PLASTIC SCINTILLATORS: SOME NOVEL APPLICATIONS

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ABSTRACT. New and improved plastics have recently become available commercially. These plastics have clearly defined properties that make them more suitable for certain applications in scintillation counting and detection. One area where new plastics have potential is in homogeneous radioimmunoassay (RIA). Here, the use of appropriately modified plastics complement the technique and thus afford a higher detection capability. I discuss other detection systems where plastics have potential applications.

INTRODUCTION

Until recently, the use of plastics in scintillation spectrometry has been limited in area of application but relatively diverse in type of plastic. Major advances in plastics technology led to new types of plastics with unusual properties and new possibilities for their use in the field of scintillation spectrometry.

Plastic scintillators can be considered as solid solutions of a fluorescent organic compound in a suitable polymer, and have, for this reason, much in common with liquid scintillators. Just as the alkyl benzenes are highly efficient liquid solvents, so the polymeric derivatives of these compounds – polyvinylbenzene (polystyrene), polyvinyltoluene, *etc.* – are very efficient plastic solvents. The commonly used primary and secondary fluors are efficient both in liquid and plastic solution. However, the efficiency of a plastic scintillator depends on its mode of preparation as well as on its components. A polymer has a higher fluorescence quantum efficiency than a liquid solvent, so there is an appreciable (10–20%) component of radiative transfer. The solvent-solute transfer is usually less efficient in plastics than in liquid solutions, so that higher solute concentrations are required to achieve the optimum efficiency. Because the solvent is rigid, no molecular diffusion occurs, and energy migration is less efficient.

Advances in the use of plastic scintillators have not matched those of liquid scintillation, which have become a first-choice solution in scintillation spectrometry. Plastic scintillators, on the other hand, have been confined to areas that require ruggedness and ease of machining to desired shapes. As a result, they are available in several forms: sheets of various thicknesses down to a few microns, rods, filaments, microbeads, capillary tubing, *etc.* Also, large-sized plastic scintillators are advantageous where the detector size makes it either impossible or impractical to use crystalline phosphors. Thus, plastic scintillators are used mainly in systems with detectors, shields and light guides, such as gamma scintillation counters, solid-state flow detectors, fiber optics and neutron-radiation flux detectors. The unusual and desirable properties of some of these new plastics have prompted a re-evaluation of their potential.

APPLICATIONS

Immunological and Cellular

Adhesive plastic scintillators may be used to discriminate between radioisotopic labels that are bound to, or close to a surface and radioisotopic labels that are relatively far from the surface. Several beta-emitting radioisotopes commonly used in research (^{3}H and ^{125}I) have energies such

Liquid Scintillation Spectrometry 1992, edited by
J. E. Noakes, F. Schönhofer and H. A. Polach. RADIOCARBON *1993, pp. 179–184*

that most or all of the energy is dissipated after traveling a fraction of a millimeter through a medium of unit density.

If these radioisotopic labels are in an aqueous solution and are more than a fraction of a millimeter from a surface covered with adhesive plastic scintillator, they will not produce scintillations, and thus, will not be detected. Labels bound to, or within, a shorter distance will produce scintillations, the magnitude of which depends on the concentration and type of radioisotope used and the precise distance from the surface. The ability to discriminate between labels close to and far from a surface can be used to perform a variety of chemical and biological assays. These assays depend on measuring the amount of label that is bound to, or accumulated within particles, cells or molecules. The subject cells or molecules must be close, or bound to the plastic scintillator surface. To make the measurement, separation of the bound from the unbound (free) label is not necessary, as the free label in solution will not contribute significantly to normal background scintillations. The elimination of this inconvenient separation step increases assay precision and simplifies assay automation.

Scintillation Proximity Assay (SPA) Signal Enhancement with Adhesive Plastic Scintillator-Coated 96-Well Microplates

Current homogeneous SPAs (Amersham International plc) employ labels (^{3}H and ^{125}I) with low-energy electron-emitting properties. When bound close to a solid scintillator surface by a binding reaction, radioactive labels are able to transfer electron energy to the scintillator to produce photons detectable with a scintillation counter. Electrons emitted from the unbound (free) labeled molecules dissipate their energy in the liquid medium and are not detected. Thus, the bound fraction is detected specifically without separation of the solution from the solid support. The introduction of the Packard TopCount™ microplate scintillation and luminescence counter complements the benefits of homogeneous SPA technology by enabling plate-based automation, scaled-down 96-well assay format and increased sample throughput.

Two types of SPA kits are available commercially. The first (primarily for radioimmunoassay (RIA)), uses an inorganic glass scintillator. Other SPA kits, designed for ligand binding assays, use plastic scintillator beads with surface-binding properties more suitable for peptide conjugation. Unfortunately, due to geometry, both solid scintillating bead types have lower photon-yielding properties when compared to conventional liquid scintillation solutions. This, combined with scaling down assays to microplate format, has resulted in fewer observed counts within a set counting period, compared to traditional liquid-scintillation-based assays. This has proven to be detrimental to the sensitivity and performance of some SPAs, particularly where color quenching lowers count rates. Increased count rates (measured in counts per minute (cpm)) have been observed with sedimentation of the beads within SPAs. Currently, it is recommended that the scintillating beads be kept in suspension to enable stable counting for all samples.

This phenomenon of increased count rate related to sedimentation of the beads warranted further investigation. Initial studies indicated that if the beads were allowed to settle for a fixed interval and were then counted, the count rate of the bound fraction increased linearly to a maximum and then leveled off; however, the non-specific binding and background counts also increased.

The suggested course for this increased count rate was that, as the beads settled close together, a radiolabel bound to one bead could transfer its energy not just to that bead, but also to an adjacent bead. The increase in recorded non-specific binding and background counts was also due to the likelihood that a free label would be trapped close enough to the settled beads to enable energy transfer. However, the increased count rate was not sufficient to enhance assay performance and

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Aug 92  15.56             TopCount - 1.01
Protocol # 27             SPA Assay (Scintillating Plastic Microbead Based)

Data Mode:             CPM
Radionuclide:          125I-Liquid       Scintillator:Liquid
                                         Energy Range:Low
Count Time:            1.00              Efficiency Mode:High
Count Termination:     no
Background Subtract    Liq-Lo-Hi
Quench Indicator:      t-SIS

Plate Size:            96                Region A:  2.90-100.00
Plate Orientation:     Normal            Region B:  2.90-256.00
Screening:             no

Count Delay:           5.00
Half Life Correction:  no
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CPM A

	1	2	Bo	4	5	6	B50	8	NSB	10	11	12
A	6.00	4.00	9258.80	8.10	8.10	7.00	2797.00	9.00	140.30	6.00	12.00	14.20
B	4.00	5.00	9991.20	7.00	4.80	8.00	2717.70	12.10	133.30	16.20	9.20	14.20
C	6.00	5.00	9414.60	6.00	4.00	10.00	2593.00	15.00	143.40	11.20	11.20	10.00
D	1.00	2.10	9823.60	5.00	6.00	4.10	2657.00	11.00	137.40	6.20	12.10	12.30
E	5.00	1.20	9593.30	7.00	5.20	5.00	2687.20	6.20	146.20	6.10	12.00	7.00
F	2.30	4.00	5.00	9.00	5.00	7.00	6.00	1.10	4.00	4.10	12.00	13.30
G	14.00	3.00	4.00	4.00	4.00	2.00	4.00	2.00	4.00	2.00	9.00	11.00
H	5.00	6.00	3.00	3.00	1.00	1.00	3.00	3.00	2.00	4.00	5.30	12.00

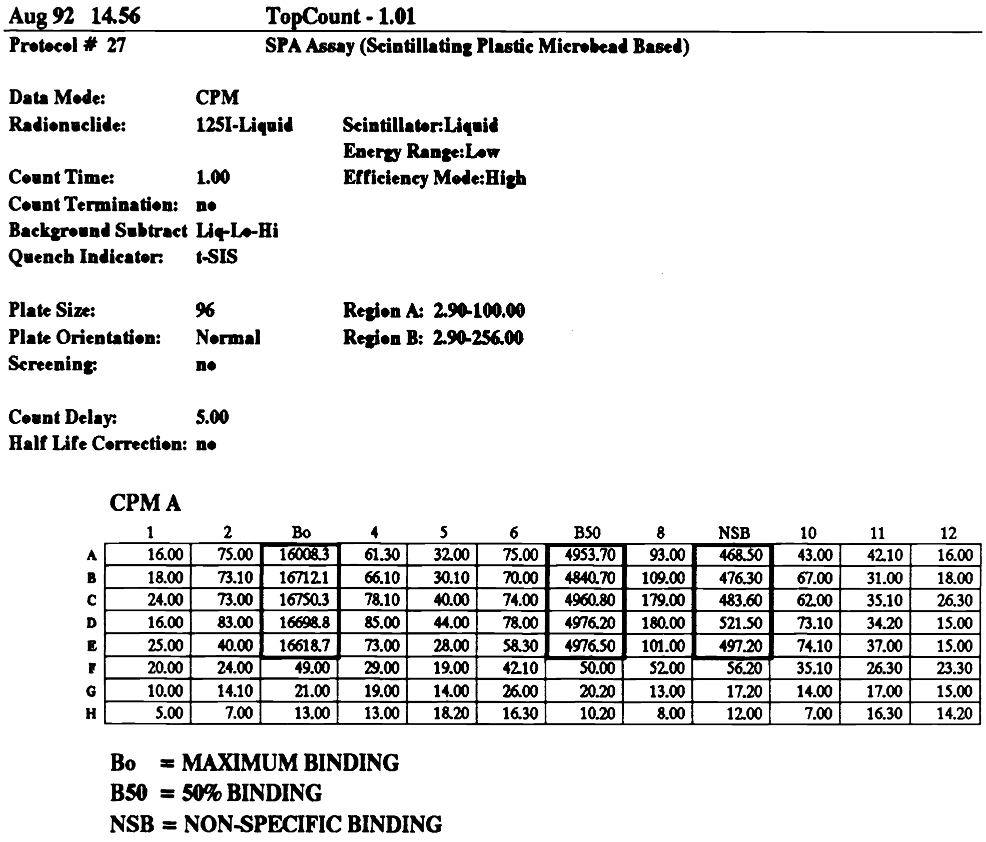
Bo = MAXIMUM BINDING
B50 = 50% BINDING
NSB = NON-SPECIFIC BINDING

Fig. 1. ^{125}I-SPA in a normal uncoated 96-well plate

reduce counting time. An adhesive plastic scintillator coated onto the bottom of the wells of microplates sufficiently enhances the required response counts. Identical ^{125}I-SPAs were made in a normal uncoated microplate (Fig. 1) and in an adhesive plastic-scintillator-coated microplate (Fig. 2). Results are presented as TopCount™ printed output. In Figures 1 and 2, columns A3–E3 show comparative count rates for the maximum binding (Bo). Columns A7–E7 show the comparative count rate for the B50 (50% binding), and columns A9–E9 show the comparative count rate for the non-specific binding (NSB). Figures 1 and 2 show a comparison of the two microplate results; the plastic-coated plate gave a significantly higher count rate. All other numbers are background count rates.

Adhesive Plastic Scintillator-Coated Well, Homogeneous Competitive Binding RIA

The plastic scintillator surface may be coated with an antibody to an analyte. Unlabeled analyte plus ^{3}H or ^{125}I- radiolabeled analyte may be incubated together with the surface in an aqueous solution. Radiolabeled analyte, which binds to the surface antibody, is detected with far greater efficiency than those free (unbound) in the bulk aqueous solution; thus, no separation of bound from free antibodies is needed. The amount of label detected is inversely proportional to the concentration of unlabeled analyte in the solution, due to competition for antibody binding sites (epitopes).



Aug 92 14.56 **TopCount - 1.01**

Protocol # 27 **SPA Assay (Scintillating Plastic Microbead Based)**

Data Mode: CPM
Radionuclide: 125I-Liquid **Scintillator:Liquid**
Energy Range:Low
Count Time: 1.00 **Efficiency Mode:High**
Count Termination: no
Background Subtract Liq-Lo-Hi
Quench Indicator: t-SIS

Plate Size: 96 **Region A:** 2.90-100.00
Plate Orientation: Normal **Region B:** 2.90-256.00
Screening: no

Count Delay: 5.00
Half Life Correction: no

CPM A

	1	2	Bo	4	5	6	B50	8	NSB	10	11	12
A	16.00	75.00	16008.3	61.30	32.00	75.00	4953.70	93.00	468.50	43.00	42.10	16.00
B	18.00	73.10	16712.1	66.10	30.10	70.00	4840.70	109.00	476.30	67.00	31.00	18.00
C	24.00	73.00	16750.3	78.10	40.00	74.00	4960.80	179.00	483.60	62.00	35.10	26.30
D	16.00	83.00	16698.8	85.00	44.00	78.00	4976.20	180.00	521.50	73.10	34.20	15.00
E	25.00	40.00	16618.7	73.00	28.00	58.30	4976.50	101.00	497.20	74.10	37.00	15.00
F	20.00	24.00	49.00	29.00	19.00	42.10	50.00	52.00	56.20	35.10	26.30	23.30
G	10.00	14.10	21.00	19.00	14.00	26.00	20.20	13.00	17.20	14.00	17.00	15.00
H	5.00	7.00	13.00	13.00	18.20	16.30	10.20	8.00	12.00	7.00	16.30	14.20

Bo = MAXIMUM BINDING
B50 = 50% BINDING
NSB = NON-SPECIFIC BINDING

Fig. 2. ^{125}I-SPA in an adhesive plastic scintillator-coated 96-well plate

Adhesive Plastic Scintillator-Coated Well, Homogeneous, Two-Site Immunoradiometric Assay (IRMA)

The plastic scintillator surface (solid phase) may be coated with a second antibody to an analyte. Unlabeled analyte is incubated with the solid phase in an aqueous solution. The tracer, ^{3}H- or ^{125}I-radiolabeled antibody, specific to a second binding site (epitope) on the analyte, is added in excess. Radiolabeled antibody (tracer) that binds to the analyte bound to the coated solid phase, is detected with far greater efficiency than those free (unbound) in the bulk aqueous solution; thus, no separation of bound from free antibodies is needed. The label is bound to the solid phase in direct proportion to the concentration of the analyte.

Similarly, antibody molecules may be bound to particles and incubated as described above. Following incubation, the particles may be brought into proximity with the plastic scintillator surface by sedimentation, by gravity, by centrifugation or by magnetic attraction (if applicable). Analyte molecules labeled with ^{3}H or ^{125}I that bind specifically to the particles are detected with greater efficiency than those free in the bulk aqueous solution; thus, no separation of bound from free antibodies is needed. Biological receptor molecules may be fixed to the surface of the plastic scintillator as in RIA, or to particles, as above, and incubated with radiolabeled ligands. The binding of the ligands to the receptors is detected, as above. Cells may be grown on the pre-sterilized surface of the plastic scintillator, or to particles as above and incubated with radiolabeled ligands. The binding of the ligands to the receptors on the cell membrane, or to receptors within the cells is detected as above.

Sealed Standards

Until now, only sealed liquid standards for liquid scintillation counting have been used. With the exception of the top end of the dynamic counting efficiency range, there is no reason why solid scintillators cannot replace liquid scintillation successfully as alternative sealed standards. The limiting factor preventing this innovation has been the availability of a suitable plastic. For optimum performance, the host plastic should have the following properties:

- A softening/melting point low enough to facilitate dispensing and revert to a rigid condition when cooled
- Transparency when solid
- Good solubility for the commonly used fluors
- Good solubility for the commercially available reference isotopic standard materials
- Good energy transfer properties in the scintillation process
- Stability for an extended period (with resistance to photochemical degradation).

If these requirements are met, solid plastic scintillators have the potential to replace the conventional liquid-sealed standards. A solid scintillator offers certain advantages over a liquid scintillator:

- The solid nature of the plastic scintillator means that handling operations are inherently safer. Accidental breakage of the "sealed" solid standard results in a more containable contamination and cleanup, as compared to a liquid standard.
- The low vapor pressures of plastic scintillators preclude the need for isolating glass vial contents by flame sealing.
- Standards encased in plastic are classified as solids, and thus, are not subject to disposal legislation associated with liquids.

To demonstrate the potential of this application, a "sealed" standard was fabricated using a suitable host plastic (α-methylstyrene/para-vinyltoluene co-polymer). Figure 3 shows a comparison with a conventional working series of liquid sealed standards. Here the ^{3}H was added in the form of tritiated n-hexadecane; t-SIE refers to the quench indicating parameter.

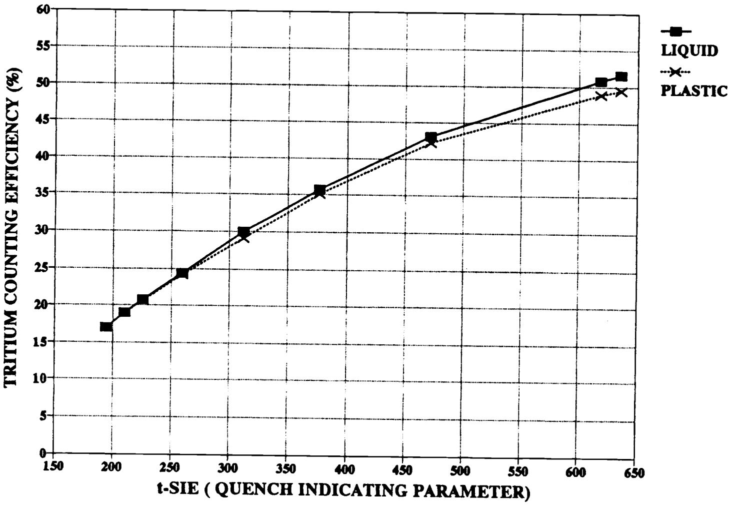


Fig. 3. ^{3}H counting efficiency *vs.* t-SIE for a series of liquid sealed standards

Finally, plastic scintillators are also being considered as suitable alternatives to liquid scintillators in large-scale detectors where plastic scintillators with low melting points offer certain advantages: long-term stability, high light output, freedom of orientation and compatibility with all construction materials.

CONCLUSION

Plastics are undergoing a renaissance in scintillation spectrometry. They are now being considered as suitable alternatives to liquid systems and as novel systems in their own right.

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