

RADIOACTIVE FLOW DETECTORS  
LIQUID OR SOLID SCINTILLATORS?

Andrew R. Reich, Radiomatic Instruments & Chemical Co., Inc.,  
Tampa, Florida 33611, U.S.A.

The detection of low energy, beta-emitting radionuclides can be accomplished in a number of well researched and documented ways. Gas proportional counting, scintillation counting and auto-radiography are the major methods in use today. The media in which the radionuclide is carried limits the choice of detection methods, especially if premeasurement sample preparation is to be avoided.

The measurement technique, for effluents of low and high pressure liquid chromatographs was almost always considered the exclusive domain of scintillation counting.

Early work on radio-liquid chromatography was reported using the technique of fraction collection and consequent aliquot counting in a liquid scintillation counter<sup>1</sup>. This method, while inherently cumbersome, was acceptable since each chromatographic run was long (2-16 hours); resolution of the chromatogram was good, and the resolution volume allowed the collection of several fractions through each peak.

Efforts to use the liquid scintillation counter as an "on-line" detector for liquid chromatography were never completely successful. The scintillators used in these early attempts were naphthalene or anthracene crystals packed in modified LSC vials with the liquid chromatography effluents fed through the packing, hopefully in a laminar flow pattern.

The results of these experiments were usually less than acceptable. The cells notoriously leaked, the fluid path always found dead spots to play havoc with the resolution, and some samples found it more advantageous to attach to the surface of the crystals than to exit with the liquid stream, thereby, causing the cell to be radioactive and necessitate its change or cleaning. However, despite all the difficulties, it was superior to the "collect, sample and count method" when this technique worked.

As high pressure liquid chromatography (HPLC) began to replace traditional liquid chromatography<sup>2</sup>, it was obvious that a method of on-line detection for radionuclides must also follow. HPLC is not only

order of magnitudes faster than LC, but the peak-to-peak resolution is infinitely better, necessitating the sampling process to increase to typically hundreds of fractions. The researcher was now faced with a paradox. The results of the HPLC runs through a mass detector (UV, EC) were available at the end of the run - typically in 20 minutes. The radioactive results, however, took 8 to 24 hours, depending on the number of fractions collected, and the availability of a liquid scintillation counter. The stage was set for the development of an on-line radioactivity detector for HPLC.

During the past five years, two schools of thought have emerged producing two different types of radio-HPLC detectors. Based on the naphthalene-in-the-vial principle, manufacturer's such as Berthold Laboratories, Nuclear Enterprises and United Technology's Packard Division developed heterogeneous scintillation detectors. In these detectors the anthracene or naphthalene crystals are replaced by other scintillators<sup>3</sup>. The design of the cell has also been changed. In order to avoid dead space and turbulence, a narrow diameter tube is used, either straight, or more popularly formed into a coil or a "U". To optimize light transmission to the photomultiplier tubes, mirrors are used. The limiting factor of these designs is the ability of the solid scintillator to transfer the low-energy beta radiation into photons. While some solid scintillators (i.e., Eu activated  $\text{CaF}_2$ ) have the ability to yield high photon efficiency, in order for them to work, the beta-emitting radionuclide has to be within microns to the scintillator. To overcome this problem, the particle size of the scintillator was reduced to be in the 25-75 micron range. Even at this size, counting efficiency for tritium is below the 10% level.

The other school of radio-HPLC detectors based their design on classical liquid scintillation counting technology. Radiomatic Instruments' FLO-ONE series and the Reeves HPLC detector are based on this principle<sup>4-5</sup>.

In a homogeneous detector, the effluent from the HPLC system is mixed with a suitable liquid scintillator before entering the counting cell. The cell design is typically a flat glass or Teflon coil tightly sandwiched between two photomultiplier tubes, making good optical contact without the use of mirrors. Depending on the chromatographic

effluent,  $^3\text{H}$  efficiencies between 25 to 50%, and  $^{14}\text{C}$  counting efficiencies up to 85% can be achieved.

On the surface, the two methods look comparable. The advantage of measuring radionuclides without the addition of scintillator in the heterogeneous system (and the consequent recoverability of the sample) is compensated by the high  $^3\text{H}$  counting efficiency achieved in the homogeneous system. The higher operating cost of the homogeneous system can be drastically reduced with the use of an effluent stream splitter, also allowing the recovery of the major portion of the sample material.

The fundamental difference between the heterogeneous and homogeneous methods is not in the area of scintillation counting, but in chromatography.

The chromatographic process is based on the selective adsorption and desorption of the sample mixture on the chromatographic column. The specificity of this process is controlled by the careful selection of adsorbing materials on the column and the eluting reagent mixture used. The heterogeneous scintillator in the chromatographic system acts as a secondary column, uncontrolled and unwanted by the researchers. The unwanted chromatographic properties of the solid scintillator are further enhanced by its particle size. In order to increase counting efficiencies, the solid scintillator has to be in sizes approaching that of the chromatographic column packing, i.e., nearing 5-10 microns, adsorbing and desorbing the sample molecules as they flow through, uncontrolled by the chromatographer.

This uncontrolled chromatography has two serious effects on the detector's operation: one is obvious, the other is hidden; both caused by the same process.

If any compound in the chromatographic effluent has more affinity to the detector's packing than to the chromatographic column's packing, it will be adsorbed. If the adsorbed compound is radioactive, the detector is said to be "contaminated"; its background is increased and for all practical purposes it becomes useless until the radioactive compound is desorbed, i.e. the cell is decontaminated. The hidden effect is caused by the adsorption of nonradiolabeled molecules on the detector. A coating of foreign material prevents the interaction between scintillator and radiolabeled compounds, thereby, drastically

reducing the efficiency of the system, causing it to "miss" radioactive peaks.

The results of both problems are the same: unreliability. Due to the great variety of compounds chromatographed, the effect on the detector is usually non-predictable. Certain classes of compounds are more prone to adsorb than others.

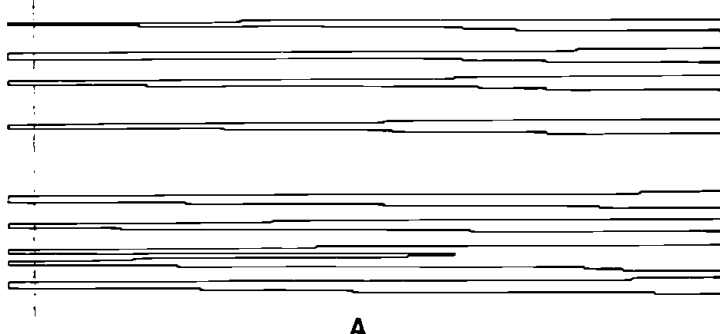
Attempts to treat the solid scintillator for inertness are met with various success. The sheer size of the surface area is sometimes sufficient to precipitate or absorb protein-like compounds. Of the three classes of solid scintillators in use today, possibly the least prone to unwanted adsorption is the plastic scintillator. A copolymer of PPO and polyethyltoluene is used in small bead sizes. The drawback of the plastic scintillator is its vulnerability to organic solvents. Glass scintillator (lithium glass activated with some doping agent) is probably the worst offender in this category. Silanizing the large surface temporarily helps, but when unwanted adsorption occurs, the best method of cleaning is cold ashing, or acid wash.

The third class of scintillators are crystalline materials like naphthalene, anthracene or the inorganic scintillator  $\text{CaF}_2$ . Adsorption on these compounds are selective and only practical experience can determine the usefulness of the detector.

Detectors using the homogeneous method are not prone to these problems. While rare, even liquid cells contaminate occasionally, however, the cause of this "real" contamination is not the same as the chromatographic process described above. Since the chromatographic effluent must be mixed with scintillator, the wrong selection of the liquid scintillator can cause the radioactive (or nonradioactive) compound to precipitate from the mixture. In this case, just as in an LSC vial, the precipitate can coat the walls of the system, making it unusable until redissolved. Samples containing raw protein and lipoproteins are the most troublesome compounds in this respect. A careful choice of liquid scintillator and mixing ratio, however, can usually solve the most stubborn problems.

When it comes to a choice of which system to be used (homogeneous or heterogeneous) the researcher must make an intelligent decision (Figure 1). For routine analytical work when the compounds to be

Inject



A

Inject



B

Figure 1. A represents 9 injections of 1000 dpm each  $^{14}\text{C}$  labeled glyceryl tripalmitate. Analyzed through a Radiomatic Instrument Model HP using 0.5 ml liquid cell. The HPLC effluent was 50% MeOH/50% water. B represents 4 injections of the same compound, analyzed with the same instrument but using a 0.25 ml solid scintillator filled cell. Note the apparent peak broadening and the deterioration of the baseline of the solid scintillator runs.

analyzed are known, and proven to be nonadsorbent on the solid cell, the cost of the heterogeneous system is an advantage even at the loss of  $^3\text{H}$  counting efficiency. The best application is amino acid analysis and quality control of synthesized radiolabeled compounds. For the general researcher working with metabolites, natural products and compounds known to have high adsorptive properties, the homogeneous system offers the advantages that overwhelm the difference in the cost of the analysis.

#### REFERENCES

1. W.R. Hende, "Radioactive Isotopes in Biological Research", John Wiley & Sons, 193-195, 1973
2. C. Giersch, "Quantitative High-Performance Liquid Chromatographic Analysis of  $^{14}\text{C}$ -Labelled Photosynthetic Intermediates in Isolated Intact Chloroplasts", J. Chrom., 172, 153-161, 1979.
3. P.A. Rodriguez, L.N. Mackey, F.B. Schroeder, "High-Efficiency Solid Scintillation Radioactivity Detection for High-Performance Liquid Chromatography", J. Chrom., 208, 1-8, 1981.
4. M. Kessler, "A Sensitive Radioactivity Detector for HPLC", Amer. Lab, August 1982.
5. A. Reich, "Solid Scintillators in High Pressure Liquid Radiochromatography", Radiomatic Instruments & Chem. Co. Inc., Technical Notes, Vol.2, 1981.