COUNTING SOLUTIONS
LSC TECHNICAL TIPS FROM PACKARD
CS-006

Safer Flow Cocktails

Jock Thomson, Packard Instrument Company, Meriden, Connecticut 06450 USA

Introduction

The use and detection of radiotracers in organic and biological chemistry has benefitted from advances in the efficiency and sophistication of both the chromatographic and the radioactive detection techniques. As a result, the chromatographic separation of radiolabeled compounds, sometimes referred to as "radiochromatography," is now carried out in many laboratories throughout the world. The basic technique involves the separation of the components of a chemical mixture by High Performance Liquid Chromatography (HPLC) followed by the detection and quantitation of the radioactivity in the sample components using Flow Scintillation Analysis (FSA). Recent technological advances in HPLC have been associated with column specificity, pulseless pumping, new and improved detectors (including hybrid methods *e.g.*, HPLC-Mass Spectroscopy) and finally, computerization for control and data handling. Likewise, the latest advances in FSA are related to cell design and type, mixing processes, scintillation measurement and detection (including TR-LSC® with MCA spectral analysis), and computerization for control and data handling. The flow scintillation analyzer can be operated with two different cell types: solid scintillation cells for heterogeneous counting and liquid flow cells for homogeneous counting. Although both techniques are possible with the same instrumentation, this application note will focus on homogeneous counting using safer liquid scintillation cocktails. Until recently, FSA has been largely neglected in the changeover to safer, high flash point cocktail systems. Radiochromatographers have tried to adapt currently available safer cocktails for FSA, but have been thwarted by viscosity, as well as mixing and sample complexity problems. The only way to resolve these problems was to design and formulate high flash point, safer flow cocktails, thus offering an alternative to the classical flow cocktails that are currently in use.

Development[1]

The effective use of any cocktail in a flow system depends upon physical and performance characteristics; therefore, the design and development of a safer flow cocktail requires the following:

- Low viscosity
- Rapid and easy mixing with the eluate
- High sample acceptance capacity
- Compatibility with complex samples and gradients
- No gel formation
- Good counting performance
- Low background contribution
- Chemiluminescence resistance
- Safety
- High flash point
- Biodegradability

Unfortunately, with the raw materials available today, a cocktail with all the characteristics listed above is not possible. However, by selecting a hybrid solvent system, viscosity problems were overcome. The end result was a range of safer cocktails with high flash point and biodegradability as premier characteristics. Careful selection of the detergent raw materials ensured rapid and easy mixing as well as performance with complex sample types and gradients. The presence of long chain alcohols within the hybrid solvent system prevented

Ratio of Cocktail:Water	Viscosity in centiStokes (cSt) at 25 °C			
	Ultima-Flo™ M	Ultima-Flo AP	Ultima-Flo AF	Ultima-Gold™
No sample	17	14	22	26
3:1	22	20	40	48
2:1	23	22	37	**
1:1	23	19	**	**
** Indicates unstable emulsion formed.				

Table 1.
Viscosity comparison with added water.

gel formation and helped to further reduce the viscosity. The use of high quality raw materials in conjunction with in-process purification and Packard's patented system for luminescence removal[2] assured minimal background contribution and acceptable counting performance. The complexity and nature of samples encountered in FSA (*e.g.*, phosphate and formate gradients), were such that different formulations were needed to effectively satisfy the sample acceptance capacities required by radiochromatographers.

Performance

The result of the above development was the evolution of the Ultima-Flo range of high flash point, safer flow cocktails which can perform as substitutes for the classical flow cocktails in all the important FSA areas. The Ultima-Flo range consists of three specialized cocktails that cover virtually all the sample types encountered in FSA:

- Ultima-Flo M (M for multiple dilute sample types)
- Ultima-Flo AF (AF for ammonium formate)
- Ultima-Flo AP (AP for ammonium phosphate)

The viscosity reduction achieved over conventional safer LSC cocktails can be best shown by comparing the Ultima-Flo range with such a cocktail. This is illustrated in Table 1.

Ultima-Flo M

The letter M in Ultima-Flo M refers to its intended use with multiple dilute sample types. In addition, Ultima-Flo M is suitable for use at ratios up to 2:1 (cocktail:sample) with methanol/water and acetonitrile/water gradients common in Reverse Phase HPLC separations. The ^{3}H counting performance of Ultima-Flo M with various sample types is shown in Figure 1.

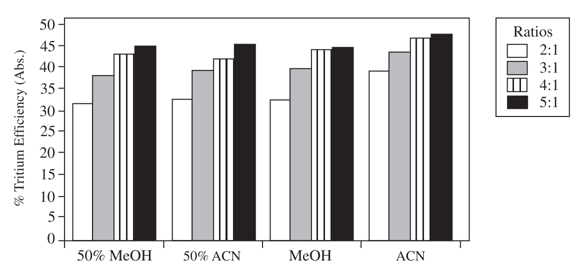

Figure 1.
Ultima-Flo M counting performance with various samples at different ratios of cocktail to sample.

Sample	Maximum Sample Uptake (%)	Optimal Mixing Ratio Cocktail:Sample
Deionized water	50.0	1:1
Methanol/water (50/50)	31.0	3:1
Methanol	50.0	1:1
Acetonitrile/water	41.2	2:1
Acetonitrile	50.0	1:1
0.2 M sodium chloride	41.2	2:1
0.15 M sodium chloride	41.2	2:1
0.05 M sodium chloride	50.0	1:1
0.1 M PBS buffer (pH 7.4)	33.3	2:1
0.01 M PBS buffer (pH 7.4)	41.2	2:1
0.01 M PBS/plasma (10%)	45.9	2:1
1.0 M sodium hydroxide	21.6	4:1
0.5 M sodium hydroxide	35.5	2:1
0.1 M sodium hydroxide	50.0	1:1
0.2 M HEPES (pH 7.2)	50.0	1:1
0.1 M HEPES (pH 7.2)	50.0	1:1
50 mM Tris-HCl	50.0	1:1
0.05 M Na_2HPO_4	50.0	1:1
0.02 M ammonium formate	50.0	1:1

Table 2.
Typical sample load capacities for Ultima-Flo M at 20 °C.

The sample acceptance capacities of Ultima-Flo M are shown in Table 2. These data clearly show the versatility of Ultima-Flo M with multiple dilute sample types.

Ultima-Flo AP

The letters AP in Ultima-Flo AP refer to its intended use with ammonium phosphate samples and gradients. Ultima-Flo AP is based on a patented safer solvent system[3] which allows for even lower viscosity than with Ultima-Flo M or AF. Where viscosity is an issue, Ultima-Flo AP is generally recommended. Ammonium phosphate gradients are used in the separation of inositol phosphates using HPLC and concentrations up to 2.0 M ammonium phosphate (pH 3.8 with orthophosphoric acid) are routine. The sample acceptance and counting performance of Ultima-Flo AP with this gradient are shown in Figures 2 and 3.

Ultima-Flo AP is also able to accept other complex samples and gradients including 0-1.0 M PBS gradients, 0-1.0 M sodium hydroxide gradients, methanol/water gradients and acetonitrile/water gradients. As a result of its wide range of sample acceptance, Ultima-Flo AP has been shown to be all purpose. The performance of Ultima-Flo AP with PBS gradient is shown in Figure 4 and with sodium hydroxide gradient in Figure 5.

The ^{3}H counting performance of Ultima-Flo AP with methanol/water and acetonitrile/water is shown in Figure 6. Table 3 shows the broad applicability of Ultima-Flo AP for a variety of samples and gradients.

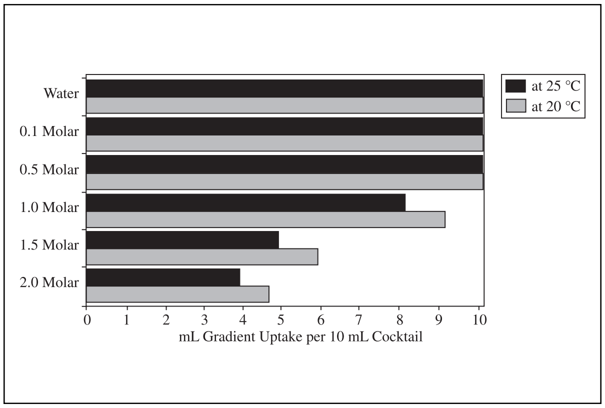

Figure 2.
Ultima-Flo AP performance with 0-2.0 M ammonium phosphate gradient (pH 3.8).

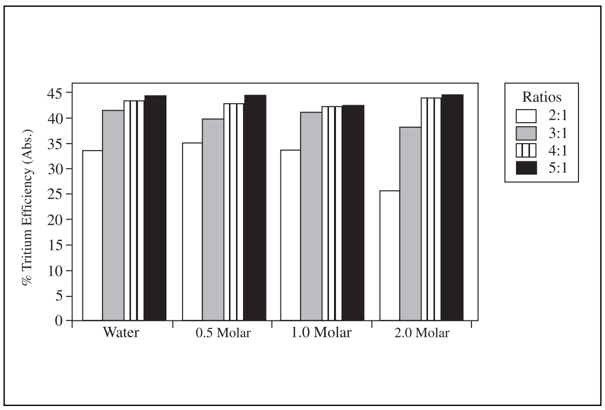

Figure 3.
Ultima-Flo AP counting performance with 0-2.0 M ammonium phosphate gradient (pH 3.8) at different ratios of cocktail to sample.

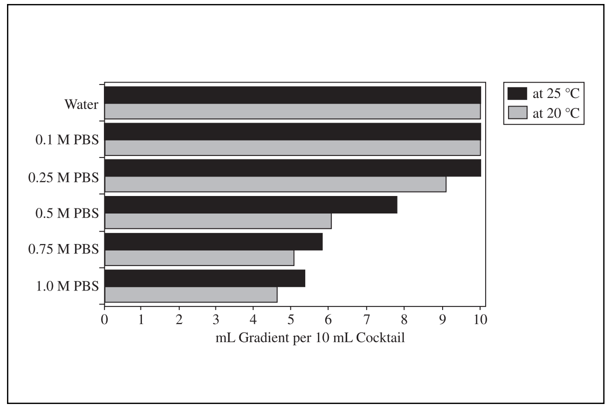

Figure 4.
Ultima-Flo AP performance with PBS gradient.

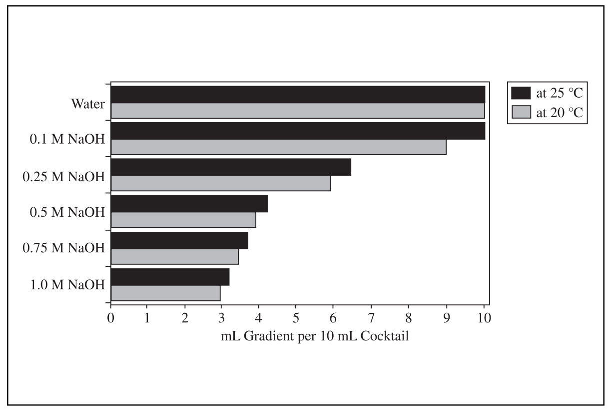

Figure 5.
Ultima-Flo AP performance with sodium hydroxide gradient.

Ultima-Flo AF

The letters AF in Ultima-Flo AF refer to its intended use with ammonium formate samples and gradients. Ammonium formate gradients are also used in the separation of inositol phosphates and concentrations up to >2.0 M ammonium formate (pH 3.8 with formic acid) have been used routinely. The use of ammonium formate eluents in HPLC is uncommon since the formate can attack stainless steel. Therefore, the samples are generally collected using a fraction collector. Ultima-Flo AF is then added to the discrete fractions of the gradient and counting is carried out by conventional LSC. The sample capacity of Ultima-Flo AF for the complete 0-2.0 M gradient is shown in Figure 7 and the ^{3}H counting performance in Figure 8.

Safety

The Ultima-Flo family satisfied the ideal development criteria. In addition, they are also safer for the user and the environment. As confirmation, all three cocktails were tested for biodegradability and their flash points were measured. This ensures that the final cocktail mixture is tested. Test results can be seen in Table 4.

Packard does not advocate drain disposal of safer cocktails. The biodegradability tests have been carried out to give an assurance that, in the event of an accidental small spillage, any waste that gets washed into the sanitary sewer system will be biodegradable. With the Ultima-Flo family of safer FSA cocktails, waste considerations are important as the HPLC

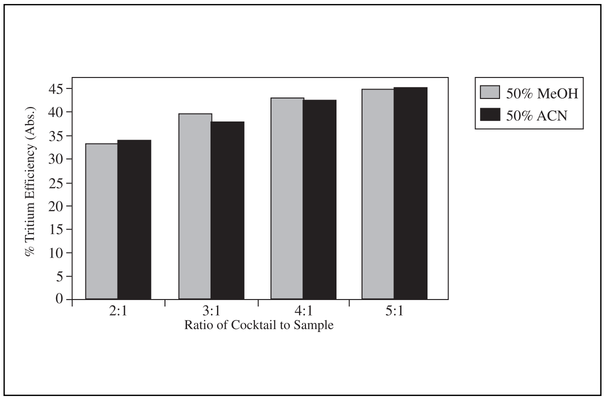

Figure 6.
^3H counting performance of Ultima-Flo AP with methanol/water and acetonitrile/water.

eluent dictates the form of the waste, in that mixing toxic, flammable eluent (*e.g.*, methanol/water) with a safer flow cocktail produces a toxic, flammable waste mixture.

Summary

All the information necessary for the correct selection of a safer flow cocktail is presented. This demonstrates that the Ultima-Flo family of FSA cocktails can be used as substitutes for the classical flow cocktails that are currently used by many researchers for radiochromatographic separations. Although the Ultima-Flo range of cocktails was designed for use in FSA, this does not preclude their use in conventional liquid scintillation counting.

Sample	Maximum Sample Uptake (%)	Optimal Mixing Ratio Cocktail:Sample
Deionized water	50.0	1:1
Methanol/water (50/50)	28.6	3:1
Methanol	50.0	1:1
Acetonitrile/water	31.0	3:1
Acetonitrile	50.0	1:1
1.0 M sodium hydroxide	23.1	4:1
0.5 M sodium hydroxide	28.6	3:1
0.1 M sodium hydroxide	50.0	1:1
1.0 M PBS	31.0	3:1
0.5 M PBS	37.5	2:1
0.1 M PBS	50.0	1:1
0.01 M PBS	33.3	2:1
0.2 M sodium chloride	33.3	2:1
0.05 M Na_2HPO_4	50.0	1:1
0.01 M PO_4/methanol (50/50)	28.6	3:1
0.01 M PO_4/acetonitrile (50/50)	33.3	2:1
0.01 M PBS/methanol (50/50)	28.6	3:1
0.01 M PBS/acetonitrile (50/50)	28.6	3:1
5.0 M guanidine	19.4	4:1
2.0 M guanidine	31.0	3:1

Table 3.
Typical sample load capacities for Ultima-Flo AP at 20 °C.

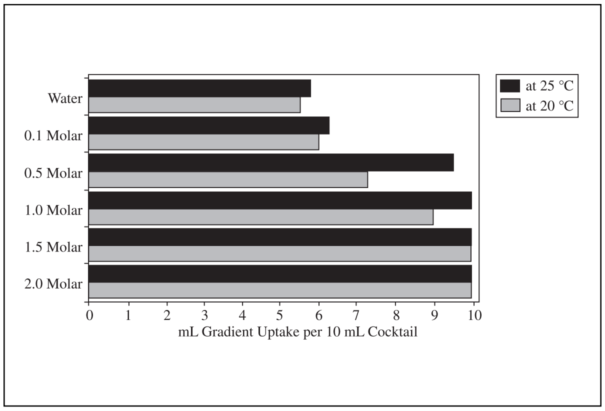

Figure 7.
Ultima-Flo AF performance with 0-2.0 M ammonium formate (pH 3.8) gradient.

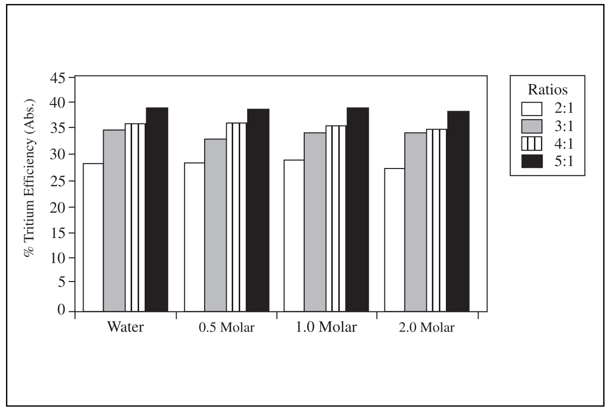

Figure 8.
Ultima-Flo AF counting performance with 0-2.0 M ammonium formate (pH 3.8) gradient at different ratios of cocktail to sample.

Cocktail	Flash Point	Biodegradability
Ultima-Flo M	120 °C (248 °F)	Readily biodegradable by ISO method 7827-1984(E) (equivalent to OECD 301E)
Ultima-Flo AP	120 °C (248 °F)	Inherently biodegradable by Zahn/Wallens EMPA test (equivalent to OECD 302B)
Ultima-Flo AF	120 °C (248 °F)	Readily biodegradable by ISO method 7827-1984(E) (equivalent to OECD 301E)

Table 4.

Their ability to accept medium to large amounts of complex sample types often makes them the cocktail of choice in certain LSC applications.

Conclusion

The Packard Ultima-Flo family of safer, high flash point FSA cocktails are suitable for use in flow scintillation counting and offer both user and environmental safety as well as performance. In particular, the broad applicability of Ultima-Flo AP for a diverse range of eluents, gradients and samples, may establish this unique cocktail as an all purpose or universal FSA cocktail.

References

1. Thomson, J. 1994. The Advent of Safer Flow Scintillation Cocktails. In Cook, G.T. et al., eds., Liquid Scintillation Spectrometry 1994, Tucson, Arizona, Radiocarbon: 257-260.
2. Hegge, Th. C.J.M. and ter Wiel, J. 1986. Mixture for use in the LSC analysis technique. U.S. patent no. 4,624,799.
3. Thomson, J. 1993. Scintillation Counting Medium and Process. European Patent Application 93.200718.0, March 1993.