After 4 months at -20°C and one filtration through 4 layers of cheese cloth, the reading was 78%. The filter

contained a big residue and gave off a strong formaldehyde smell.

### VACUUM DISTILLATION:

The samples that we distilled were mixtures of all the samples used for the freeze separation tests, mainly containing used acetone from plastination. They were monitored with the hydrometer (Table 3).

### PHYSICAL EXTRACTION:

When monitored with the hydrometer, the purity of one liter samples of distilled acetone, held at room temperature after addition of 200 g of molecular sieves, increased from 96% to 99%.

## DISCUSSION

The described method has been used in our plastination lab for one year and it has proven to be very efficient. This process may seem to be time consuming, but we have not found it so. It just takes a small space in the freezer because our used acetone is always kept at -20°C. After each impregnation, before changing the pump oil, we take a few days to recycle acetone. The most contaminated acetone is taken from the freezer, filtered before it warms up and distilled.

Because of the big difference between the vapor pressure of the acetone (446.7 torrs at 45 °C and 20.16 torrs at -20°C; Weast, 1973), we can condense almost all the vapors. The risks of damaging the pump are negligible because we send less acetone through it during 2 days of distillation than during 3 weeks of impregnation. We have never lost more than 18 ml of solvent during one run which is less than 0.6% of the volume distilled.

We also consider our distillation process as a very efficient method. We typically obtain 97% purity which compare favorably with the maximum purity obtainable by distillation (98.7%; Weast, 1973). Finally, we consider it as a completely safe method because it is all done in a well-ventilated plastination lab with the same equipment that is used for the dehydration and impregnation process.

The first and third steps are optional; however, because they do not require any manipulation there is no reason to by-pass them. The first one helps to reduce the volume of acetone to be distilled and to keep the distillation equipment clean, mainly by removing the fat. Freeze-separation can also be used to take water from acetone but we believe that the

disadvantages are greater than the advantages because it involves a long term storage of contaminated acetone at -20 °C and a lot of room in your freezers. The third one can also be avoided if you use new acetone for the last dehydration bath. The molecular sieves leave very small particles in suspension in the acetone. It gives a light coloration to the acetone. These particles can be removed by filtration through a very dense filter or by redistillation. We do not remove them and they have not caused us any problem.

Finally, we reached our goals which were to avoid paying for discarding used acetone and to minimize the use of new acetone. We also consider that by reducing the volume of solvent, we make our small contribution for the protection of our planet.

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#### REFERENCES

- HENRY R: Communication presented at the second interim conference on plastination, Chaffey College, August 1991.
- von HAGENS G: Heidelberg Plastination folder: Collection of all technical leaflets for plastination. Anatomisches Institut 1, Universitat Heidelberg, 1985.
- WEAST BCD: Handbook of Chemistry and Physics: a ready-reference book of chemical and physical data, 54th edition, C.R.C. Press, 1973.

**TABLE 2**  
**FREEZE-SEPARATION OF FAT**  
**FROM ACETONE**

Sample #	Acetone purity (%) measured with hydrometer			
	Day 0	Day 2	Day 4	Day 6
	1	94	95	95
2	96	98	98	98
3	95	95	95	95

**TABLE 3**  
**VACUUM DISTILLATION OF ACETONE AFTER**  
**FREEZE-SEPARATION**

Sample #	Acetone purity (%) measured with hydrometer			Rate (ml/hr)
	Beginning	Distillate	Residue	
1	75	96	58	565
2	80	97	74	630
3	75	97	66	600
4	62	96	43	
5	76	97	59	
6	83	97	70	
7	56	97	36	

**TABLE 1**  
**FREEZE-SEPARATION OF FAT**  
**FROM ACETONE**

Sample #	Acetone purity (%) measured by evaporation			
	Day 0	Day 2	Day 4	Day 6
1	82.6	95.9	96.0	95.8
2	87.3	98.1	98.3	98.1
3	91.6	95.1	95.0	94.7