Design and Validation of a Radiochromatogram Scanner with Analog and Digital Output

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We have designed and built a versatile radiochromatogram scanner for chromatographic and electrophoretic strips of variable width and length and for chromatographic columns. The scanner's variable speed and collimator width and the availability of digital output give it a wide dynamic range. To every extent possible, it was constructed from general laboratory equipment and surplus parts that can be found in many nuclear medicine departments. A series of validation experiments was performed to demonstrate the capabilities of the system and shows its excellent spatial resolution for such gamma emitters as Tc-99m. Quantitative results are available immediately without the need to integrate peaks by planimetry or to cut and count each activity peak.

Radiochemical purity, the fraction of radioactivity present in the specified chemical form, is a major factor contributing to the reproducibility of a nuclear medicine diagnostic procedure. At one time the responsibility for radiochemical quality control was generally borne by the commercial manufacturer of the radiopharmaceutical. However, with the increased use of Tc-99m-labeled radiopharmaceuticals from commercial or home-made kits and preparations of other short-lived radiopharmaceuticals by independent investigators, the responsibility for quality control has shifted to individual nuclear medicine laboratories where the preparations are made.

Open bed chromatographic techniques on paper or thin-layer chromatographic (TLC) media and electrophoresis have become the methods of choice for determining radiochemical purity because of their speed and ease (*1*). The activity distribution on the chromatography or electrophoresis strips is usually quantitated using a radiochromatogram scanner designed for detecting radiation of the type and energy level in question, or by cutting the strips into multiple segments and individually counting each segment.

Not all radiopharmaceuticals can be tested adequately using open bed techniques. In some instances, such as in the separation of Tc-99m-labeled proteins and reduced hydrolyzed Tc-99m, column chromatography may be more helpful. When column chromatography is needed, fractions of eluate may be collected and subsequently counted but this is a tedious method. Column chromatography followed by scanning of the intact column has been developed (*2, 3*). If a radiochromatogram scanner that could also scan columns were available, this simple and rapid technique could be used for routine radiochemical quality control of Tc-99m-labeled radiopharmaceuticals.

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Existing Methods

For most of the commonly used Tc-99m radiopharmaceuticals, rapid and simple miniaturized paper and thin-layer chromatographic systems have been described in the literature and many are now commercially available. In order to quantitate the two common radiochemical impurities in Tc-99m radiopharmaceuticals, pertechnetate and reduced hydrolyzed technetium, these chromatographic strips are commonly cut into two pieces, which are counted individually. The Squibb Q.C. Analyzer is designed specifically for this purpose. When cutting the strips in two, based on the expected migration rate of the components in the sample, one may run into problems caused by variations in running time and rate of migration of chromatographic and electrophoretic systems (*1*). Without knowing for sure how the activity is distributed, the quality control technologist can never be certain that this cut evenly divides the radioactivity peaks. An alternative method to determine the radioactivity profile is to cut the strips into many short segments and count each segment individually; this is a rather time-consuming and cumbersome technique.

Instead of cutting strips, the radioactivity profile can be determined by simple commercial radiochromatogram scanners especially designed for short strips. These systems utilize Geiger counters and detect primary beta particles and Compton electrons. They usually lack sufficient lead shielding to be useful for radionuclides of higher gamma energy than Tc-99m. Furthermore, collimator width and scan speed are usually not adjustable. The ADC Qualitygraph strip scanner has digital data output but only records total counts for the entire strip and counts over one impurity peak. The systems designed for scanning miniaturized chromatography strips are not useful for either longer strips or electrophoresis strips. A few compa-

nies (KESCO, Panax) have introduced instruments with scintillation detectors and sufficient collimation and shielding to be useful with moderate- to high-energy gamma emitters. These instruments are relatively expensive, especially if equipped with digital data output.

Because of the lack of appropriate commercial instrumentation, several groups have designed their own radiochromatogram scanners. Sunderland (*4*) used a shielded G-M tube integrated with a ratemeter and a strip-chart recorder. The chromatographic strip was attached to the chart paper in a way that pulled it across the narrow opening in front of the detector. English (*5*) used the scintillation camera and persistence scope as a detector and monitoring device. Williams (*6*) adapted a multichannel analyzer to serve as a radiochromatogram scanner. Above a collimated base he mounted a motor drive with a rubber friction wheel to advance strips. Using the multiscaling mode, counts were recorded into memory as a function of time, which could be related to position on the strip by the rate at which it moved.

If open bed techniques are not suitable for radiochemical quality control of a radiopharmaceutical, column chromatography may be the method of choice. Persson, (*2, 3*) developed a gel permeation, column chromatographic technique suitable for radiochemical analysis of Tc-99m-labeled radiopharmaceuticals. The activity distribution in the column was measured by scanning with the same techniques developed for strips or by using the gamma camera to image the whole column (*7*). Although the latter is acceptable when the bands of radioactivity are well separated, the camera does not allow as much resolution as a scanning device.

We felt that in an active radiopharmacy laboratory a scanning instrument was needed that was versatile enough to handle strips of variable sizes as well as columns, and that could be used for routine and investigational quality control. The ideal radiochromatogram scanner should have a well-shielded detector so that it can be used for radioisotopes with gamma energies higher than that of Tc-99m. Both scan speed and collimator width should be variable to give a wide dynamic range. The instrument should record analog output as a rapid check of radiochemical impurities and digital output, preferably with integration, to give quantitative results. The latter is particularly important for the small and often wide impurity peaks that are seen in some radiopharmaceutical preparations. Since a versatile and inexpensive radiochromatogram scanner meeting the above criteria was not, to our knowledge, commercially available, we designed our own. By using a detector and some electronic components from a dual-probe counting system that was no longer being used in our clinic, the cost was modest.

Instrument Design

Figure 1 shows a schematic drawing of our system. A standard NIM bin and the following electronic components were available from the dual-probe system: high

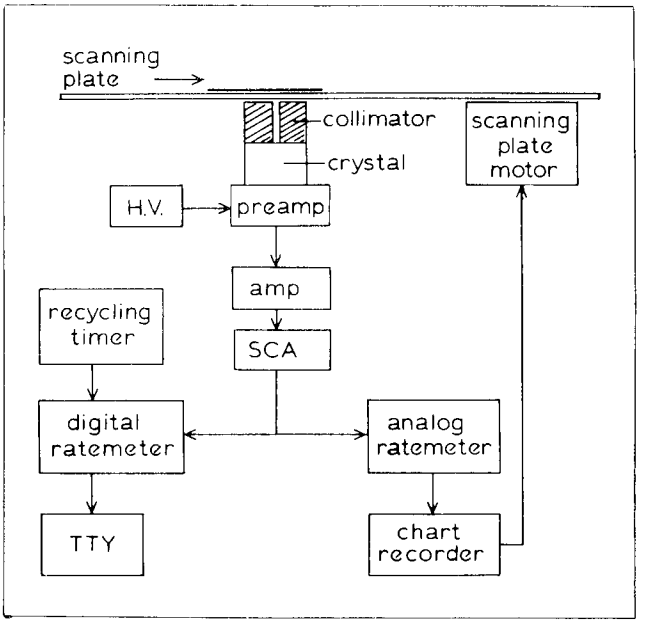

FIG. 1. Block diagram of the NaI radiochromatogram scanner.

voltage power supply, linear amplifier, single-channel analyzer (SCA), and linear ratemeter with variable time constant. We added a four channel digital ratemeter, a variable timer and a teletype, giving us digital data printed at preselectable intervals as well as total accumulated counts. We also added a strip chart recorder (Heath-Schlumberger model SR-204, Heath Co., Benton Harbor, MI) to give analog output. The strip chart recorder has a stepper motor with variable speed of 0.01–10 cm/min. For the movement of the scanning plate to be controlled by the strip chart recorder, a second stepper motor was incorporated into the recorder circuitry. The additional stepper motor was given its own peripheral driver to prevent exceeding the current capacity of the strip chart drive. Increased reliability can be achieved by the inclusion of a latch to buffer the existing circuitry from the additional driver. Modification of the strip chart recorder required one additional stepper motor (No. 420-88, also from Heath Co.), one dual peripheral positive driver (device No. 75452), one dual J-K master-slave flip-flop (TTL No. 7473), and two 2μF capacitors.

The NIM bin, NIM electronics, digital ratemeter, and strip chart recorder were all mounted in a relay rack inside a home-built, counter-high cabinet. The detector is a 2-in. NaI (Tl) crystal with ½-in. lead shielding on all sides except for the top. The detector is also housed inside the cabinet with the crystal mounted 12 mm below the countertop, which has a circular opening in it. The removable collimator sits on top of the crystal flush with the table top. The lead collimator is 12 mm thick, 9.5 cm in diameter, and has an opening of 4.5 cm across that can be adjusted up to a maximum width of 4 mm. Chromatographic strips and columns are mounted on a ⅛ in. × 2½ in. × 24 in. Plexiglas scanning plate that rests on the table top and moves across

the collimator opening guided by two narrow Plexiglas strips. Two nylon gears, powered by the second stepper motor, extend through the countertop. These gears drive the movable Plexiglas, which has mated perforations on both sides. A clutch mechanism is included to disengage the gears so that the chart paper and the Plexiglas scanning plate may be moved independently.

Instrument Validation

Dynamic range: To determine the total amount of activity that could be scanned without significant deadtime losses, 10μl drops of Tc-99m were applied to several strips of Whatman No. 1 paper. The total activity on the strips at the onset of the experiment ranged from 160 to 590μCi as determined with a dose calibrator. These strips were scanned several times over a 2-day period. The collimator opening was 3 mm, the scan speed 2 cm/min, the single-channel analyzer window 100–200 keV, and the printout interval 0.2 min. Identical experiments were performed using a collimator opening of 1 mm with all other parameters unchanged. The results are illustrated in Figs. 2 and 3. With a 3 mm opening, deadtime losses of 5% or more were observed with total spot activity levels above 25μCi. When the collimator width was reduced to 1 mm, the same degree of count loss did not become apparent until the total activity was 250μCi.

Several scans were obtained for the above strips with a collimator width of 3 mm after the activity level had decayed to 0.5–2.4μCi. The total integrated background-corrected counts detected in each peak at this time were in the range 4,100 to 19,500. Based on two scans of each strip at these activity levels, the mean number of counts/μCi using the conditions listed above was 8443±270 (1s.d.).

We have subsequently used this instrument in routine quality control of Tc-99m and I-123 radiopharmaceuticals and have compared the results to those obtained by cutting the strips into segments for individual counting in a well counter as described below. Digital data are both accurate and reproducible and detect low level impurities that were not seen on the strip chart output. Even when the total counts in the impurity peak is a few hundred counts after background correction, the impurity can be quantified.

The counting efficiency for a low energy photon emitter, I-125, was determined in a similar experiment. A strip was prepared with two 0.7–0.8-cm wide spots of I-125 centered 4.5 cm apart and with activity levels of approximately 0.4 (1) and 1.2μCi (2), respectively. This strip was scanned several times using scan speeds of 1 and 2 cm/min and collimator widths of 1 and 3 mm. The SCA window was 20–50 keV. As seen in Table 1 total counts/μCi of I-125 varied from 2,000 to 30,000 depending upon the collimator width and scan speed.

Spatial resolution: In order to measure how well the scanner could separate spots of radioactivity located close together, we spotted a Whatman No. 1 paper strip with a total of eight Tc-99m spots along its length. The centers of the first four spots were 2 cm apart, the last four spots were 1 cm apart. The ratio of activity levels for the four spots in each series was 1:2:3:4. The volume was kept constant, resulting in spots with a diameter of 7–8 mm. Each spot was dried rapidly with a flow of warm air. The strip was scanned at 2 cm/min, using a collimator opening of 1.5 mm. The single channel analyzer window was set at 100–200 keV. The excellent resolution is illustrated in Fig. 4.

The same experiment was performed with I-131 and activity ratios of 1:2.5:5:10 for the two series of four spots each. The smallest spot contained about 0.4-μCi I-131. The single channel analyzer window was set at 344–384 keV, collimator opening was 1 mm, and scanning speed was 2 cm/min. As seen in Fig. 5 (A), the peaks that were spaced 1 cm apart could not be separated. The spots that were spaced 2 cm apart were poorly separated, and the lowest activity spot could not be identified because of

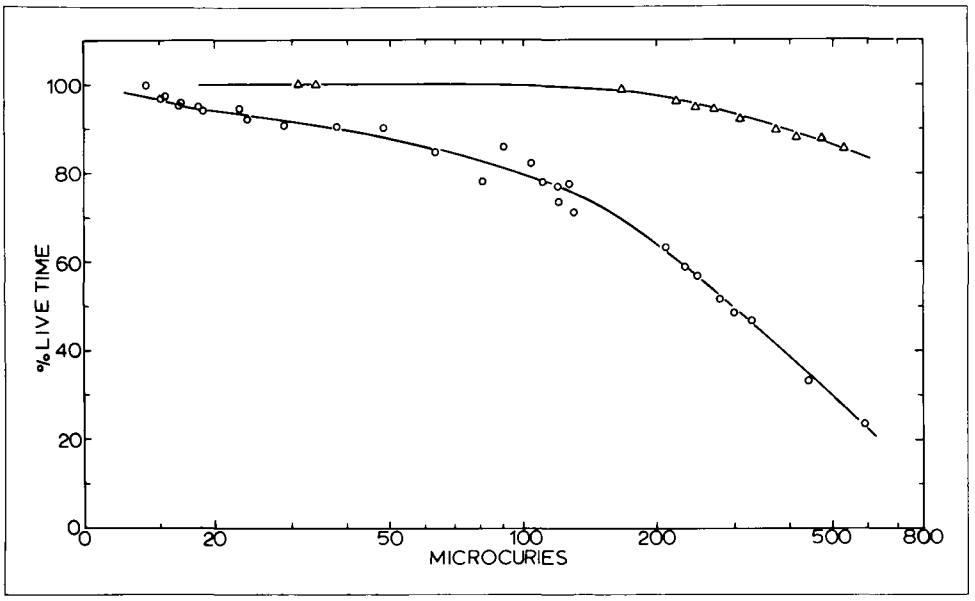

FIG. 2. Percent live time (% of actual counts observed) against μCi of Tc-99m in peak using collimator widths of 1 mm (△) and 3 mm (o).

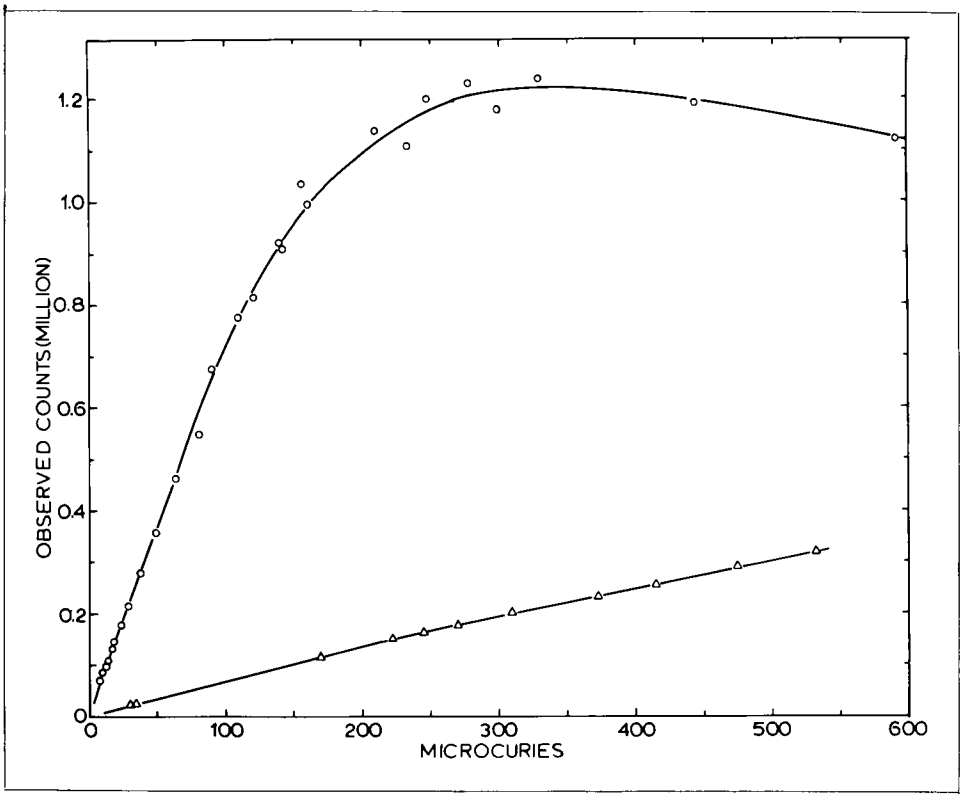

FIG.3. Observed counts (million) against μCi of Tc-99m in peak using collimator widths of 1 mm ($\triangle$) and 3 mm (o).

TABLE 1. Counts from Scanning I-125-Strip

Collimator Width (mm)	Scan Speed (cm/min)	Counts in I-125 peak 1(0.4μCi)	2(1.2μCi)	Counts/μCi 1	2
1	2	808	2,228	2,020	1,857
1	1	1,592	4,748	3,980	3,957
3	2	5,955	18,401	14,860	15,334
3	1	12,258	36,065	30,645	30,054

the high activity peak preceding it by 2 cm. A repeat scan, Fig. 5 (B), of the strip using a radiochromatogram scanner with 1 cm wide gas-flow detector and no lead collimation showed much improved resolution. Radionuclides that are beta emitters and have high energy gamma peaks are probably best studied with instruments equipped with beta detectors, unless the NaI detector can be constructed with even more lead shielding than in our instrument.

Comparison with other methods: Routine use of the radiochromatogram scanner in our department involves determining the activity distribution on cellulose acetate strips following electrophoresis of [^{123}I] iodide and radioiodinated protein solutions such as fibrinogen. In our electrophoretic system we achieve good separation of iodate and iodide in [^{123}I] NaI solutions. A third peak, probably caused by small amounts of periodate, is usually retained at the origin. Iodine-123-labeled fibrinogen remains close to the origin and is tested for free iodide following purification. The electrophoresis strips are scanned at 2 cm/min with a window of 100–200 keV and counts are printed every 0.2 min. The total counts and percent activity in each peak are then calculated after correcting for background activity.

A comparison of the results obtained in the above manner (method 1) with those using our previous method was made on three different batches of [^{123}I] NaI and purified I-123-labeled fibrinogen. The previous method (2) consisted of scanning the strips using a commercial scanner with gas-flow detector and only analog output. The scanning speed was synchronized with the chart paper, so that we could identify the location of individual peaks. Strips were cut accordingly, and each segment was counted in a gamma well. Since the activity level needed for scanning was considerably higher than what could be counted in a well counter, we usually had to wait 1–2 days before obtaining final results. The results from both methods correlated well (Table 2), even for peaks containing as little as 0.5% of the total activity on the strip.

Discussion and Conclusions

We have designed and built a versatile radiochromatogram strip scanner that is well suited for detection of gamma emitters. Because we incorporated several components that were no longer in use, we were able to do this at a modest expense. This instrument is useful for scanning strips of variable widths (up to 4.5 cm) and lengths as well as chromatographic columns. The latter is only possible with a flat and rigid scanning plate. A clutch mechanism

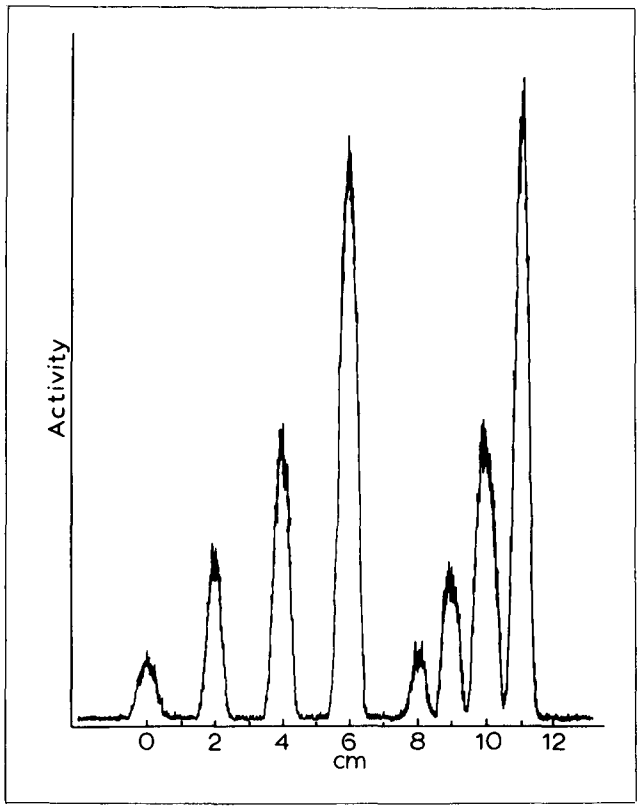

FIG. 4. Resolution of Tc-99m peaks. Scan speed 2 cm/min. Collimator width 1.5 mm.

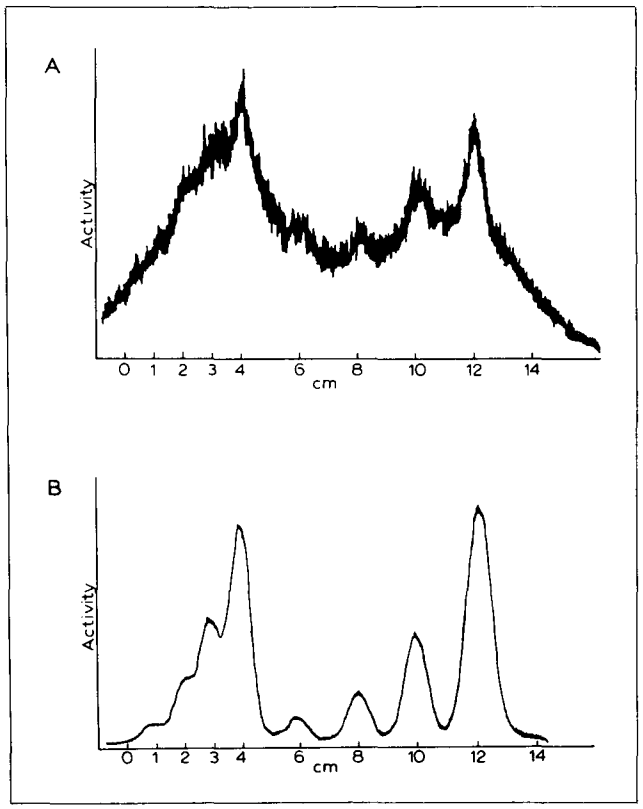

FIG. 5. Resolution of I-131 peaks: (A) Scanned at 2 cm/min using new scanner with NaI detector and 1 mm wide collimator opening; (B) Scanned at 5.4 cm/min using instrument with gas-flow detector.

TABLE 2. Comparison of Two Methods for Determination of Activity Distribution on Electrophoresis Strips

		% Activity Distribution	
Test Samples from Different Batches	Component	Method 1	Method 2
[^{123}I] NaI	origin	0.5	0.6
	iodate	2.2	1.6
	iodide	97.3	97.8
[^{123}I] NaI	origin	0.7	0.7
	iodate	1.8	1.4
	iodide	97.5	97.9
[^{123}I] NaI	origin	1.9	2.0
	iodate	12.1	11.2
	iodide	86.0	86.8
I-123 fibrinogen	fibrinogen	99.7	99.7
	iodide	0.3	0.3
I-123 fibrinogen	fibrinogen	98.0	95.6
	iodide	2.0	4.4
I-123 fibrinogen	fibrinogen	97.7	98.3
	iodide	2.3	1.7

allows for manual scanning in order to quickly check the activity level when setting up for scanning a sample. An alternative design would be to keep the object to be scanned stationary and to move the detector. This approach is common in commercial instrumentation but the added structural engineering required to support a shielded crystal results in a much larger and more expensive instrument with no advantages if scanning is to be limited to one dimension. Our detector system is easily adapted for counting stationary samples in test tubes by ensuring constant geometry. Because both scan speed and collimator opening are variable, we can count relatively low activity samples with good statistics by increasing the collimator opening or decreasing the speed; the latter will not reduce spatial resolution. High activity samples can be counted without significant deadtime losses by decreasing the collimator width. The dynamic range of this scanner for Tc-99m is about 50nCi to 250μCi and sensitivity for I-125 is equally good. The SCA makes it possible to select the best window to count multiple energy peaks. The detector has enough lead shielding for medium energy gamma emitters such as Tc-99m and I-123, but is not well suited for strip scanning the multiple high energy photons of I-131 or the annihilation radiation of positron emitters. For the latter, a second detector operated in coincidence could be positioned directly above the primary detector and scanning plate.

Digital printout eliminates the need to quantitate the activity in individual peaks by such less accurate techniques as planimetry. It is possible to accurately assess radiochemical purity within minutes after preparation and the problem of activity levels too high for counting in a conventional well scintillation counter is avoided. Radio-

chromatogram scanners with similar capabilities are now becoming available commercially. However, the price for new equipment of this type makes the alternative of building your own system one that should be considered.

Schematics, photographs of the printed circuit boards, and other construction details are available on request from Kenneth A. Krohn.

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