that the Astra-8 is an excellent instrument for urine chemistry determinations.

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References


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Counting Efficiency of Some Commercially Available Liquid Scintillators Compared

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We investigated four commercially available liquid scintillators as to overall counting efficiency and quenching effect of added phosphate-buffered isotonic saline. They differed significantly in these respects. Evidently, characteristics of the scintillator used in radioimmunoassays where β-particle emitters are counted should be carefully examined if results are to be optimal.

Additional Keyphrases: quenching by aqueous buffers - radioimmunoassay - quality control

Radioimmunoassay is used extensively in all branches of laboratory medicine today. Valid results depend on stringent quality control. In many of these procedures, weak β-particle emitters such as tritium are used, requiring liquid scintillation counting, which suffers from the inherent problem of quenching—i.e., fewer photons are emitted by the scintillator and photon energy shifts to lower levels. The magnitude of quenching depends on the exact composition of the scintillator and the form and concentration in which the radioactive specimen is introduced into the solution.

In routine radioimmunoassays of some steroids, our laboratory uses tritium as the labeling isotope, in a medium of phosphate-buffered isotonic saline. Addition of a 500-μL sample to 10 mL of a commercially obtained scintillator currently used produced turbidity of the counting solution, resulting in a precipitate, which settled during 4 h. Was quenching affected by this phenomenon and could a sample be counted 4 h after the standards and validly compared to the same standard curve? In an attempt to answer these questions we compared four commercially available scintillators with respect to total counts yielded, changes in energy spectra and channel ratios, and changes of these parameters with time after addition of the sample.

Materials and Methods

The buffer was phosphate-buffered saline (PBS), pH 7.0, containing, per liter, 60 mmol of Na2HPO4, 30 mmol of NaH2PO4, 160 mmol of NaCl, and 15 mmol of NaN3. We used 10 mL of each of four commercial scintillation fluids: Ready-Solv GP (from Beckman Instruments, Inc., Scientific Instruments Division, Irvine, CA 92713), Instagel, Monophase, and Pico-Fluor (all from Packard Instrument Co., Downers Grove, IL 60515).

We added 20 μL of tritium hexadecane (Packard Source Reagent, 5.04 × 106 ± 3% dpm/g; Packard Instrument Co.) to each vial and measured the radioactivity with a Model SL-30 liquid scintillation spectrometer (Intertechnique, 78370 Plaisir, France). Energy spectra were determined with a Model 6420 multichannel analyzer (EGSG Ortec Inc., Oak Ridge, TN 37830). Counts were obtained after each 50-μL increment of buffer and the energy spectra were determined after each total increment of 200 μL, i.e., the energy spectra were determined seven times during addition of buffer. After a total of 1200 μL had been added, the radioactivity in the vials was counted repeatedly for 1 min during the next 4 h, at the end of which the energy spectra were redetermined. The radioactivity in the vials was then counted for 1 min at regular intervals during the following 48 h. The counter is equipped with three channels: A, B, and C. With no buffer added to the scintillators the A and B channels were set by adjusting the upper and lower discriminators to yield about the same number of counts in both channels. The fixed tritium (3H) setting was used for channel C, a setting that is routinely used for 3H counting. The channel ratio A to B was calculated for every 50-μL increment of buffer. Maximum counting efficiency was determined by integrating the areas below the energy spectra and was therefore not affected by window settings.

Results

The count rate determined before buffer was added proved Instagel to be the most efficient (57879 ± 249 cpm); Monophase exhibited significantly lower counts (44695 ± 362 cpm). All scintillation fluids except for Ready-Solv GP became
opaque after addition of 200 μL of buffer. In Monophase and Pico-Fluor this opacity cleared after 400 μL buffer was added, but it persisted in Instagel until a precipitate finally settled after 4 h. Ready-Solv GP remained clear throughout the experiment. The counting efficiency of Instagel was not affected by the turbidity and subsequent precipitate formation. The counting efficiency of all scintillators decreased as more buffer was added. In the range of volumes utilized in practice (500–900 μL) Ready-Solv GP exhibited the highest relative counting efficiency of the four compared and was the least affected by changes in buffer volume (Figure 1).

Figure 2 illustrates the change in channel ratios with the addition of successive increments of buffer; the shifts in energy spectra during the same experiment are compared in Figure 3. A significant difference in the percentage channel ratio change can be observed, which is most marked in the case of Monophase, with Ready-Solv GP least affected by changes in buffer volume. A change in energy spectra to lower levels is also evident. Pico-Fluor proved to be the most inefficient in these experiments, showing the lowest total counts and the most pronounced shift in energy spectrum.

**Discussion**

The overall counting efficiency of different scintillation fluids varies significantly, both in terms of total counts yielded and change induced by addition of aqueous buffer solutions.

![Graph showing influence of buffer volume on relative counting efficiency](image)

**Fig. 1.** Influence of buffer volume on relative counting efficiency

A constant number of dpm, as tritium hexadecane, was added to each fluid and the relative efficiency of counting calculated after every 50-μL increment of buffer.

The highest counting efficiency will result in the highest overall precision. Ready-Solv GP and Instagel yielded the best results while Pico-Fluor and especially Monophase were unsatisfactory. Each scintillator behaved differently when aqueous buffer solutions were added. Except for Ready-Solv GP, all scintillators examined became opaque, with formation of precipitates. Although we were primarily concerned about this effect in terms of quenching and therefore changes in counting efficiency with time, this did not appear to influence the results in the case of Instagel. However, one cannot assume that this will be the same for all fluids, because Ready-Solv GP, in which this phenomenon did not occur, also showed the least change in spectral energy and channel ratio. Should co-precipitation of labeled isotope also result, serious errors may be introduced. The effect of adding increasing volumes of buffer to different scintillators is dramatic, both in terms of relative counting efficiency and percentage channel ratio change.

In these respects Ready-Solv GP was clearly superior to the others investigated. It is vitally important to evaluate for each scintillator a range of sample volumes that can be accommodated without significantly changing counting efficiency. This becomes particularly important in an assay where the total number of counts per tube is relatively low, because the coefficient of variation will be large, resulting in poor precision.

The properties of the scintillator used in radioimmunoassays should be carefully evaluated. The exact composition of commercially available scintillation liquids is not made known to the user and thus it is impossible to predict their behavior (2). This particular variable in the quality control of radioimmunoassays should perhaps receive more attention.

**References**
