On-Line Radioisotope Detection for Capillary Electrophoresis

Stephen L. Pentoney, Jr., and Richard N. Zare*

Department of Chemistry, Stanford University, Stanford, California 94305

Jeff F. Quint

Beckman Instruments, Inc., Scientific Instruments Division, Fullerton, California 92634

Two simple, on-line radioactivity detectors for capillary electrophoresis are described. The first capillary electrophoresis/radioisotope detector system utilizes a commercially available semiconductor device, which is positioned external to the separation channel and which responds directly to impinging γ or high-energy β radiation. The second capillary electrophoresis/radioisotope detector utilizes a commercially available plastic scintillator material that completely surrounds (360°) the separation channel, thereby improving the efficiency of detection. The system performance is evaluated for both detection schemes by using synthetic mixtures of ^{32}P-labeled sample molecules. The efficiency of the semiconductor detector (planar geometry) is determined to be approximately 26%, whereas that of the plastic scintillator is found to be approximately 65%. The detection limits are determined to be in the low-nanocurie range for separations performed under standard conditions (an injected sample quantity of 1 nCi corresponds to 110 × 10^{-18} mol of ^{32}P). The lower limit of detection is extended to the subnanocurie level by use of flow (voltage) programming to increase the residence time of labeled sample components in the detection volume.

The highly efficient separations afforded by capillary electrophoresis (CE) are a direct result of employing extremely narrow separation channels. Effective dissipation of heat generated by the passage of electrical current through the separation medium occurs only when the capillary inner surface area to internal volume ratio is sufficiently large (typically 10^4 to 10^5 m^{-1}). For this reason capillary tubes with internal diameters ranging from 200 μm to as small as 10 μm (1) have been selected for CE separations.

Early in the development of capillary electrophoresis, it was noted that the successful detection of separated sample components present within the narrow confines of these capillary tubes posed a major challenge (2). In response to this challenge, much research has been directed toward the development of sensitive and selective detectors for capillary electrophoresis. The reported CE detector technology has been largely borrowed from the field of high-performance liquid chromatography (HPLC), especially from microcolumn HPLC. Successful extension of the various HPLC detection schemes to capillary electrophoresis has generally involved miniaturizing existing technology while at the same time striving for improved sensitivity.

One detection scheme that is used widely in HPLC but has received little attention in capillary electrophoresis applications is radioisotope detection (3–6). The availability of an on-line radioisotope detector for CE would be especially appealing for several reasons. First, state-of-the-art radiation

detection technology can offer extremely high sensitivity. Second, radioisotope detection affords unrivaled selectivity because only those sample components that have been radiolabeled yield a response at the detector. Third, the radiolabeled molecule possesses the same chemical properties as the unlabeled molecule, thereby permitting tracer studies to be conducted. Fourth, radioisotope detection can be directly calibrated to provide a measurement of absolute concentration of the labeled species. Finally, a capillary electrophoresis system in which radioactivity detection is coupled with more conventional detectors would add extra versatility to this new separation technique.

Radioisotope detection of ^{32}P, ^{14}C, and ^{99}Tc has previously been reported by Kaniansky et al. for isotachophoresis (7, 20). In their work, isotachophoretic separations were performed by using 300 μm internal diameter fluorinated ethylene-propylene copolymer capillary tubing and either a Geiger-Mueller tube or a plastic scintillator/photomultiplier tube combination to detect emitted β particles. One of their reported detection schemes involved passing the radiolabeled sample components directly through a plastic scintillator. Detector efficiency for ^{14}C-labeled molecules was reported to be 13–15%, and a minimum detection limit of 0.44 nCi was reported for a 212 nL cell volume.

Altria et al. reported the CE separation and detection of radiopharmaceuticals containing ^{99m}Tc, a γ emitter with a 6-h half-life (8; see also ref 9). Their design involved passing a capillary tube ($\cong$2 cm long) through a solid block of scintillator material and detecting the light emitted as technetium-labeled sample zones traversed the detection volume. Unfortunately, detection limits and detector efficiency have not been reported.

We report here the design and characterization of two simple on-line radioisotope detectors for capillary electrophoresis. The first detector utilizes a commercially available semiconductor device that responds directly to emitted β particles that pass through the walls of the fused silica separation channel. A similar semiconductor detector for γ-emitting radiopharmaceuticals separated by high-performance liquid chromatography has previously been reported by Needham and Delaney (10). Alternatively, a second detector utilizes a commercially available plastic scintillator material that completely surrounds (360°) the detection region of the separation channel. Light emitted by the plastic scintillator is collected and focused onto the photocathode of a single, cooled photomultiplier tube. The detectors described in the present work are applicable to both high-energy β emitters and γ emitters. We report here on their application to the detection of ^{32}P-labeled molecules separated by capillary electrophoresis.

EXPERIMENTAL SECTION

On-Line Radioactivity Detectors. The first on-line radioactivity detector (see Figure 1) consisted of a Model S103.1/P4 spectroscopic grade cadmium telluride semiconductor detector and a Model CTC-4B radiation counting system (Radiation Monitoring Devices, Inc., Watertown, MA). The cadmium tel-
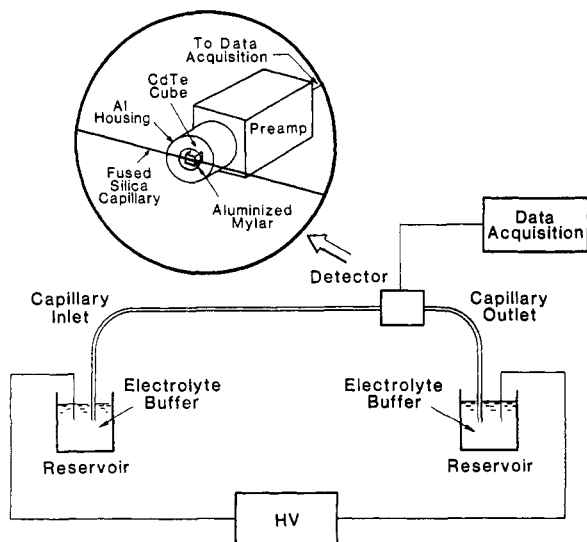
Figure 1. Experimental setup of the capillary electrophoresis/semiconductor radioisotope detector system. The inset shows the positioning of the CdTe probe with respect to the capillary tubing. The 2-mm Pb aperture is not shown in this illustration.

Figure 2. Exploded diagram of the plastic scintillator radioisotope detector. The fused silica capillary is exposed to a 2-mm section of the plastic scintillator that is located in between the press-fit aluminum mounting rods.

luride detector probe consisted of a 2-mm cube of CdTe, which was set in a thermoplastic and positioned behind a thin film of aluminized Mylar at a distance of approximately 1.5 mm from the face of an aluminum housing (see Figure 1). A 2 mm wide Pb aperture (0.008 in. thick) was used to shield the CdTe detector element from radiation originating from regions of the capillary adjacent to the detection volume. The aluminum housing incorporated a BNC-type connector that facilitated both physical and electrical connection to a miniature charge-sensitive preamplifier. The CdTe probe and preamplifier assembly were mounted on an x-y translation stage, and the face of the aluminum housing was brought into direct contract with the polyimide-clad fused capillary/Pb aperture assembly. The CdTe detector was operated at the manufacturer's suggested bias voltage of 60 V, and the detector signal (the creation of electron-hole pairs produced as β particles were decelerated within the semiconductor material) was amplified by the charge-sensitive preamplifier and sent through a 6-ft. cable to the counting a display electronics of the CTC-4B counting unit. Although the CTC-4B is capable of room-temperature energy discrimination, all experiments reported here were performed with a relatively large energy window. The upper energy discriminator setting was 1 MeV (the maximum setting for the CTC-4B), and the low energy setting was 0.01 MeV.

The CdTe radioactivity detector was computer-interfaced to a laboratory personal computer by using the open collector output of the CTC-4B counting unit. The open collector output was tied high by way of a 1-KΩ pull-up resistor so that the unit provided a negative going transistor-transistor logic (TTL) pulse for each count measured. This TTL signal was sent to a photon counter (Model 1109, EG&G Princeton Applied Research), and counting intervals (typically 1 s) for run-time data acquisition were preselected by way of front panel thumbwheel switches on the photon counter. The binary coded decimal (BCD) output of the photon counter was read at the end of a preset counting interval (strobe sent by the 1109 counter) by a laboratory computer (IBM PC-XT) using a 32-bit digital I/O board (Model DT2817, Data Translation, Inc., Marlboro, MA). Data acquisition and storage were accomplished with software that was written in-house (BASIC). Migration times and peak widths reported here were determined manually from scale-expanded portions of the recorded electropherograms.

The second on-line radioactivity detector consisted of a plastic scintillator material (BC-400, Bicron Corp., Newbury, OH), which was machined from 1 in. diameter rod stock into a $^5/_8$ in. diameter (front face) solid parabola (see Figure 2). A special rotating holder was constructed for the plastic scintillator, and the curved outer surfaces were coated by vaccum deposition with a thin film of aluminum in order to reflect the emitted light toward the front face of the scintil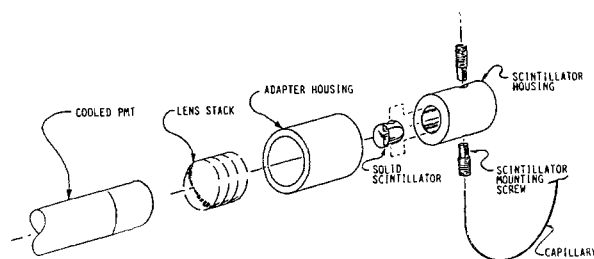lator. A 2-mm detection length was defined within the parabola by 0.250 in. outer diameter aluminum mounting rods, which were press-fit (coaxial to the separation capillary) in the sides of the scintillator as illustrated in Figure 2.

The light emitted by the scintillator as radiolabeled sample passed through the detection region was collected and focused onto the photocathode of the cooled photomultiplier tube (Centronic No. 4283) by a condenser lens combination (Physitec, No. 06-3010, focal length 16 mm). Photon counting was accomplished with a Model 1121A discriminator control unit and a Model 1109 photon counter (EG&G Princeton Applied Research, Princeton, NJ). The background count rate observed under typical operating conditions for this system was approximately 12 counts/s. This detector system was observed to yield a large response (at the onset and completion of each separation), which was associated with the application or termination of high voltage. Although the cause of this response is not yet fully understood, the signal was observed to damp out within the first 1–2 min of a run and therefore posed no interference to the separations reported here.

Apparatus. The experimental setup of the capillary electrophoresis system was similar to that described previously (11, 12) and is illustrated in Figure 1. For the semiconductor detector, a 2-mm section of a 100 μm internal diameter, 100 cm long, fused silica capillary tube (TSP 100/365 Polymicro Technologies, Inc.) was exposed to the CdTe semiconductor by placing the Pb foil aperture directly in between the face of the detector housing and the fused silica capillary at a distance of 75 cm from the inlet end of the capillary tube. For the plastic scintillator detector, a 2-mm section of a 100 μm internal diameter, 140 cm long, fused silica capillary was exposed to the scintillator by passing the capillary through a 400-μm hole that was drilled through the aluminum mounting rods and the central 2-mm portion of the parabola at a distance of 75 cm from the capillary inlet. This resulted in a detection volume of approximately 15 nL for both detectors.

Each end of the capillary tubing was dipped into a 4-mL glass vial containing approximately 3 mL of electrolyte–buffer solution. Connection to high voltage was provided by a strip of Pt foil submersed in each of the buffer reservoirs. The injection end of the capillary was connected to ground while the detection end was held at a high negative potential, typically –20 kV. Plexiglass shielding (0.25 in. thick) was placed around the ground buffer reservoir because the top of this vial was quickly contaminated by sample solution carried on the outside of the capillary tube during the sample injection procedure. This contamination, if unshielded, leads to unnecessary operator exposure to radiation.

The current through the system was monitored as a potential drop across a 1-KΩ resistor in the ground side of the circuit. The capillary system and detector were enclosed in a Plexiglass box to prevent operator exposure to high voltage. Electroosmotic flow rates reported here were measured in a manner similar to that described by Huang et al. (13). The capillary tube was filled with running buffer diluted by 10%, the buffer reservoirs were filled with running buffer, and the current was monitored as one tubing volume was displaced by supporting buffer under the influence of the applied potential. Sample introduction was accomplished in all experiments reported here by using hydrostatic pressure.

The high-voltage power supply (Model MG30N100, Glassmann High Voltage, Whitehouse Station, NJ) was continuously programmable from 0 to –30 kV by means of an external 0–10-V direct current (dc) signal voltage. The flow programming experiments reported here were accomplished by manually reducing the

program voltage to the high-voltage supply.

Reagents. Aqueous ethanol solutions of the triethylammonium salts of adenosine 5'-[α-^{32}P]triphosphate (ATP), thymidine 5'-[α-^{32}P]triphosphate (TTP), and cytidine 5'-[α-^{32}P]triphosphate (CTP) were purchased from Amersham (Arlington Heights, IL). Radioactive sample concentrations reported for detector efficiency determination were adjusted from the manufacturer's specifications after subjecting several diluted aliquots of the stock solution to liquid scintillation counting. The concentration was further corrected for radiochemical purity according to the manufacturer's specifications because liquid scintillation counting measures the total sample activity and does not account for the presence of radiolabeled impurities. Stock solutions were stored at −15 °C in order to minimize sample loss due to hydrolysis. Injected sample solutions were prepared in 0.25-mL plastic vials by diluting stock solutions with buffer and were also stored at −15 °C.

Water used to prepare solutions was freshly deionized and distilled with a water purifier (Model LD-2A coupled with a Mega-Pure Automatic Distiller, Corning Glassworks). The supporting electrolyte for all experiments reported here was a borate buffer (pH 8.1, 0.20 M) prepared from reagent-grade sodium borate decahydrate and boric acid (J. T. Baker).

RESULTS AND DISCUSSION

Successful detection of ^{32}P-labeled molecules separated by capillary electrophoresis using the above detection schemes in which a sensor is positioned external to the separation channel is made possible by several factors. These include (1) the large energy associated with β decay of ^{32}P (1.7 MeV), (2) the high sensitivity and small size of commercially available semiconductor detectors, (3) the availability of efficient solid scintillator materials and sensitive photomultiplier tubes, (4) the short lengths of fused silica (capillary wall thickness) and aqueous electrolyte through which the radiation must pass before striking the detector, and (5) the relatively short half-life of ^{32}P (14.3 days).

The process of β decay for ^{32}P may be written as (*14*)

$$^{32}_{15}P \rightarrow {}^{32}_{16}S + \beta^- + \bar{\nu} \qquad (1)$$
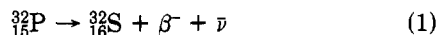
where β^- represents the negatively charged β particle and $\bar{\nu}$ is the antineutrino. ^{32}P is an example of a "pure β emitter" that populates only the ground state of the product nucleus. Each β decay transition is characterized by a fixed decay energy that is shared between the β particle and the antineutrino. As a result, the β particle is emitted with an energy that varies from decay to decay and ranges from zero to the "β end-point energy", which is numerically equal to the transition decay energy. A β energy spectrum for ^{32}P shows a maximum particle energy of 1.7 MeV and an average particle energy of approximately 0.57 MeV. The penetrating ability of β particles through various media may be obtained from literature range–energy plots in which the product of particle range and medium density ("mass thickness") is plotted against particle energy. Such plots are especially useful because they may be used to predict the penetration length at a given particle energy in media other than that used to obtain the original plot (*14*). From such plots, one would predict that the average β particle energy ($\simeq$0.57 MeV) produced by decay of ^{32}P would correspond to a range of approximately 2000 μm in water and approximately 950 μm in fused silica. Thus, ^{32}P decay would be detectable by a sensitive device positioned external to the fused silica capillary tubing (of the dimensions normally selected for capillary electrophoresis separations).

Because the CdTe detector was not visible through the aluminized Mylar film, it was necessary to check for proper alignment of the capillary tube with respect to the CdTe cube. This was accomplished by filling the detection volume with radioactive material and monitoring the signal level as the detector was translated with respect to the capillary. The observed signal was not very sensitive to positioning when the capillary was offset over a range of ±1.5 mm for the center

Table I. Injection Data for CdTe Radioisotope Detector CE System

injection no.	DPM
1	101 885
2	110 449
3	101 884
4	111 375
5	103 512
6	113 018
7	109 432
8	104 581
9	106 740
10	107 357
av	107 023
std dev	3996
% RSD	3.7
injection vol	84 nL

Table II. Injection Data for Plastic Scintillator Radioisotope Detector CE System

injection no.	DPM
1	89 732
2	95 220
3	90 353
4	94 939
5	90 564
6	90 039
7	94 648
8	96 628
9	95 212
10	93 150
av	93 049
std dev	2621
% RSD	2.8
injection vol	72 nL

of the aluminum housing but dropped off rapidly at greater distances. All experiments reported here were performed with the capillary positioned at the center of the aluminum housing, as indicated in Figure 1.

Detector Efficiency. In order to calculate the efficiency of the on-line radioactivity detectors for ^{32}P, it was necessary to determine the volume of sample that was injected onto the capillary tube by the gravity flow technique. The volume of sample introduced by hydrostatic pressure was determined as follows: A plug of ^{32}P-labeled ATP was introduced onto the capillary by raising the sample vial 6.5 cm above the high-voltage reservoir for 30 s. The end of the capillary was then returned to the anode reservoir, and electrophoresis was performed for 5 min at high voltage. This 5-min high-voltage period served to transfer the sample plug toward the detector and away from the injection end of the capillary as if an actual separation were being performed. At the end of the 5-min period the voltage was switched off and the electrolyte within the capillary tube was driven, via syringe, into a liquid scintillation vial located beneath the capillary outlet. This rinsing process was continued until approximately eight capillary volumes of electrolyte were collected. The collected sample plugs were mixed with scintillation cocktail and subjected to liquid scintillation counting. The injection volumes were determined by relating the activity of the sample plugs to that of the injected sample solution. The injection volumes and repeatability of the manually performed hydrostatic injections for the two detector efficiency determinations are shown in Tables I and II.

The results from replicate capillary electrophoresis runs in which a standard solution of adenosine 5'-[α-^{32}P]triphosphate was injected into the capillary are shown in Tables III and IV. The tables list the migration time, peak width, peak area, and detector efficiency. Representative electropherograms

Table III. CdTe Radioisotope Detector Efficiency Data

run no.	elution time, min	peak area, counts	residence time, min	efficiency
1	18.33	1519	0.049	27.6%
2	17.96	1372	0.048	25.4%
3	18.30	1461	0.049	26.5%
4	18.07	1511	0.048	28.0%
5	17.98	1407	0.048	26.1%
6	17.48	1601	0.047	30.3%
7	17.76	1197	0.047	22.7%
8	17.32	1195	0.046	23.1%
9	17.91	1392	0.048	25.8%
10	17.37	1266	0.046	24.5%
11	17.77	1359	0.047	25.7%
av	17.84	1389		26.0%
std dev	0.34	132		2.2%
% RSD	1.9	9.5		8.4%

Table IV. Plastic Scintillator Radioisotope Detector Efficiency Data

run no.	elution time, min	peak area, counts	residence time, min	efficiency
1	20.65	3375	0.055	72.7%
2	20.62	3088	0.055	66.5%
3	20.55	3119	0.055	67.2%
4	20.54	3091	0.055	66.5%
5	20.69	2953	0.055	63.6%
6	20.84	2881	0.056	60.9%
7	21.22	2883	0.057	59.9%
8	21.00	2952	0.056	62.4%
9	20.84	3213	0.056	67.9%
10	20.88	3122	0.056	66.0%
av	20.78	3068		65.4%
std dev	0.22	156		3.8%
% RSD	1.06	5.07		5.8%

corresponding to the two detector efficiency determinations and the conditions under which the separations were performed are shown in Figures 3 and 4. The efficiencies reported in the tables were calculated by using the following equation:

$$\text{NOC} = \{\text{DPM}_{peak}\} \{\text{residence time}\} \{\text{efficiency}\}$$
$$= \{\text{DPM}_{peak}\} \{\text{detector length/zone velocity}\}$$
$$\{\text{efficiency}\}$$
$$(2)$$

where NOC represents the number of observed counts integrated over a peak, DPM_{peak} represents the number of radioactive transformations occurring each minute in the injected sample plug, the residence time is the amount of time (in minutes) a radioactive molecule within a given sample zone spends in the detection volume, and the efficiency is the fractional number of events sensed by the detector. The efficiencies for the on-line detectors described here are a function of detector collection geometry, i.e., positioning of the CdTe probe or plastic scintillator with respect to the capillary, as well as particle flux attenuation by the capillary wall (a fraction of the emitted β particles are of sufficiently low energy to be stopped by the capillary wall material). Note that the residence time within the detector must be determined for each component in a mixture because separated sample zones travel with different velocities according to their individual electrophoretic mobilities. This is in sharp contrast with radio HPLC detection in which the residence time for each sample component is the same and is given by the ratio of the detector cell volume to the mobile phase flow rate. The residence time for a particular sample component separated by capillary electrophoresis is easily obtained from its mi-
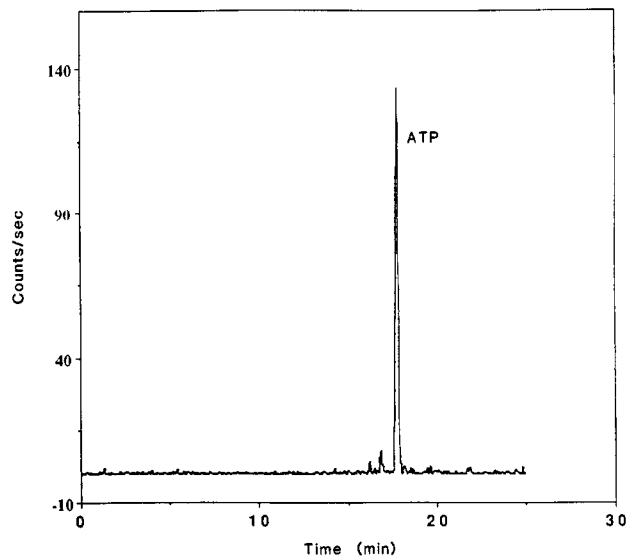

Figure 3. Capillary electropherogram of adenosine 5'-[α-^{32}P]triphosphate obtained by injecting approximately 51 nCi (7 $\times$ 10^{-8} M solution) onto the capillary and applying a constant potential of -20 kV. The separation was continuously monitored with the CdTe semiconductor radioisotope detector. Data was subjected to a 5-point sliding smooth. Electrolyte was 0.2 M borate buffer, pH = 8.1.
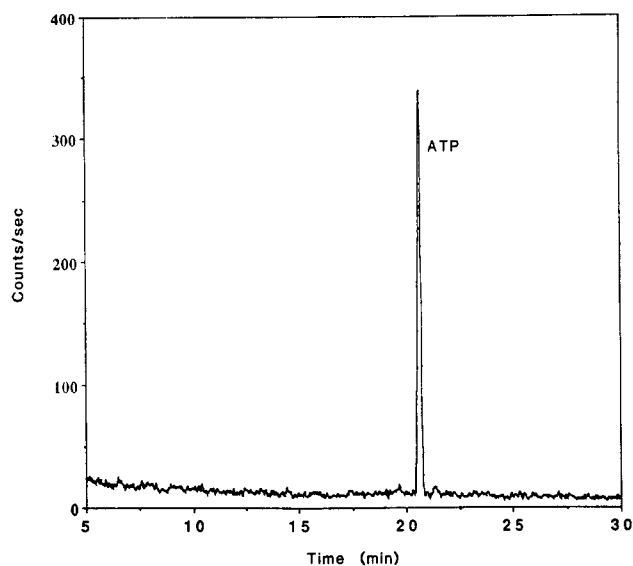

Figure 4. Capillary electropherogram of adenosine 5'-[α-^{32}P]triphosphate obtained by injecting approximately 38 nCi (6 $\times$ 10^{-8} M solution) onto the capillary and applying a constant potential of -25 kV. The separation was monitored with the plastic scintillator radioisotope detector. Data was subjected to a 5-point sliding smooth. The electrolyte was the same as in Figure 3.

gration time and from the length of capillary to which the detector is exposed.

Results obtained for the replicate runs shown in Tables III and IV indicate that the efficiency of ^{32}P detection for the on-line CdTe radioactivity detector is approximately 26%, while the efficiency of the plastic scintillator radioactivity detector is approximately 65%, reflecting the improved geometry of the latter device. The background noise level of the CdTe detector system is a function of the low energy discriminator setting. The value of 0.01 MeV selected for all experiments reported here gave a background count rate of approximately 10 counts/min while leaving a wide energy window open for detection. Comparison of signal-to-noise ratios in the two electropherograms indicates that the two detectors exhibit quite similar sensitivities despite the fact that the efficiency of the plastic scintillator detector is a factor

Table V. Capillary Electrophoresis Separations Performed under Standard (Runs 1-6) and Flow Programmed (Runs 7-12) Conditions[a]

run no.	peak width, min	peak area, counts	voltage program
1	0.38	985	20 kV constant
2	0.34	1098	
3	0.43	1236	
4	0.38	1016	
5	0.48	1078	
6	0.35	968	
av	0.39	1064	
7	0.87	2404	20 kV initial,
8	0.79	2705	10 kV during
9	0.71	2081	detection period
10	0.81	2448	
11	1.12	2695	
12	0.80	2673	
av	0.85	2501	

[a] Peak area ratio, 2.4; current ratio, 2.4; voltage ratio, 2.0.

of 2.5 times greater than that of the semiconductor detector. This is caused by the extremely low background noise level of the CdTe device compared to that of the cooled photomultiplier tube used in this work.

Flow Programming. Equation 2 suggests that the number of counts measured (the detector sensitivity) over a sample peak may be increased by lengthening the residence time of the sample in the detection volume. This is equivalent to increasing the counting time on a liquid scintillation counter and this concept has been recognized in both radio HPLC applications (see, for example, ref 15) and radioisotope detection in isotachophoresis (7, 20). In capillary electrophoresis, the velocity of a sample zone may be reduced and its residence time increased by simply reducing the applied potential as the zone passes through the detection volume. The most efficient implementation of this flow programming concept would involve reducing the zone velocities only while the sample was present within the detection volume and operating at a relatively high potential at all other times. To our knowledge, this type of flow programming has not previously been explored in capillary electrophoresis. Although it is demonstrated only for radioisotope detection here, this methodology should be applicable to other modes of sample detection in CE.

The flow programming concept is demonstrated in Table V, which lists the peak width and peak area for five capillary electrophoresis separations performed with and without flow programming. Separations 1-5 were performed at a constant potential of -20 kV using the CeTe radioisotope detector, while in runs 6-10 the potential was reduced to -10 kV as soon as signal was detected above the detector background level. Because the zone velocity is directly proportional to the applied field strength, the average temporal peak width and area (number of counts observed) for the five flow programmed runs were approximately doubled. This improvement in sensitivity is, however, accompanied by an increase in analysis time as well as a small loss in resolution due to zone broadening. The magnitude of the resolution losses incurred during flow programming will be strongly dependent upon the amount of sample injected and the additional run time associated with the flow programming process. For injected sample plug lengths that are several times larger than the length associated with diffusional broadening (typical operating conditions), the resolution loss will not be significant. In the limit of injected sample plugs with no initial width (δ function), the additional peak variance increases linearly with programming time (ignoring analyte-wall interactions) and
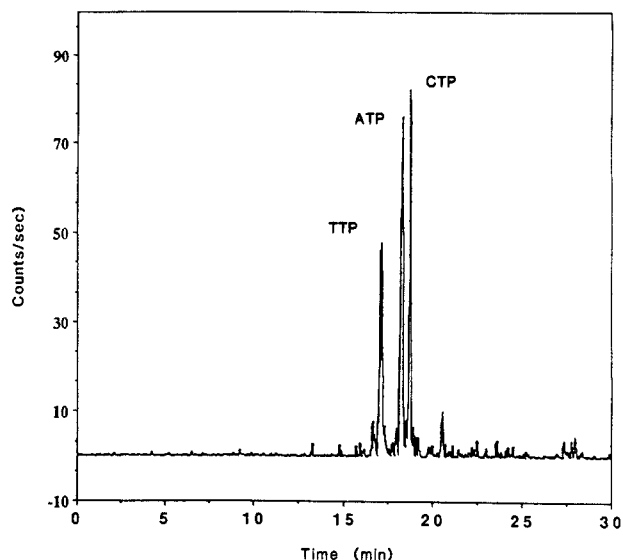

Figure 5. Capillary electropherogram of thymidine $5'$-[α-^{32}P]triphosphate, cytidine $5'$-[α-^{32}P]triphosphate, and adenosine $5'$-[α-^{32}P]triphosphate obtained by injecting approximately 19 nCi (2 × 10^{-8} M solution) of each component onto the capillary and applying a constant potential of -20 kV.
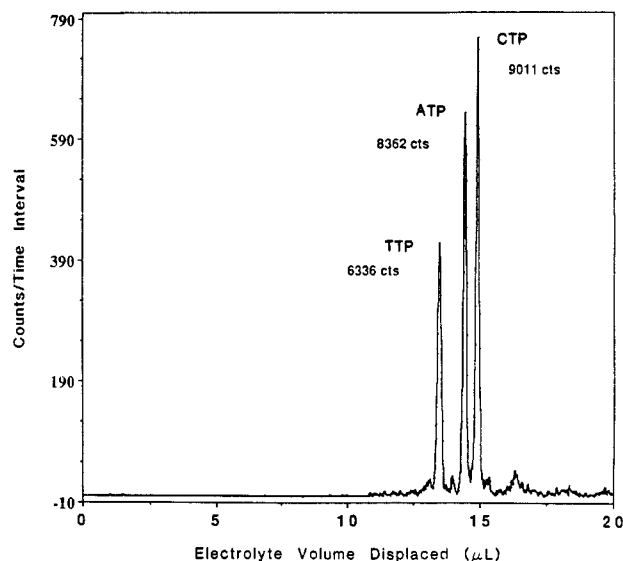

Figure 6. Flow programmed capillary electropherogram of thymidine $5'$-[α-^{32}P]triphosphate, cytidine $5'$-[α-^{32}P]triphosphate, and adenosine $5'$-[α-^{32}P]triphosphate obtained by injecting approximately 19 nCi (2 × 10^{-8} M solution) of each component onto the capillary. The separation was flow programmed by applying a constant potential of -20 kV until radiolabeled sample approached the detection volume and then reducing the potential to -2 kV as the sample zones traversed the detection region. Note that the detector signal has been plotted as a function of electrolyte volume displaced, resulting in a time-compressed abscissa over the flow programmed region of the electropherogram. The operating current was 38 μA at -20 kV and 3.8 μA at -2 kV. The data was subjected to a 5-point sliding smooth.

the resolution loss will become significant.

A striking example of increased sensitivity gained through the application of flow programming is illustrated in Figures 5 and 6. In Figure 5, a synthetic mixture of thymidine $5'$-[α-^{32}P]triphosphate (^{32}P-TTP), cytidine $5'$-[α-^{32}P]triphosphate (^{32}P-CTP), and adenosine $5'$-[α-^{32}P]triphosphate (^{32}P-ATP) was injected with each component present at a concentration of approximately 2 × 10^{-8} M ($\cong$19 nCi injected) by using hydrostatic pressure and separated under the influence of a constant -20-kV applied potential. In Figure 6 the sample solution and injection volume were the same as in Figure 5, but the residence time of each component was increased by

reducing the applied potential from -20 to -2 kV as radioactive sample was passing through the detection volume. At the same time, the counting interval was increased proportionately (from 1 to 10 s) and the detector signal was plotted as a function of electrolyte volume displaced through the capillary tube. Note that this results in a time-compressed abscissa over the flow programmed period of the electropherogram (the entire separation required about 70 min in this case). It is important to point out that the lower limit of detection for radioisotope detection refers to the lowest sample activity contained within a peak that may be detected and accurately quantified. From the data presented in Table V and Figures 3-6 it is apparent that the lower limit of detection for this system is greatly dependent upon the conditions under which the analysis is performed and that detector sensitivity may be extended by an order of magnitude or more by using flow programming.

The sensitivity gain afforded by this flow programming methodology will ultimately be limited by practical considerations of analysis time and resolution losses caused by diffusional broadening of the sample zones. Simplicity and consideration of analysis time, however, still make flow counting detection for capillary electrophoresis an attractive alternative to the quantitatively superior batch counting approach in which fractions are collected and subjected to conventional counting techniques (15). The batch counting approach, provided that sufficiently small fractions may be collected, does offer the advantage of decoupling separation considerations from measurement time. Considering only the limitation imposed by diffusional spreading of sample zones during the flow programmed portion of a run, it is possible to predict the extent to which detector sensitivity may be improved by flow programming. For an injection volume of 84 nL, as used in this work, and a maximum allowable increase in zone variance defined to be 10%, approximately 84 min of flow programming would be permitted (this calculation assumes a rectangular injection profile and a solute diffusion coefficient of 10^{-6} cm^2/s and neglects both diffusional broadening prior to flow programming and velocity-dependent analyte–wall interactions). This 10% increase in variance would be accompanied by a 250-fold increase in the number of counts observed over a peak. Because the sensitivity of radioisotope detection is governed by counting statistics, a 16-fold increase in the signal-to-noise ratio $(NOC/(NOC)^{1/2})$ would result. Thus, a lower limit of detection of about 10^{-11} M would seem to be a conservative extrapolation. Obviously, the limitations imposed by diffusional broadening would become more severe if the initial injection volume were reduced.

In an automated implementation of the flow programming methodology, that is, with the high-voltage power supply under computer control, there is a further limitation imposed upon achievable sensitivity gains. There must be enough sample present to generate a signal that is sufficiently large to exceed the detector background level under standard (non-flow-programmed) conditions in order to initiate the flow programming procedure. In certain instances, however, prior knowledge of migration times for the compounds of interest would permit this limitation to be overcome.

CONCLUSION

Two simple on-line radioisotope detectors for capillary electrophoresis have been described and characterized for the analysis of ^{32}P-labeled analytes. The minimum limit of detection for these systems was shown to be strongly dependent upon the conditions under which the analysis is performed. For standard CE separations that are performed at a relatively high (constant) voltage, the minimum limit of detection was

found to be in the low-nanocurie (injected sample quantity) range, corresponding to an analyte concentration of about 10^{-9} M. The lower limit of detection for this type of detection system was extended to the sub-nanocurie level ($\cong 10^{-10}$ M) by application of flow programming methodology, which served to increase the residence time of labeled sample components within the detection volume. Thus radioisotope detection, when applicable, has a sensitivity which is superior to that of most other detection schemes and which is comparable to that of electrochemical detection (16, 17) and laser-induced fluorescence detection (11, 18, 19).

One improvement to the current systems would involve automation of the flow programming methodology, and such efforts are currently under way in this laboratory. A second improvement over the current semiconductor system would involve optimizing the detector geometry by capturing a larger solid angle with the CdTe detector. The performance of the plastic scintillator radioisotope detection scheme would be greatly improved by reducing the background noise level through the use of a quieter photomultiplier tube or two photomultiplier tubes operated in the coincidence mode. In certain instances it would be desirable to reduce the effective detection volume of these systems in order to increase resolution. This could be accomplished by installing a narrower aperture in the semiconductor detector or machining a smaller detection region in the plastic scintillator detector. In either case, detector sensitivity will be reduced because the detection volume and effective residence time will be decreased. Hence, there is once again a practical trade-off between detector sensitivity and resolution.

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