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CONTINUOUS DETECTION OF RADIOACTIVE EFFLUENTS IN LIQUID CHROMATOGRAPHY BY HETEROGENEOUS OR HOMOGENEOUS SCINTILLATION COUNTING

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SUMMARY

Beta radioactivity in liquid chromatography effluents can be monitored by either heterogeneous or homogeneous scintillation counting. The two systems have been compared and appropriate flow cells have been developed.

With heterogeneous counting, the solution is led through a U-shaped flow cell filled with glass scintillator beads. This system is best for preparative chromatography when activities are high and losses are unacceptable. The activity per peak should exceed 10 nCi for ^{14}C and 1 μCi for ^{3}H.

With homogeneous counting, part of the effluent is mixed with a scintillator solution and passed through an empty helical flow cell. This system is best for detection of ^{3}H, low activities, and in dual-label experiments, or when complete recovery is not vital. The activity per peak should exceed 2 nCi for ^{14}C and 5 nCi for ^{3}H.

INTRODUCTION

Recent advances in liquid chromatography include the development of various sensitive effluent detectors[1]. The continuous monitoring of ^{14}C- and ^{3}H-labelled compounds has, however, not yet reached the same degree of sophistication. We therefore investigated the methods reported in the literature and tried to optimize them and to overcome their particular disadvantages. We assumed that the best method of measuring radioactivity in solution is liquid scintillation counting.

Among the first to deal with the continuous detection of tritium and radiocarbon in solution were SCHRAM AND LOMBAERT[2]. Their cell consisted of polyethylene tubing filled with anthracene and bent into a spiral shape. The counting efficiencies for tritium and carbon-14 were 2% and 44%, respectively. Because anthracene and polyethylene were used, radioactivity could be measured in dilute aqueous solutions only. The applicability becomes more general when beads of lithium–cerium glass are used as the solid scintillator[3]. This material dissolves only in hydrofluoric acid. The counting efficiency, however, is low: 20% for ^{14}C and 0.3% for ^{3}H. A scintillator recommended for organic solvents is europium-activated calcium

fluoride in crystalline form[3,4]. The efficiencies cited are reasonable: 17–50% for ^{14}C and 1.6% for ^{3}H.

In the method of HUNT[5], the column effluent is mixed with a suitable liquid scintillator counting solution until a homogeneous mixture is obtained, and this mixture is passed through an empty cell. A disadvantage of this method is that the effluent is lost for further use. If, however, the eluted compounds must be recovered, a stream splitter can be used with a splitting ratio of, *e.g.*, 4:1. The mixing chamber used by HUNT[5] is rather large (about 1 ml), which may cause loss of resolution.

The only commercially available scintillation flow cells are of the anthracene-filled type with volumes of 1–4 ml (ref. 6). As these cells did not meet our requirements, we decided to develop our own flow cells. In doing so, we investigated the heterogeneous counting system, with various solid scintillators, and the homogeneous counting system, for both ^{14}C and ^{3}H activity. Important considerations are the size and the shape of the cell; the larger its effective volume, the higher is the sensitivity but the lower is the resolution. The optimum size will depend on the flow-rate and the proximity of the peaks. A practical minimum for the residence time in the cell is usually about half a minute.

METHODS

The counter was a Tracerlab Coruflow, Model SCE-542, with twin spectrometers and lin/log rate meters. It consists of a counting chamber with two low-noise, high-gain photomultiplier tubes in a lead shield. The photomultipliers are connected in a coincidence circuit. The instrument was used at room temperature. The dual spectrometer/rate meter unit has two independent channels, which have positions for display of linear, logarithmic or integrated counting rates. A two-pen recorder (Servogor 2, RE 520) was connected to the spectrometer and, if desired, to another flow detector, such as an ultraviolet monitor.

The counting efficiency (E) was calculated for each of the flow cells investigated with the aid of eqn. 1:

$$E\ (\%) = \frac{c}{d'} \cdot \frac{F}{V} \cdot 100 \qquad (1)$$

where c represents the integrated counts of a peak, d' the absolute activity (disintegrations/min), F the flow-rate (ml/min) through the cell and V the effective volume (ml) of the cell. If a solution of known activity per millilitre (D') is circulated through the cell, eqn. 1 simplifies to:

$$E\ (\%) = \frac{c'}{D'V} \cdot 100 \qquad (2)$$

where c' represents counts/min. The effective volume of a cell is determined from the weight of water required to fill it, or by the time of migration of the front of a coloured solution pumped through it at a known rate.

HETEROGENEOUS COUNTING SYSTEM

The heterogeneous counting system is shown schematically in Fig. 1. An ultra-

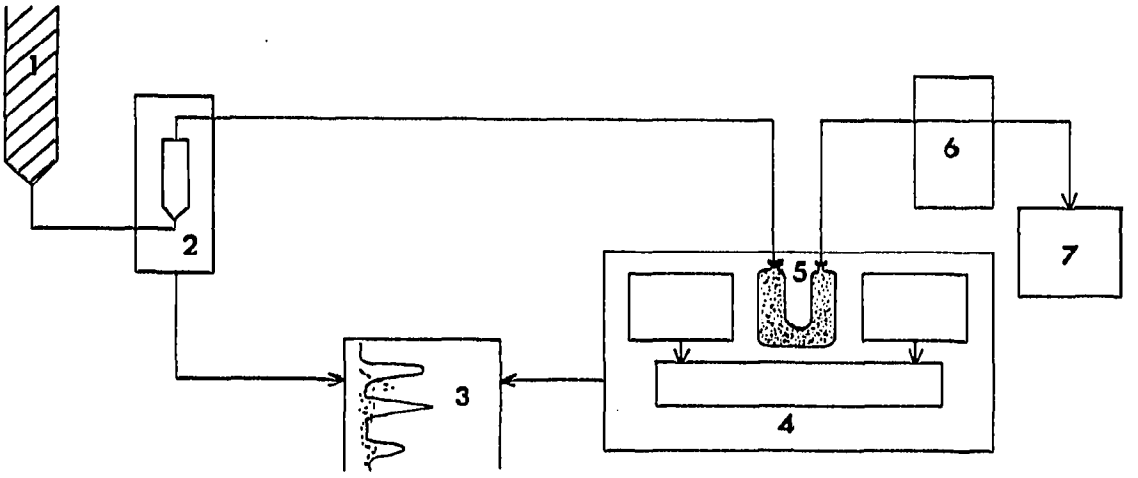

Fig. 1. Schematic representation of the heterogeneous counting system. 1 = Column; 2 = ultraviolet detector, 254 nm; 3 = two-pen recorder; 4 = liquid scintillation spectrometer; 5 = U-shaped flow cell filled with glass scintillator beads; 6 = pump (optional), 1.5 ml/min; 7 = fraction collector.

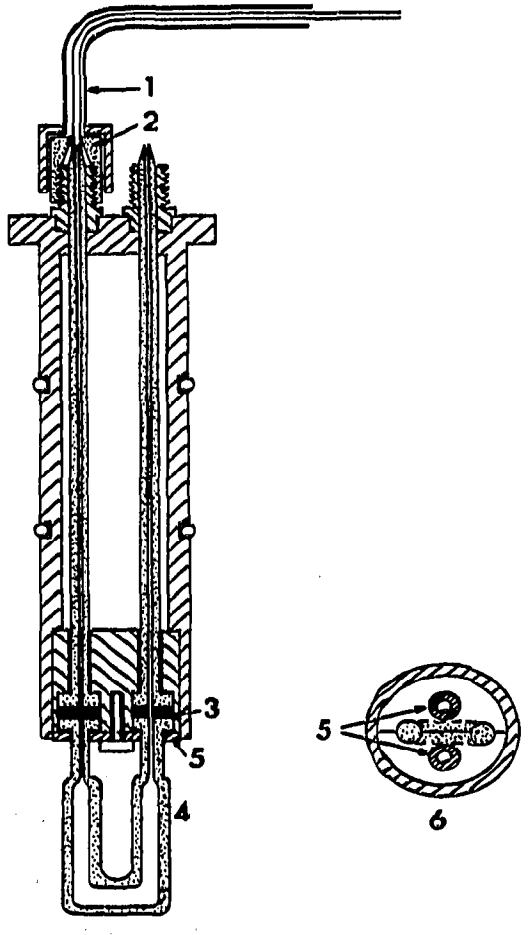

Fig. 2. Probe with scintillator flow cell for the heterogeneous counting system. 1 = Metal cap and bent sleeve for light-proofing; 2 = swage lock; 3 = Viton O-rings; 4 = interchangeable borosilicate glass cell; 5 = semi-circular collar disks; 6 = bottom view of cell and probe.

violet spectrometer (LKB Uvicord 4701 A) with a 0.1-ml cell is included as an independent flow monitor. The pump (LKB 12000) is optional, and may be included to ensure a constant flow-rate. All the flexible tubing, except that in the pump, is made of PTFE and all rigid tubing is made of glass. The probe with its dismountable flow cell is shown in Fig. 2. The cell can be detached by releasing the screws in the two semi-circular collar disks, which press the cell on to two Viton rubber O-rings. PTFE tubes are connected to the glass inlet and outlet tubes by swage locks. Close-fitting metal caps with bent sleeves preclude the entrance of light through the transparent tubes.

Various shapes were tried for the borosilicate glass cell. Coiled or helical cells were difficult to fill and, once filled, tended to become clogged. Flat, U-shaped cells were easy to handle and, moreover, gave the highest counting efficiencies without loss of resolution. The efficiencies of different cells filled with various solid scintillators are summarized in Table I.

TABLE I

COUNTING EFFICIENCIES FOR VARIOUS FLOW CELLS

Shape of cell	*Effective volume (µl)*	*Scintillator*	*Counting efficiency (%)*	
			^{14}C	^{3}H
U	350	Anthracene	37	1.0
U	160	Anthracene	31	1.0
Coil	500	Anthracene	20	0.6
U	350	PPO[a]	43	1.8
U	500	Butyl PBD[b]	40	1.7
U	480	Glass[c]	17	0.2
Coil	430	Glass[c]	5	<0.1
U	190	CaF_2 (Eu-activated)	38	0.5

[a] 2,5-Diphenyloxazole.
[b] 2-(4′-*tert.*-Butylphenyl)-5-(4″-biphenylyl)-1,3,4-oxadiazole.
[c] Cerium-activated lithium glass NE 901 (250–1000 µm), from Nuclear Enterprises Ltd., Edinburgh, Great Britain.

PPO and butyl PBD were dissolved in toluene. After evaporation of the solvent, the resulting dry cake was broken and sieved, and the 700–1000 µm particle fraction was funnelled into the cells. A small plug of quartz-wool was used to keep the scintillator in position.

Anthracene, PPO and butyl PBD are known to dissolve in organic solvents. Anthracene dissolves appreciably in 50% ethanol, while particles of PPO and butyl PBD are broken down by this solvent and clog the cell. PPO dissolved to some extent in 0.05 *N* HCl, as was evident from the ultraviolet spectrum of solvent that had passed the cell. Moreover, PPO adsorbed radioactive materials such as nucleotides, which resulted in tailing peaks and an increase in the background. Butyl PBD and anthracene dissolved slightly in 2 *N* HCl. Europium-activated calcium fluoride is known to dissolve in solutions of ammonium salts. Nucleotides were highly adsorbed by calcium fluoride; this is not unexpected, in view of the low solubility of calcium salts of nucleotides. The only universally applicable solid scintillator proved to be cerium-

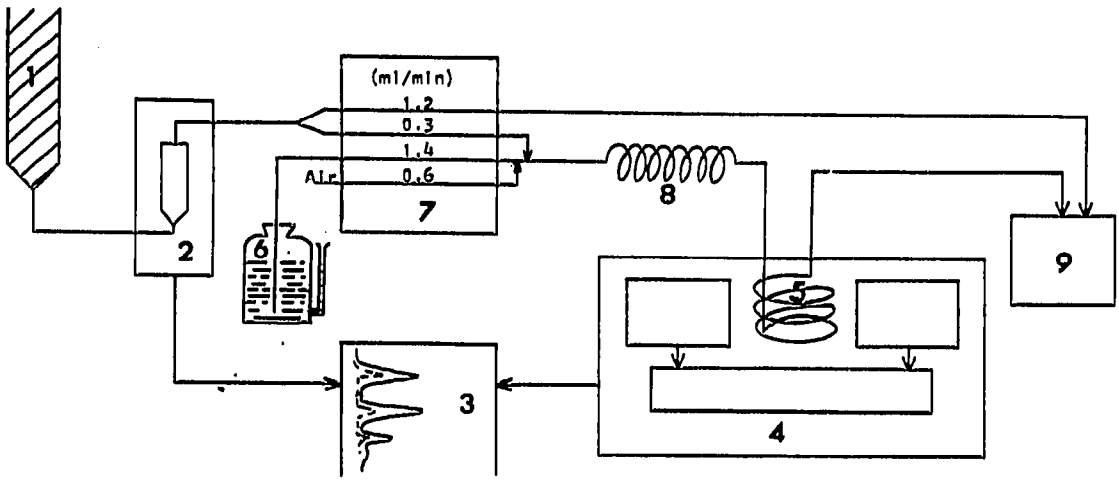

Fig. 3. Schematic representation of the homogeneous counting system. 1 = Column; 2 = ultraviolet detector, 254 nm; 3 = two-pen recorder; 4 = liquid scintillation spectrometer; 5 = helical flow cell; 6 = scintillator solution reservoir; 7 = proportioning pump; 8 = mixing spiral; 9 = fraction collector.

activated lithium glass beads, in spite of the relatively poor counting efficiency. No tailing of peaks or increase in background were observed. A minor inconvenience is the strong phosphorescence of the glass, which makes it necessary to store the cell in the dark for some days before use. Provided that excessive light is avoided during installation of the cell, adaptation is rapid. The resulting background is about 140 counts/min in the ^{14}C channel.

HOMOGENEOUS COUNTING SYSTEM

The system developed for homogeneous counting is shown in Fig. 3. Part of the column effluent is dissolved in a scintillator solution, which is subsequently passed through an empty flow cell. Emulsions of the type obtained in toluene–Triton X-100 mixtures are also regarded as being homogeneous in this context. A Technicon Auto-Analyzer proportioning pump, placed after the ultraviolet monitor, regulates the flow-rates. The total flow-rate of the effluent is 1.5 ml/min, of which 1.2 ml/min (80%) is usually collected unchanged. The remainder is added to a stream of scintillator solution (1:1 v/v toluene–Triton X-100 containing 8 g/l of PPO and 0.2 g/l of POPOP for aqueous effluents), which is interspaced with air bubbles to prevent tailing due to the difference in laminar flow-rates during transport. The scintillator solution and air were proportioned at flow-rates of 1.4 and 0.6 ml/min, respectively. The scintillator solution is transported through toluene-resistent Acidflex tubing (Technicon, Rotterdam). The mixture is homogenized in a mixing spiral, 70 cm long and of I.D. 2 mm. As mixing takes place between two successive air bubbles, *i.e.*, in about 30 μl of solution, resolution is not affected. The solution is then passed through a helical flow cell of I.D. 2 mm and volume 1.4 ml. The net flow-rate through the cell is 2.3 ml/min. The solution emerging from the flow cell can be collected if a more accurate determination of the radioactivity of the peak is required. One can, of course, use a different splitting ratio or even no splitting at all. For higher flow-rates, a larger cell should be used.

The counting efficiency with the toluene–Triton scintillator solution was about

80% for ^{14}C and 30% for ^{3}H. The background was about 65 counts/min. The system has also been used in combination with an AutoAnalyzer, in which event the counting mixture was passed through a 2.8-ml double-helical flow cell at a rate of 4.6 ml/min. A similar flow system has also been applied for the continuous detection of radioactive effluents from a gas chromatograph[7].

COMPARISON BETWEEN THE TWO COUNTING SYSTEMS

Liquid chromatography of an aqueous solution of [^{14}C]oxalic acid, [8-^{14}C]inosine 5'-monophosphate and [U-^{14}C]-guanosine 5'-monophosphate (each 80 nCi) over a strongly acidic cation-exchange resin (Dowex 50 X4 in the H^+ form, 450 mesh) was

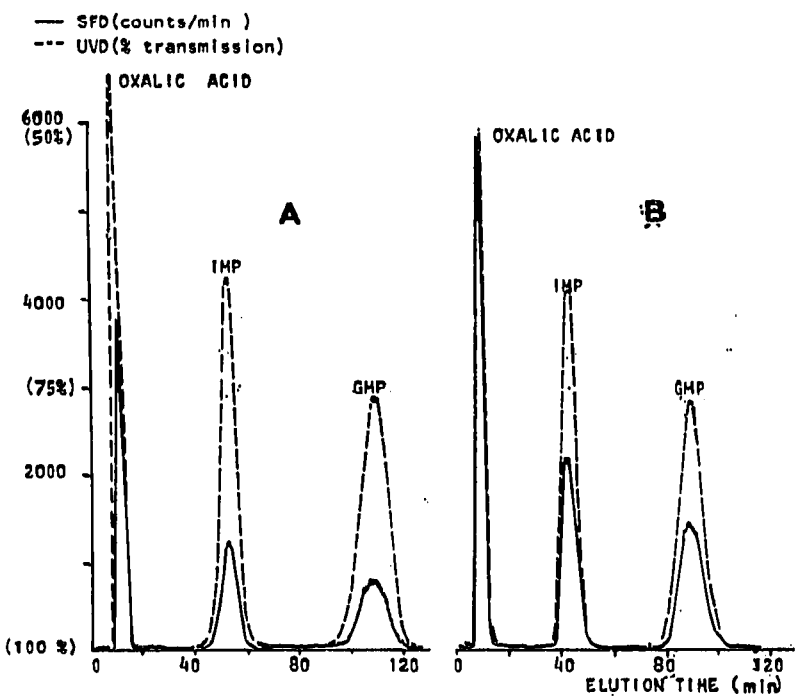

Fig. 4. Response to effluent of scintillation flow detector (SFD) and ultraviolet detector (UVD) after ion-exchange chromatography of ^{14}C-labelled oxalic acid, inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP). (A) Heterogeneous counting: 0.4-ml cell filled with glass scintillator beads, flow-rate 1.5 ml/min. (B) Homogeneous counting: 20% of effluent added to toluene–Triton scintillator solution; 1.4-ml cell, flow-rate 2.3 ml/min.

performed in duplicate and monitored with both the heterogeneous and homogeneous counting systems. The column was eluted with 0.05 *N* HCl. For heterogeneous counting (Fig. 1), the U-shaped flow cell was filled with cerium-activated lithium glass beads. The responses of the ultraviolet detector (UVD) and the scintillation flow detector (SFD) are plotted against elution time in Fig. 4A. For the homogeneous counting (Fig. 3) 20% of the column effluent was split off and added to a toluene–Triton X-100 scintillator solution. The plots of UVD and SFD response against elution time are shown in Fig. 4B.

For both homogeneous and heterogeneous counting, the correlation between the SFD and UVD responses was good. The SFD responses were of similar size for

the two systems. The lowest detectable ^{14}C-activity per peak was estimated at 10 nCi for both the heterogeneous system and the homogeneous system with an effluent splitting ratio of 4:1. In neither system was there any tailing or loss of resolution.

CONCLUSIONS

The main advantage of the heterogeneous counting system is that the effluent is fully recovered. Care should be taken in the choice of the solid scintillator; the only scintillator that does not dissolve in any common solvent and on which no materials are adsorbed is cerium-activated lithium glass. In view of the low counting efficiency of this scintillator, the system is most suitable for monitoring the separation of compounds of high activity, *e.g.*, in the purification of synthesized or commercial radioactive materials. The activity per peak should exceed 10 nCi for ^{14}C and 1 μCi for ^{3}H. In many isotope dilution analyses with ^{14}C-labelled compounds, the radioactivity will be high enough for this system.

In the homogeneous counting system, part of the effluent is "lost" when it is dissolved in the counting solution. The counting efficiency, however, is better than in the heterogeneous counting system, especially for ^{3}H. When no splitter is used, the activity per peak may be as low as 2 nCi for ^{14}C and 5 nCi for ^{3}H. This system can therefore be used with success in dual-label experiments, subject to the appropriate choice of spectrometer channels. Aqueous effluents can be dissolved in toluene–Triton X-100 and non-aqueous effluents in toluene scintillator solutions. When these solutions, interspaced with air bubbles, are pumped through toluene-resistent Acidflex tubing, no loss in resolution or tailing occurs. The homogeneous counting system is therefore most suitable for detection of ^{3}H, low activities, and for dual-label experiments.

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