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HIGH-EFFICIENCY SOLID SCINTILLATION RADIOACTIVITY DETECTION FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The design and optimization of a detector to monitor ^{14}C radioactivity in the effluent from a high-performance liquid chromatograph is described. The technique of solid scintillation with cerium-impregnated lithium silicate glass scintillating material is employed. The optimized detector provides a counting efficiency for ^{14}C β-radiation greater than 70% which is comparable to counting efficiency typically achieved with liquid scintillation techniques. The high counting efficiency was achieved by optimizing the detector electronics and by reducing the particle size of the scintillating glass in the flow cell to more closely approximate the average range of ^{14}C β-radiation in aqueous solution.

INTRODUCTION

Investigations of the absorption, distribution, and biotransformation of test compounds administered to animals normally involve the use of radiolabeled materials, with the determination of the radiolabeled metabolites often accomplished using a separation scheme such as high-performance liquid chromatography (HPLC), followed by radioassay. Collection of column effluent fractions followed by liquid scintillation counting has been used extensively, but has the disadvantages of being time consuming, requires manual manipulation of samples, compromises resolution, and necessarily contaminates the separated metabolites with the scintillation solution. A continuous flow-through radioactivity detector minimizes these disadvantages. Several continuous-flow radioactivity monitors have been described in the literature[1–4]. Homogenous detectors, where a liquid scintillation solution is mixed with the column effluent stream, generally have higher counting efficiencies than heterogeneous radioactivity detectors[5], but require more complex instrumentation[6] and are more expensive to operate due to the large volume of scintillation cocktail used. Heterogeneous radioactivity detectors generally have lower counting efficiencies, but are simpler instrumentally, less expensive to operate, are non-destructive, thereby allowing subsequent isolation and identification of the compounds in solution, and are subject to fewer quenching effects. The primary disadvantage of hetero-

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geneous radioactivity detection has been low counting efficiency. This paper describes the design of a heterogeneous radioactivity detector that provides counting efficiencies comparable to homogeneous detectors.

EXPERIMENTAL

The radioactivity detector described here consists of a flow cell packed with the solid scintillating material, a scintillation spectrometer, and a rate-meter. A diagram of the flow-through cells is shown in Fig. 1. The two variations in shape are indicated by Roman numerals, variations in internal diameter are indicated in the text by the letters A = 0.9 mm, B = 1.8 mm, C = 2.4 mm.

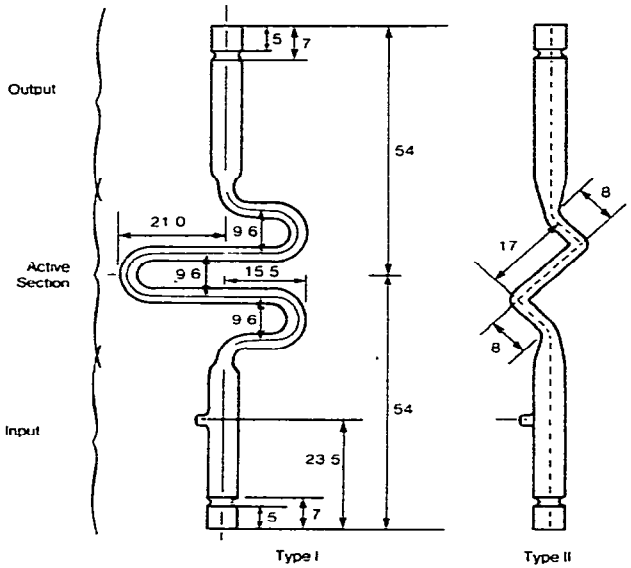

Fig. 1. Diagram of flow-through cell. Dimensions in mm. Output: 6 mm O.D., 2 mm I.D. Input: 6 mm O.D., 0.9 mm I.D.

Borosilicate glass or quartz cells were mounted in an aluminum cell holder to protect the cell and fix the position of the cell in relation to the photomultiplier tubes (PMTs). PTFE tubing (1/16 in. O.D. × 0.01 in. I.D.) was connected to the cells via LDC Corporation's glass to Cheminert® connectors. Outside the cell holder, the PTFE tubing was adapted to stainless-steel tubing, 1/16 in. O.D. × 0.01 in. I.D. using Altex adaptors. The stainless-steel tubing passed through the light-tight enclosure using Swagelok stainless-steel bulkhead fittings.

Two types of lithium glass scintillator particles, NE901 and NE913, were purchased from Nuclear Enterprises (Edinburgh, Great Britain, and San Carlos, CA, U.S.A.). We also purchased similar glasses, GS1 and KG3L, from Koch-Light Labs. (Scintillator Division, Colnbrook, Great Britain). These were sold as "crushed material ground to a required maximum size in the range 0.25 mm to 0.30 mm". The glass particles, when viewed microscopically, consisted of irregularly shaped chips ranging in size from <1 μm to 500 μm for the longest dimension.

Sieving the scintillator particles segregated them into six size ranges named according to the sieve hole sizes: >210 μm, 125–210 μm, 90–125 μm, 63–90 μm, 38–63 μm, and <38 μm. Microscopic examination (at 111 ×) showed the longest dimension of any one particle to be two or three times the sieve size it passed through.

A dry packing technique was used to fill the flow cell with sieved scintillation glass.

The "active" cell volume is that volume which is both exposed to the PMTs and filled with the scintillator. The cell volume was determined by measuring the empty cell volume and subtracting the volume of the scintillator used to fill the cell. The volume of the scintillator was calculated by dividing the weight of the scintillator by its density. The cell volume was also determined by filling the cell at a known rate with radiolabeled material and monitoring the detector output. The volume was calculated as the product of the flow-rate and the time required to reach a stable detector output.

The scintillation spectrometer was constructed using conventional single photon counting techniques[7,8] in a coincidence mode to discriminate against low-level and non-coincident noise pulses. The photomultiplier tubes were 9635QA tubes with B-235 housings from EMI Gencom (Plainview, NY, U.S.A.). The tubes were operated with the cathode at ground and the anode at high positive voltage (1350–1500 V). The anode signal was a.c. coupled through a 0.01-μF capacitor. The 9635QA PMTs had a typical transient response of approximately 25 nsec. Fig. 2 is a schematic diagram of the coindence detection circuit for the radioactivity detector. The current-to-voltage converters were 46J amplifiers from Analog Devices (Norwood, MA, U.S.A.). The voltage comparators were μA760 from Fairchild Semiconductor (Mountain View, CA, U.S.A.) and were operated at a 35 mV discrimination level. The 74121 monostable multivibrators from Texas Instruments (Dallas, TX, U.S.A.) were operated with 70-nsec output pulses. The 7408 (AND) gate from Texas Instruments eliminates the non-coincident pulses. The gate output was monitored with an

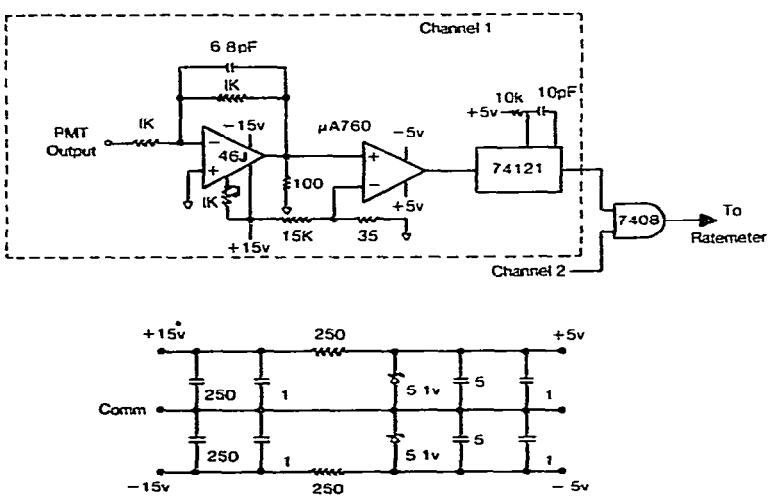

Fig. 2. Schematic of signal conditioning and coincidence circuit. Only one complete channel is shown. Resistance values are in Ω. Capacitance values are in μF unless shown differently.

Ortec (Model 449) rate meter. Two Analog Devices (Model 920) and two ± 5 V Zener power supplies were used to provide maximum isolation between channels.

Measuring efficiency and figure of merit

Efficiency (E) was measured by two methods:

(1) When the cell was filled with radiolabeled sample:

$$E = \frac{[\text{Observed output (cpm)} - \text{Background (cpm)}]}{\text{Sample activity (dpm)}}$$

(2) When a small slug of sample was pumped through the cell at known, constant rate:

$$E = \frac{\text{Observed output (cpm)}}{\text{Sample activity (dpm)}} \times \frac{\text{flow-rate}}{\text{cell volume}}$$

Figure of merit (FM) was computed: $FM = E^2/BG$, where BG is background in cpm.

RESULTS AND DISCUSSION

A cell suitable for monitoring radioactive materials in HPLC effluents should have a small volume and should provide good flow characteristics to avoid degrading chromatographic performance. In addition, a cell packed with a solid scintillator operates most efficiently when a maximum area of scintillator is exposed to the radiation: because the average range of ^{14}C β-radiation in aqueous solution is about 14 μm (ref. 9), no point in solution should be more than 14 μm from the glass scintillator surface. However, the packing density of the scintillator must be optimized to facilitate the transfer of light pulses to the PMTs.

To optimize the cell design we studied the effect of varying the size and shape of the cell and the effect of construction materials and scintillator type. The performance of the cell was evaluated with solutes dissolved in eluents that differed in refractive index, UV absorbance, pH, and chemical quenching effects.

Effect of changing cell size and shape

The efficiency as a function of size (active volume) and shape is shown in Table I.

TABLE I
COUNTING EFFICIENCY AS A FUNCTION OF CELL SIZE AND SHAPE

	Cell			
	Type IA 2.4 mm I.D.	*Type IB 1.8 mm I.D.*	*Type IC 0.9 mm I.D.*	*Type II 1.8 mm I.D.*
Active volume (μL)	270	130	30	60
Efficiency, NE913, 38–63 μm	44.4 ± 1.1%	54.5 ± 0.5%	45.3 ± 2.5%	62.3 ± 2.4%

Of the type I cells, IB (1.8 mm I.D.), consistently outperformed IA (2.4 mm I.D.) and IC (0.9 mm I.D.). The 1.8 mm I.D. was carried over into the design of type II cells. These cells were designed when we found the center of the PMT windows were approximately twice as sensitive as the peripheral areas. Cells taking advantage of this fact consistently outperformed earlier models.

Effect of construction materials, scintillator type and particle size

We observed no difference in efficiency with cells made of quartz or Pyrex glass. This might be expected since the scintillator emits in the region of 395 nm, according to Nuclear Enterprises and Koch-Light Labs. The effect of scintillator type and size is shown in Table II. Based on manufacturer's specifications and our own observations, we judged NE901 to be comparable to GS1; likewise, NE913 was comparable to KG3L. NE901/GS1 outperformed NE913/KG3L in terms of efficiency. However, when comparing *FM*, the advantage was not so clear because NE913/KG3L has inherently lower background levels than NE901/GS1. Results were ambiguous because background levels tended to vary by as much as 30%; but we believe NE901/GS1 has a slight advantage in *FM* and therefore, an overall advantage.

TABLE II
EFFICIENCY AS A FUNCTION OF SCINTILLATOR TYPE AND SIZE, TYPE II CELL

Scintillator size	*NE901*		*NE913*		*GS1*		*KG3L*	
	Efficiency (%)	*FM*	*Efficiency (%)*	*FM*	*Efficiency (%)*	*FM*	*Efficiency (%)*	*FM*
38–63 μm	73.0 ± 3.4	65	62.3 ± 2.4	111	71.8 ± 1.2	99	51.9 ± 1.7	67
63–90 μm	66.7 ± 2.2		57.3 ± 2.3	75				
90–125 μm	63.0 ± 1.6	75	44.0 ± 1.1	39	63.6 ± 1.4	75	46.8 ± 1.9	48
Unsieved			40.7 ± 1.5	36				

Table II also shows the effect of particle size on efficiency. In all cases, the smallest size range produced the best results. We tried obtaining smaller fractions than the 38–63 μm fraction, however, the fragile nature of sieves available below 38 μm and problems with fines made the effort impractical. Reproducible efficiencies were obtained when the cells were packed with 38–63-μm particles. Repacking the same cell three times resulted in efficiencies of 71.6 ± 0.3%. Packing five different cells of the same nominal dimensions (type II) gave efficiencies of 68.8 ± 2.4%.

Cell masks reduced background noise due to PMT crosstalk. Cut-out paper masks lowered the background to about half of its unmasked value. Paraffin masks, molded around the cells, reduced the background even more but with a reduction of efficiency. For this reason, paper masks were used throughout this work.

Evaluation of cell performance

Because the refractive index of the solvent might affect the amount of light transmitted out of the packed cell, we investigated the importance of this effect in the counting efficiency of the system using water and ethanolamine (EA) solutions in varying ratios. No significant counting dependency on refractive index was found as shown in Table III. The effect of pH and absorbance of the solution were studied separately.

TABLE III
EFFICIENCY AS A FUNCTION OF REFRACTIVE INDEX; TYPE I CELL PACKED WITH NE901, 90–125 μm

Test No.	*Concentration of EA (%)*	*Density (g/cm^3)*	*Absorbance 400 nm, 1 cm*	*pH*	n_D	*Efficiency (%)*
1	0	1.000	0.000	6	1.220	50.4 ± 1.1
2	1	1.008	0.002	11.2	1.223	49.6 ± 0.8
3	32	1.027	0.022	12.3	1.272	49.3 ± 0.6
4	60	1.006	0.054	13.5	1.410	49.9 ± 0.7
5	100	1.017	0.084	—	1.453	51.4 ± 1.5

Eluents or materials in the eluent that absorb light at 400 nm have a significant effect on efficiency. As the absorbance increases, the efficiency decreases. Table IV shows results of using *p*-nitrophenol in water as an eluent. Eluents over a wide pH range do not affect efficiency as shown in Table V. Chloroform, a liquid scintillation quencher, was used as an eluent for a few experiments. Efficiencies using chloroform were no different than when using water as the eluent. Using a type II cell packed with NE901, 38–63 μm, five experiments yielded 77.7 ± 1.6% efficiency with chloroform as the eluent; two tests with water as the eluent yielded 74 ± 5.1%.

TABLE IV
EFFICIENCY AS A FUNCTION OF ABSORBANCE; TYPE II CELL PACKED WITH NE901, 38–63 μm

Test No.	*Concentration of p-nitrophenol (%)*	*Absorbance, 400 nm, 1 cm*	*pH*	*Efficiency (%)*
1	0.00	0.00	8.3	80
2	0.001	1.2	8.3	61
3	0.002	2.4	8.3	53
4	0.0027	3.0	8.3	48

TABLE V
EFFICIENCY AS A FUNCTION OF ELUENT pH

Cell	*Experimental sequence*	*Eluent*	*Efficiency (%)*
Type IA, NE901 (90–125 μm)	1	0.1 *F* HNO_3	48.3 ± 0.2
	2	Water, pH 6	48.5 ± 0.7
	3	0.005 *F* NaOH	45.9 ± 0.9
	4	Water, pH 6	45.3 ± 2
Type IA, NE901 (90–125 μm)	1	Water, pH 6	53.0 ± 2.9
	2	0.005 *F* NaOH	54.9 ± 2.8
	3	Water, pH 6	52.8 ± 1.2
	4	0.1 *F* Acetic acid	55.0 ± 0.6

The scintillator glasses were subject to reactions resulting in loss of scintillation activity or irreversible uptake (adsorption) of chemicals. Labeled phosphates and acetates were irreversibly adsorbed as evidenced by stepwise increases in the background counts following direct injection of these materials into the flow cell. Also, extended use of this detector for HPLC profiling of radiolabeled urinary metabolites resulted in the irreversible adsorption of non-labeled urinary materials as evidenced by yellowing of the scintillator glass. Attempts to remove adsorbed materials with solutions of salts or dilute acids, or with organic solvents, were mostly ineffective. Neither was silanization of the scintillator glass before or after contamination effective in preventing adsorption or in desorbing the contaminants. The most effective means of removing the contaminants was overnight exposure of the cell to ozone in a low temperature asher. In short, when the response characteristics of the detector change, the simplest procedure is to repack the cell.

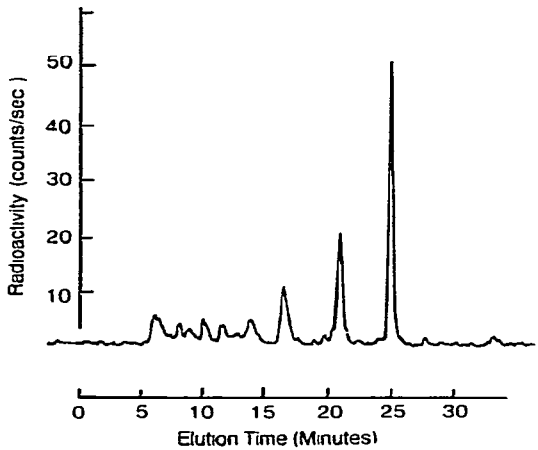

Fig. 3. Radiochromatogram of alkylethoxylate urinary metabolites. C_{18} column, acetonitrile–acetate buffered water, step from 10% to 20% at 10 min. Flow-rate: 1 ml/min. Type IB cell. Radioactivity injected: 110,000 dpm.

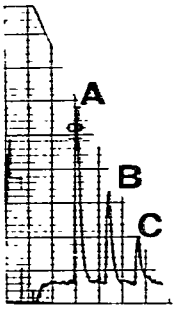

Fig. 4. Radiochromatogram of ^{14}C-labeled hexadecane. Type IB cell packed with NE901, 38–63 μm. Propanol flow-rate: 0.6 ml/min. Detector sensitivity: 100 counts/sec full scale. Time base: 1 div/min. Detector time constant: 10 sec. ^{14}C Activity injected: A = 10^4 dpm; B = $5 \cdot 10^3$ dpm; C = $2.5 \cdot 10^3$ dpm.

This radioactivity monitor has been used primarily for the qualitative profiling of urinary metabolites of decylmethylsulfoxide, alkylethoxylates and zinc pyridinethione. Fig. 3 is a radiochromatogram of $C_{13}E_6$ alkylethoxylate urinary metabolites. The high resolution and sensitivity evident in this chromatogram demonstrate the feasibility of profiling samples containing less than 100,000 dpm of ^{14}C activity per injection. The detection limit for ^{14}C was estimated from the data shown in Fig. 4. It should be possible to detect $3 \cdot 10^{-10}$ Ci (*ca.* 660 dpm) in a chromatographic peak 30 sec wide.

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