Distinguishing between ethanol and isopropanol in natural history collection fluid storage

Teresa Mayfield

Field Museum, Division of Amphibians and Reptiles, 1400 S. Lake Shore Dr., Chicago, IL 60605-2496

Introduction

Natural history specimens in fluid storage are typically maintained in either ethanol (grain or ethyl alcohol) (CH_3CH_2OH), isopropanol (isopropyl alcohol or propan-2-ol) [$(CH_3)_2CHOH$] or formalin (CH_2O). If not previously documented, identifying storage fluids can be surprisingly difficult and potentially hazardous especially in a collection where all three fluids are used. Switching specimens from one alcohol to another is not recommended (Simmons, 2002) nor are the effects of alcohol mixtures on specimens well documented.

In assessing a herpetological collection, it became apparent based on unused alcohol on hand and the odor of opened jars that some specimens were preserved in ethanol, while others appeared to be in isopropanol or denatured ethanol (ethanol that has been made unfit for human consumption by adding another chemical or chemicals to it). Using olfaction to drive preservative choice during activities such as topping-off jars or packing specimens for shipment is problematic. Specimens that were properly switched from isopropanol to ethanol may retain the odor of isopropanol. Denatured ethanol may smell similar to isopropanol. Toxic effects, as listed in the Material Safety Data Sheets (MSDS), range from respiratory irritation to central nervous system depression, and must be considered.

Gas chromatography is an option to test storage fluids, but most museums do not have the equipment or the budget for such tests. In order to avoid human overexposure yet still ensure proper identification, a search was mounted for a simple and inexpensive test to distinguish between ethanol and isopropanol. In this paper, formalin is only looked at as a potential contaminant.

Searches through Google and Google Scholar using multiple variations of "distinguish between ethanol and isopropanol" generally resulted in highly technical chemistry or DNA extraction literature. Eventually, Wikipedia's general article on isopropyl alcohol was uncovered. The article asserts that isopropyl alcohol can be separated from an aqueous solution through the addition of sodium chloride; however the addition of sodium chloride will not result in separation for ethanol and methanol [CH_4O]. Further research from this point of departure demonstrated that this process, known as salting out, is well documented in the chemistry community and can be found in various chemistry demonstrations. Salting out will occur in aqueous solutions of ethanol when potassium carbonate (K_2CO_3) is added (North Carolina State University Department of Chemistry). Isopropyl alcohol is miscible in ethanol therefore; a mixture of these two alcohols could confound certain salting out tests.

It is surprising that salting out tests haven't become part of fluid storage collection management procedures. One possibility is that the effect of contaminants leaching from specimens or a mixture of alcohols might disrupt the salting out process.

Methods

A series of samples were developed to test the effectiveness of salting out in determining alcohol type. Samples were made from fresh solutions of 70% ethanol, 50% isopropanol, 70% denatured ethanol and 10% buffered formalin (10% formalin brought to a neutral pH through the addition of sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4) and methyl alcohol). Other samples of 70% ethanol and 50% isopropanol were taken directly from reptile specimen jars. Formalin and glycerin ($C_3H_8O_3$) were added as contaminants to some of the fresh solutions. The salts used were sodium chloride in the form of table salt and potassium carbonate.

The experiment was conducted in a manner derived from a North Carolina State University chemistry demonstration (North Carolina State University Department of Chemistry). Each test utilized 20mL of fluid solution and 3g of salt combined in a glass vial. This solution was then shaken by hand for approximately 30 seconds. For tests including contaminants, 1mL of a specific contaminant was added to the unshaken fluid and salt combination. The vials were then mixed as previously described.

In order to determine the volume of ethanol in a mixture of ethanol and isopropanol that would render isopropanol completely miscible and disrupt the salting out process, 70% ethanol was added in 1 mL increments to a mixture of 20mL of 50% isopropanol and 3g of sodium chloride until salting out no longer occurred.

Results

Table 1 includes the results for single storage fluids and contaminants. All alcohols salted out with potassium carbonate, but only isopropanol salted out with sodium chloride. The formalin solution did not salt out with either. Contaminants did not affect the salting out reactions.

Table 1 – Salting out of single storage fluids with and without contaminants

Salt	Potassium carbonate				Sodium chloride		
Contaminant	None	Formalin	Glycerin		None	Formalin	Glycerin
Preservative							
70% ethanol	Y	Y	Y		N	N	N
70% ethanol from specimen jar	Y				N		
70% denatured ethanol*	Y				N		
50% isopropanol	Y	Y	Y		Y	Y	Y
50% isopropanol from specimen jar	Y				Y		
10% buffered formalin	N				N		

*Denatured ethanol includes 1 part methanol (CH_4O), 1 part ethyl acetate ($C_4H_8O_2$), 1 part methyl iso-butyl ketone ($C_6H_{12}O$) and 1 part hydrocarbon solvent per 100 parts ethanol. Other denaturants may be used and results could be affected.

The results for an alcohol mixture are recorded in Table 2. Isopropanol failed to salt out only when the total volume of alcohol in a mixed alcohol solution consisted of about 25% or more ethanol.

Table 2 – Salting out of mixtures of 50% isopropanol and 70% ethanol using sodium chloride

20 mL of 50% isopropanol with the addition of	Salting out occurs?	Ethanol as a % of total alcohol volume
1 mL 70% ethanol	Y	6.5%
2 mL 70% ethanol	Y	12.3%
3 mL 70% ethanol	Y	17.4%
4 mL 70% ethanol	Y	21.9%
5 mL 70% ethanol	Y	25.9%
6 mL 70% ethanol	N	29.6%

Thus a 50% isopropanol and 70% ethanol mixture composed of about 25% or more ethanol as a percentage of total alcohol volume will not salt out with sodium chloride Interpreting such mixtures as consisting solely of ethanol would result in the use of ethanol in collection management procedures such as jar topping. The consequences of storage in a mixture of alcohols over a long period of time are not well documented. However, given that in this scenario the specimens are already stored in a mixture, further mixing through the addition of small amounts of ethanol would likely affect little change. If there is still doubt about the alcohol that has been used, and a decision is made to transfer the specimen to a known alcohol, Simmons (1999) suggests that the transfer should be done in stages.

An additional benefit of the salting out test is that it visibly demonstrates the alcohol concentration of the solution with the less dense alcohol layer above the denser salt water layer. When a 70% ethanol solution is salted out with potassium carbonate, the aqueous layer comprises approximately 30% of the total volume and the alcohol layer approximately 70% (Figure 1). Likewise, when a 50% isopropanol solution is salted out with sodium chloride, the two layers comprise approximately 50% of the total volume (Figure 2).

70% ethanol solution salted out with potassium carbonate

Figure 1

50% isopropanol solution salted out with sodium chloride

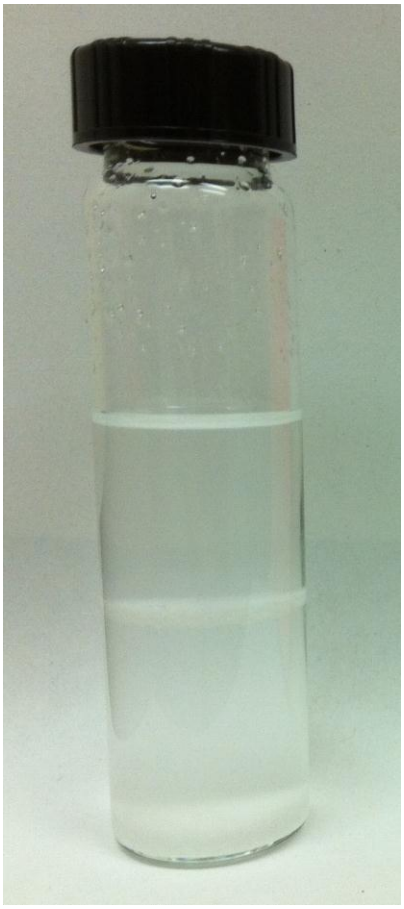

Figure 2

Consequences for Collection Management

Properly documenting the storage fluid used with biological specimens is as important as documenting details about the specimen itself. Not only should the container be labeled, but the collection catalog for each specimen should clearly state the storage fluid as well as the original preservation method. This information must be on all loan documentation to ensure proper treatment by the borrower. Unfortunately, not all collections are properly documented. Specimens received from older institutions or non-museum sources and dormant collections often showcase these problems.

When there is confusion about alcohol identity, the addition of 3g of sodium chloride to 20 mL of storage fluid shaken for thirty seconds will help identify isopropanol. This is not a definitive test if the alcohol is an isopropanol and ethanol mixture and displays the properties of one or the other depending on their relative concentrations. Although not definitive, the test is useful in determining which alcohol to use when topping off or packaging specimens for shipping. It can

also help avoid the mixing of alcohols and assist in properly labeling containers so as to avoid confusion about preservatives in the future.

Author notes on the use of this test

I have used this test to evaluate the alcohol in over 100 jars and it has made the task of curating specimens much easier. I have discovered that in the case of isopropanol being highly concentrated, salting out may be hard to distinguish because the water layer is "hidden" within un-dissolved salt at the bottom of the test vial. If all of the salt has fallen to the bottom of the test sample, there are no distinguishable layers, and the solution above the salt is clear, the addition of 1 or 2 ml of distilled water will lower the concentration of the alcohol and make the layers created in the salting out process visible. If the addition of sodium chloride to a storage fluid reveals no distinguishable layers and the solution above the un-dissolved salt is cloudy, it is an indication that the preservative is miscible with salt water and is likely ethanol. In this case, the addition of distilled water will simply reduce the amount of un-dissolved salt in the vial.

Works Cited

North Carolina State University Department of Chemistry. (n.d.). *Chemistry Lecture Demonstrations.* Retrieved Aug 22, 2012, from NCSU Project: http://ncsu.edu/project/chemistrydemos/Organic/SaltingOut.pdf

Simmons, J. E. (1999, May). Storage Concerns for Fluid-Preserved Collections. *Conserv O Gram*(11/3), 4pp.

Simmons, J. E. (2002). Herpetological Collecting and Collections Management Revised Edition *Herpetological Circulars of the Society for the Study of Amphibians and Reptiles*(31), 153pp.

Wikipedia. (n.d.). *Isopropyl alcohol*. Retrieved July 25, 2011, from Wikipedia: http://en.wikipedia.org/wiki/Isopropyl_alcohol

Special thanks to Alan Resetar and Kathleen Kelly at the Field Museum in Chicago and Karen Morton at the Museum of Nature and Science in Dallas for their advice and assistance in completing this document.