Oxidizer Application Note

A Comparison of Sample Oxidation and Solubilization Techniques

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Introduction

The primary objective of all sample preparation methods is to obtain a stable homogeneous solution suitable for analysis by Liquid Scintillation Counting (LSC). There are no absolutes in sample preparation; whichever method produces a sample that lends itself to accurate and reproducible analysis is acceptable. However, there will be occasions when more than one method will be both suitable and available and the selection of either method will depend on other factors. It is precisely this situation that occurs when considering solubilization and sample combustion for sample preparation. Both techniques are routinely used to process samples that are not directly soluble in LSC cocktails. There are numerous sample types that fall into this category and typical examples include tissue, muscle, kidney, liver, feces, blood, plant material, etc. Many of these samples are encountered in ADME (absorption, distribution, metabolism and excretion) studies in which the biological behavior and potential toxicological effects of a test substance are investigated.

What is Solubilization?

Solubilization is the action of certain chemical reagents on organic materials (such as animal or plant tissue) that effects a structural breakdown (or digestion) into a liquid form that can then be directly dissolved in a liquid scintillation cocktail. Typical solubilizers include organic and inorganic alkalis which act by the process of alkaline hydrolysis, and certain mineral acids which effect solubilization by acidic oxidation. The solubilization process usually involves heating the sample/solubilizer at elevated temperature (40° to 65°C) for periods ranging from <1 to 24 hours, until a homogeneous mixture is formed. Certain samples that remain colored after solubilization are optionally treated with hydrogen peroxide, and following this a recommended LSC cocktail is added and the sample is ready for analysis by LSC.

Figure 1. *Commonly used solubilizers and recommended LSC cocktails.*

What is Sample Combustion?

The principle of sample oxidation is that the sample is combusted in an oxygen rich atmosphere and any hydrogen present is oxidized to water while any carbon is oxidized to carbon dioxide. If Tritium is present then the combustion product will be $^{3}H_2O$ and if ^{14}C is present then the combustion product will be $^{14}CO_2$. In the Model 307 Sample Oxidizer, the water is condensed in a cooled coil and then washed into a vial where it is mixed with an appropriate LSC cocktail. The CO_2 is trapped by vapor-phase reaction with an amine and the resulting product is mixed with an appropriate LSC cocktail. At the end of the combustion cycle, two separate samples (a Tritium sample and a ^{14}C sample) are trapped at ambient temperature, thus minimizing cross contamination.

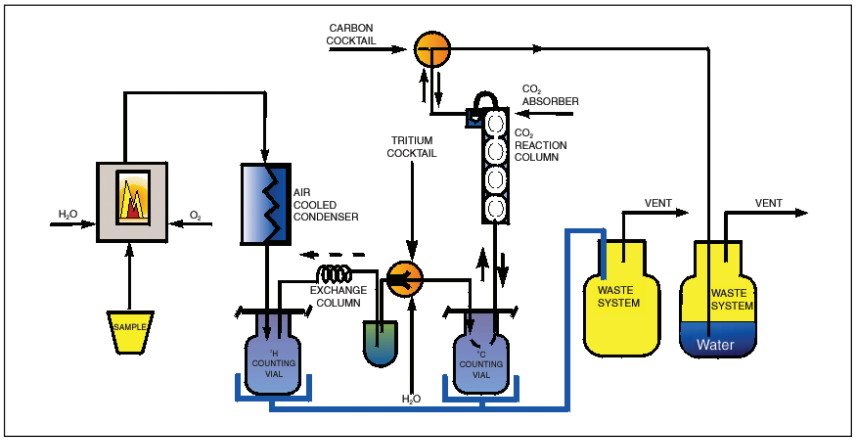

Figure 2. *Non-catalytic combustion of organic samples by flame oxidation to $^{3}H_2O$ and $^{14}CO_2$.*

Advantages and Disadvantages

The oxidation process described above can be compared with the steps in a typical solubilization method in the schematic below:

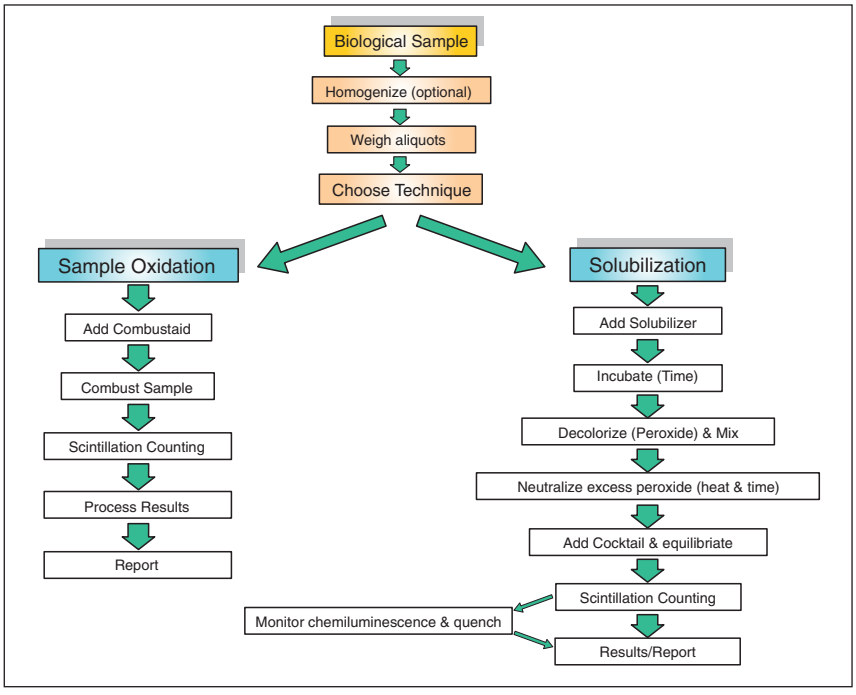

Figure 3. *Sample Oxidation and Solubilization techniques compared.*

As can be seen from Figure 3, there are more steps involved in solubilization as compared to combustion and while this may seem less attractive, there are other important factors to consider before deciding which methodology is best.

Solubilization methods and suitability

The decision to use solubilization as the preferred method of sample preparation depends primarily on the nature of the sample and the number of samples that need to be processed. When the number of samples is low, then solubilization is usually the method of choice. Solubilization is suitable for many organic sample types but certain of these are prone to problems such as color formation, limited sample size and time to complete solubilization. These include biological samples such as whole blood, plasma, serum, liver, kidney, fatty tissue and most plant materials. Many other biological sample types including muscle, whole tissue, brain, stomach, intestines, nerve cells, cornea and cartilage can be easily and rapidly processed. There is no major cost involved in setting up to do solubilization. The cost of 1.0 L solubilizer reagent and 10.0 L appropriate LSC cocktail is relatively small and this is sufficient for 1,000 analyses, assuming 1.0 mL solubilizer used with 10.0 mL cocktail. The method is relatively straightforward and is shown in Figure 4 below.

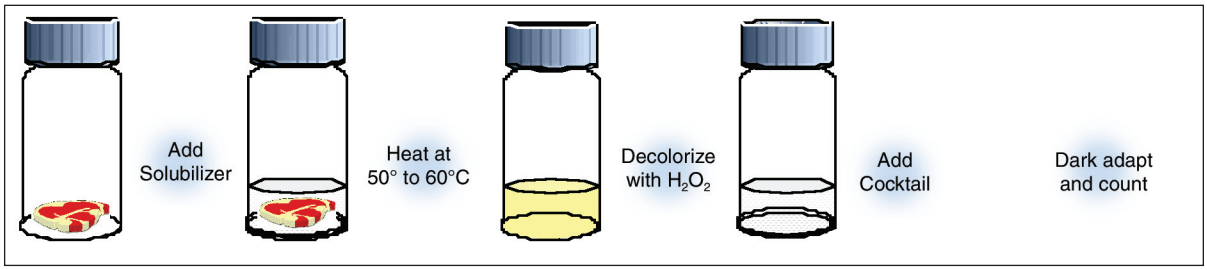

Figure 4. *Stepwise process for solubilization.*

To process a sample, simply add the solubilizer and heat at 50° to 60°C until the sample is dissolved. After solubilization, the sample may be colored and this color can usually be removed or reduced by treatment with hydrogen peroxide. The final step is to add the recommended LSC cocktail and the sample is ready for counting. Using this method many samples can be processed simultaneously and then counted sequentially.

The typical advantages of solubilization are:

- Capital outlay is low.
- Homogeneous samples are produced.
- Many samples can be processed simultaneously.
- Color quench is corrected using a quench curve.
- It is suitable for a diversity of isotopes.

The disadvantages of solubilization are:

- Sample sizes are generally ≤ 200 mg.
- Time to complete solubilization can vary from a few hours to many hours.
- Certain samples require modified techniques or longer solubilization times.
- With acidic solubilizers loss of radioactivity by volatilization may occur.
- Excess peroxide must be destroyed after decolorization.
- Chemiluminescence may be present.
- If ^{3}H and ^{14}C are both present in the sample then dual label DPM calculation is necessary.

Solubilization has been in use for many years and experienced researchers are typically able to optimize the methodologies through attention to detail and judicious selection of reagents and cocktails. Examples of sample types, reagents and cocktails are shown below:

Sample Type	Solubilizer	Max. sample size	Suitable LSC Cocktails
Muscle	Soluene®-350	150 mg 200 mg	Pico-Fluor™ 40 Ultima Gold™ or Hionic-Fluor™
	SOLVABLE™	150 mg 200 mg	Pico-Fluor 40 Ultima Gold or Hionic-Fluor
Liver	Soluene-350	100 mg	Hionic-Fluor
	SOLVABLE	50 mg 100 mg	Ultima Gold Hionic-Fluor
Kidney	Soluene-350	100 mg	Hionic-Fluor
	SOLVABLE	100 mg	Hionic-Fluor
Heart	Soluene-350	100 mg	Hionic-Fluor
	SOLVABLE	150 mg	Hionic-Fluor
Sinew	Soluene-350	150 mg	Hionic-Fluor
	SOLVABLE	150 mg	Hionic-Fluor
Brains	Soluene-350	150 mg	Hionic-Fluor
	SOLVABLE	150 mg	Hionic-Fluor
Stomach	Soluene-350	100 mg	Hionic-Fluor
Feces	Hypochlorite	150 mg	Hionic-Fluor
	Soluene-350	20 mg	Hionic-Fluor
	SOLVABLE	20 mg	Hionic-Fluor
Blood	Soluene-350	0.4 mL	Hionic-Fluor
	SOLVABLE	0.5 mL 0.2 mL	Hionic-Fluor or Pico-Fluor 40 Ultima Gold
Plant Material	Soluene-350	< 50 mg	Hionic-Fluor
	SOLVABLE	< 50 mg	Hionic-Fluor
	$HClO_4/H_2O_2$	200 mg	Hionic-Fluor
	$HClO_4/HNO_3$	200 mg	Hionic-Fluor
	Hypochlorite	200 mg	Hionic-Fluor

Table 1. *Sample types that can be processed by solubilization.*

Sample Combustion methods and suitability

Since sample combustion is suitable for any organic and even some inorganic samples, the selection of this method is usually governed by the number of samples that need to be processed. When the sample load exceeds 50 per day, then sample oxidation becomes the method of choice for many sample types. In manual mode, the Model 307 oxidizer can process 100 samples daily, while the robotic version can process 240 samples in 8 hours. High temperature flame combustion at 1,300°C enables wet, dry or freeze-dried samples up to 1.5 g to be processed. For those samples containing dual label $^{3}H/^{14}C$, the combustion cycle produces single label ^{3}H and ^{14}C samples in separate vials with no cross contamination.

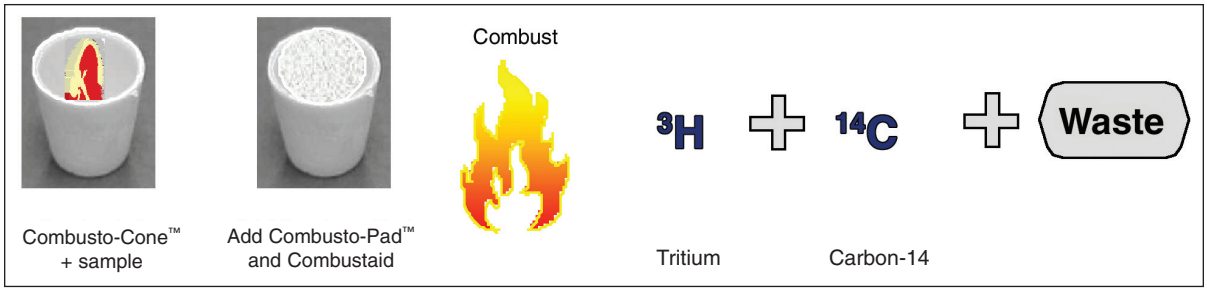

Figure 5. *Schematic of steps involved in sample combustion.*

Advantages of sample combustion:

- Sample processing time is rapid.
- Robotic sample processing is possible.
- Sample can be wet, dry or freeze-dried.
- Any sample containing H and/or C can be combusted.
- It is ideally suited for both single and dual label ^{3}H and ^{14}C.
- Sample sizes up to 1.5 g are possible.
- Radioactive recovery is excellent (>97%).
- Memory effect is <0.08%.
- There is no loss of radioactivity by volatilization.
- There is no chemiluminescence interference.
- There is no color quench interference.

Disadvantages of sample combustion:

- Initial capital investment.
- It is only suitable for ^{3}H and ^{14}C.
- Need a gas supply (oxygen and nitrogen).
- Must be operated in a fume hood.
- Reagents are corrosive and flammable.

The flame combustion technology has proven to be a simple and reliable means of sample preparation and can process a diverse array of samples with a high degree of precision and accuracy. The technique requires a minimal amount of time and sample handling, and eliminates any potential interference from color quenching or chemiluminescence. In addition, with the System 387 robotic option, the entire procedure can be automated to process up to 80 samples per run, without supervision. The diversity of samples that can be processed using flame combustion is shown in Table 2 below.

• Liver	• Whole blood	• Bone	• Gels
• Spleen	• Lung	• Egg shell	• Plastics
• Skin	• Heart	• Plant tissue	• Filters
• Plasma	• Fat	• Bacteria	• Crude oil
• Muscle	• Intestines	• Insects	• TLC's
• Kidney	• Hair	• Glands	• Toluene
• Brain	• Adipose	• Water	• Synthetic fibers
• Feces	• Bladder	• Urine	• Soil

Table 2. *Sample types that can be processed by flame combustion.*

Conclusion

Both solubilization and flame combustion are viable sample preparation techniques for a diverse array of animal and plant tissues. Flame combustion and solubilization procedures each have specific advantages and some disadvantages. Solubilization is more suited to those situations where the sample numbers are low. There can be problems such as color formation, chemiluminescence and sample size limitations when solubilizing with certain sample types, but by careful attention to detail and experience, these can be overcome. Flame combustion, operated in either manual or robotic format, provides a very powerful tool to rapidly process many different sample types with a high degree of precision and accuracy. The technique requires a minimal amount of time and sample handling and eliminates color quench and chemiluminescence interferences. The only limitation is the initial capital investment and the restriction to ^{3}H and ^{14}C. The Model 307 manual oxidizer is well suited for sample loads of up to 50 per day, but where high sample throughput is critical, the System 387 robotic option, with its capability of processing 240 samples per day, is the ideal solution.

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