Imaging Scanners for Radiolabeled Thin-Layer Chromatography

C.O. Rock, S. Jackowski and S.D. Shulman[1]
St. Jude Children's Research Hospital and [1]Bioscan

Over the past thirty years, thin layer chromatography (TLC) has become a routine and versatile analytical technique. While it appeared to some that TLC might be replaced by high-performance liquid chromatography (HPLC), TLC continues to grow in overall use. There are several reasons for its persistance among analytical techniques: 1) Easy, cost effective separations; 2) improved quality of commercial TLC plates; 3) greater selection of adsorbants; 4) improved instrumentation for post-development analysis; 5) large body of literature on separation techniques. The above conditions permit reliable and rapid quantitative analysis of a large number of samples per plate development for an initial investment that is modest compared to that required for many other analytical techniques.

TLC and HPLC now co-exist and play mutually supporting roles. Initial separations can be performed with TLC to find out what classes of compounds are involved, and then HPLC studies can provide higher resolution separations of closely related molecular species. In many instances, TLC provides useful insight into the appropriate HPLC solvent systems to be used. TLC also provides a routine, low cost assay method once all the system components have been identified by HPLC or other techniques.

One major problem encountered with the use of TLC in the research laboratory is in the detection and quantitation of radiolabeled compounds. Two traditional techniques, autoradiography and liquid scintillation counting (LSC), both have limitations. For autoradiography, exposure times range from days to weeks, and the technique does not provide quantitative results unless pre-flashed film is used and exposures are in the linear range for the emulsion. Liquid scintillation counting (LSC) requires that the radiolabeled material be removed from the TLC plate and placed into LSC vials. This procedure, usually known as "plate scraping," yields excellent quantitative data but is very time consuming, destroys the sample, requires a large number of vials to provide reasonable spatial resolution on the lane, and is a potential safety hazard.

To improve the speed of quantitative TLC analysis of radiolabeled compounds, Imaging Scanners have been developed that provide high sensitivity digital data from an entire chromatographic separation in one measurement without mechanical scanning (1,4). The concept is shown schematically in Figure 1. The detector is a windowless imaging proportional counter that views the entire lane simultaneously to provide a complete picture of the radiolabeled compounds separated on that lane. This type of detector uses a special gas mixture (90% argon, 10% methane) to measure the ionization produced by either beta- or gamma-rays emitted during radioactive decay. The ionization is collected at the high voltage anode wire and sensed by the electronic circuitry. The key to the imaging capability is the resistive anode which divides the collected charge in proportion to the position of the ionizing particle along the anode. A position for each event is then calculated by the microcomputer, and the number of

events are accumulated and stored in specific memory locations corresponding to that position.

The imaging capability provides a factor of 100 improvement in sensitivity over traditional mechanical scanners and allows this instrumentation to overcome the limitations inherent in counting low energy beta emitters from the surface of TLC plates. Especially with tritium (^{3}H), the typical 250 micron Silica Gel layers are transparent to only 1-2% of the total tritium betas. Even at this low efficiency, the Imaging Scanner easily quantitates a component labeled with 500 DPM or less of tritium in 10 min. With ^{14}C or higher energy isotopes, the corresponding sensitivity is 100 DPM or less detected in 10 min.

The Imaging Scanner's sensitivity makes it much faster than autoradiography (minutes instead of days), and its speed, quantitative capabilities, and lack of required sample preparation make it more attractive than LSC in many TLC applications. To provide good quantitative data, however, the Imaging Scanner must be used in conjunction with uniform TLC plates. Because low energy betas from near the surface of the layer have a higher probability of reaching the detector, the layer must be of uniform thickness all along the lane being analyzed. Fortunately, with modern, commercial plates, such uniformity is now routinely available.

In many types of research laboratories, Imaging Scanners have almost completely replaced the older method of "plate scraping" and scintillation counting. A brief review of the older technique shows why just the time and supplies saved make this switch "cost-effective." Both the Imaging Scanner and "plate scraping" start with a developed, dried TLC plate. For the scanner, the next few minutes are all that is needed to have complete quantitative data in the researcher's hands. With "plate scraping," the lanes must first be marked into segments to be scraped. For samples with possible unknown components, the lane is generally divided into 20-40 segments. The technician must then scrape the Silica Gel from each of these regions into separate scintillation vials. (The scraping should be done in an enclosed box or hood because of the danger of inhaling airborne silica particles and radioactive material.) Once the material is in 20-40 separate vials, the samples must be suspended in an LSC "cocktail" added to each of the vials. The vials must then be loaded into the counter, counted, unloaded, and disposed of in accordance with the regulations for low level *liquid* radioactive waste. The LSC data must then be manipulated to show the distribution of activity on individual lanes and to give integral results for the peaks of interest.

In the lipid metabolism laboratory of the Biochemistry Department at St. Jude Children's Research Hospital, TLC is one of the main analytical methods, and the use of the Imaging Scanner provides a dramatic demonstration of its potential for increasing the speed of data acquisition and analysis. Tritium is the most common isotope used, and the windowless detector and sophisticated software allow the detection of this low energy isotope as well as all others. Even with a typical tritium detection efficiency of 1-2% on TLC plates, a statistically significant number of total counts can easily be accumulated with analysis times of 10-60 minutes per lane. The data are stored on disk and can be retrieved later for graphic manipulation or further data analysis.

An example of the application of the Imaging Scanner technique to the evaluation of the distribution of tritium label between various phosphatidylinositol species (c.f. Reference 2) is shown in Figure 2. The scanning

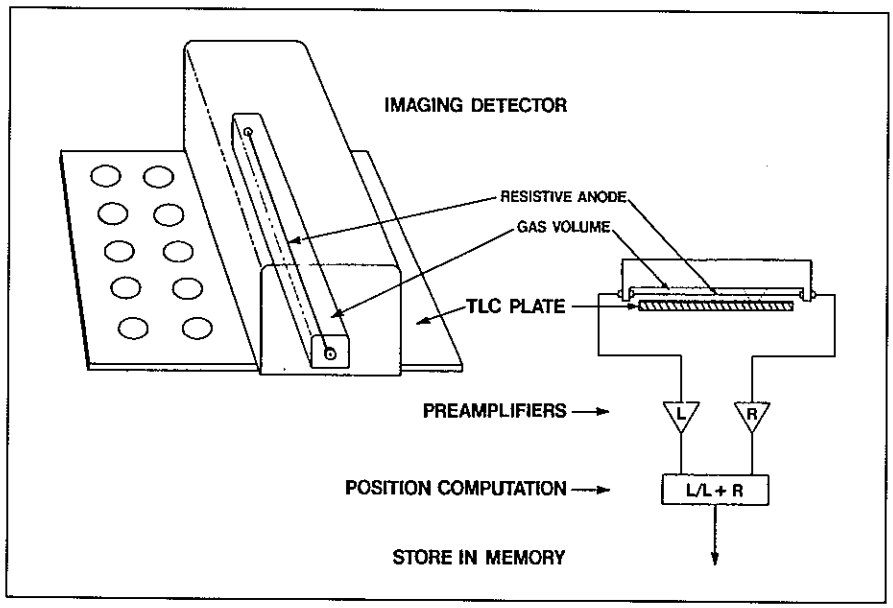

Figure 1. Schematic diagram of a resistive anode imaging proportional counter used to quantitate radioactivity on TLC plates.

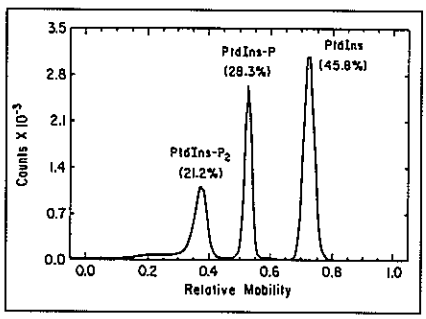

Figure 2. Typical Imaging Scanner analysis of the distribution of tritium label between various phosphatidylinositol species.

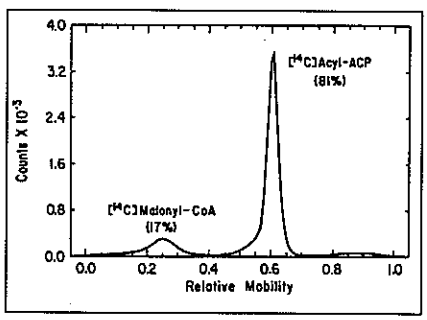

Figure 3. Typical Imaging Scanner analysis of the enzymatic activity of a cell-free extract of *E. coli*.

resolution is better than 1 mm, far superior to anything that can readily be achieved by "plate scraping." Most importantly, the Imaging Scanner analysis is non-destructive so that *samples can be recovered for further use or analysis.* This feature, combined with ability of the scanner to give precise compound locations on the TLC plate, makes it particularly useful with preparative TLC for the cleanup of extracted or synthesized compounds that will be used in further experiments.

Enzyme assays are easily quantitated using the Imaging Scanner. In Figure 3, the enzymatic conversion of [2-^{14}C]malonyl-CoA to [^{14}C]acyl-acyl carrier protein by a cell-free extract of *E. coli* is illustrated (3). An aliquot of the reaction mixture was removed and the Imaging Scanner used to determined the percentage of radioactive label transferred from CoA to acyl carrier protein. Because the scanner gives a complete picture of each lane it is particularly useful when: 1) There are several products of the reaction, 2) the compounds of interest are not well separated, 3) small (10 cm) high performance TLC (HPTLC) plates are used, and 4) something goes wrong in either the enzyme reaction or in the chromatographic separation. For routine assays, the software also allows preset integration regions to be selected so that reaction products will be automatically reported as a percent of the total activity on the lane.

Direct laboratory experience shows that the absolute counting efficiency of the Imaging Scanner depends on both the isotope and the type of TLC plate being used. For example, HPTLC plates with very thin layers will count at a higher efficiency than plates with standard 250 μ layers. Direct experience also shows that counting efficiency is uniform over the entire length of high quality commercial plates.

A typical procedure for quantitation of the constituents of a mixture is to remove two aliquots, one to be spotted on a TLC plate and the other to be quantitated by LSC to obtain the absolute specific activity of the total sample. The Imaging Scanner is used to analyze the TLC lane and determines the relative distribution of radioactivity among the individual components separated on the lane. Absolute radioactivities for individual components are then obtained by multiplying the percent of total radioactivity from the scanner results by the absolute total radioactivity obtained by LSC analysis of a single liquid sample.

There are some pitfalls in obtaining quantitative results from the scanner. Especially at the origin, a significant self-absorption or quenching effect can occur due to the presence of buffer salts, protein, or other materials that do not migrate. Similarly, components

which do not migrate out of a pre-adsorbent layer are detected with a somewhat higher efficiency due to the low density material used in these regions. These quenching effects will be most severe with tritium labeled compounds. The effect can be eliminated by spotting "clean" samples and by using standard plates with a uniform layer over the entire active surface.

Because of the above problems and the intrinsically low counting efficiency of tritium on the plate, we recommend that each new type of assay be verified by standard LSC techniques before being put into routine use with the Imaging Scanner alone. For very low activity tritium samples (less than a few hundred DPM per peak), LSC techniques will give higher statistical precision simply because of their higher counting efficiency.

In summary, the Imaging Scanner provides quantitative results quickly with concise reports of peak position (R_f), peak area, and percent of total activity. Graphic output allows prompt visualization of radioactivity distributions, precise location of compounds for recovery, and comparison of multiple sample runs. Recent developments allow the quantitative evaluation of 2-dimensional TLC separations. This technique gives TLC separations a resolving power comparable to HPLC.

In addition to decreasing analysis time and effort, the Imaging Scanner saves a great deal of labor and disposable LSC supplies while reducing radioactive waste disposal problems. For an active laboratory, such savings can be substantial.

With these capabilities, the Imaging Scanner has become an indispensable instrument in lipid biochemistry, drug metabolism, toxicology, radiosynthesis, nuclear medicine, and other areas requiring the quantitative analysis of radiolabeled compounds on TLC plates, gels and blots.

REFERENCES

1. **Baird, W.M., L. Diamond, T.W. Borun and S. Shulman.** 1979. Analysis of metabolism of carcinogenic polycyclic hydrocarbons by position-sensing proportional counting of thin-layer chromatograms. Anal. Biochem. *99*:165-169.
2. **Jackowski, S., C.W. Rettenmier, C.J. Sherr and C.O. Rock.** 1986. A guanine nucleotide-dependent phosphatidylinositol 4,5-diphosphate phospholipase C in cells transformed by the v-*fms* and v-*fes* oncogenes. J. Biol. Chem. *261*:4978-4985.
3. **Jackowski, S. and C.O. Rock.** 1987. Acetoacetyl-acyl carrier protein synthase, a potential regulator of fatty acid biosynthesis in bacteria. J. Biol. Chem. *262*:7927-7931.
4. **Shulman, S.D.** 1983. A review of radiochromatogram analysis instrumentation. J. Liquid Chrom. *6(1)*:35-53.

Address correspondence to:

S.D. Shulman
Bioscan, Inc.
4590 MacArthur Blvd.
Washington, DC 20007