PHOTODENSITOMETRIC INVESTIGATION OF THE CONDITIONS OF TRITIUM DETERMINATION ON PAPER CHROMATOGRAMS BY FLUOROGRAPHY

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During the determination of tritium radioactivity on paper chromatograms by fluorography quantitative measurements were taken of the dependence of the blackening on the type of paper used, on the sample radioactivity per area unit, concentration of the scintillating substance, reflector, and the wave-length shifter. On the basis of the measured relationships working conditions are proposed for the assay of radiochemical purity of tritiated compounds.

Paper chromatography of tritiated organic compounds is used especially in the investigation of their radiochemical purity during the preparation and storing. Continuous determinations of tritium radioactivity distribution directly on paper are carried out with a windowless flow counter[1], by scintillation technique[2] or by autoradiography[3]. The main advantage of autoradiography consists in its simplicity and cheapness and in the possibility of measuring large areas simultaneously. The limitation of its utilisability is given predominantly by the absorption of emitted low-energy electrons in the paper and the relatively low efficiency of detection of these electrons in the photographic emulsion[4,5]. Wilson[6] demonstrated that the undesirable influence of both these effects may be decreased by the saturation of the paper with the scintillating substance. The method, for which the designation fluorography was accepted, is used in determinations of tritium radioactivity not only in paper chromatography[7], but in thin-layer chromatography as well[8,9].

In this paper the effect of single factors affecting the increase in the blackening of X-ray films during fluorography of tritiated substances present on paper chromatograms is expressed quantitatively by means of photodensitometry. Experimental conditions are investigated with special regard to the determination of radiochemical purity of the tritiated compounds.

EXPERIMENTAL

A strip of chromatographic Whatman No 1 or No 3 paper was drawn slowly through an aqueous solution of cytidine-[^{3}H] (99·2 mCi/mmol), so that the paper contained approximately 10 or 50 μCi/cm^2 resp. The values of radioactivity per area unit of the saturated paper were determined in eluates both spectrophotometrically and by measuring the radioactivity in a liquid scintillation spectrometer (Tri-Carb, Model 3375). The homogeneity of planar distribution of radioactivity

was checked after drying using a 2π windowless flow counter provided with a shifting device (Frieseke and Hoepfner, German Federal Republic). From the dry radioactive strip zones were cut which were immersed into the scintillation solution for one minute. The liquid scitillator was prepared on dissolution of PPO or POPOP in analytical grade toluene. After drying in air the zones were enclosed together with an X-ray film (Medix Rapid, Foma, Czechoslovakia) into a case for X-ray films. As a reflector an aluminum foil was used, placed on paper zones. Exposition was carried out at room temperature for 4 days, development at 18°C for 6 minutes (X-ray developer, stabiliser, Foma, Czechoslovakia). After development the blackening was evaluated on a photodensitometer (MF 2, USSR).

RESULTS AND DISCUSSION

The blackening due to both values of planar sample radioactivity is dependent on the type of chromatographic paper (Table I). For all concentrations of PPO used, the blackening is stronger in the case of paper Whatman No 1, because in comparison with Whatman paper No 3 (the mass per area unit of which is approximately double) the absorption of low-energy electrons in it is lower. In accordance with this, when equal concentration of PPO is used the increase in blackening with respect to normal autoradiography is in all instances stronger in the case of Whatman No 3 paper. If various working conditions for tritium detection on paper chromatograms are compared from the point of view of the attainable detection sensitivity[10,11], it is essential to mention which type of paper was used for the measurement.

TABLE I
Blackening as a Function of the PPO (g/l) Concentration

PPO concentration	Blackening[a]	S_i/S_0[b]	PPO concentration	Blackening[a]	S_i/S_0[b]
Whatman No 1; 9·9 μCi/cm²			Whatman No 1; 51 μCi/cm²		
0	0·256		0	0·550	
2	0·301	1·176	2	0·732	1·331
5	0·542	2·109	5	1·214	2·207
20	1·131	4·418	20	1·920	3·491
50	1·853	7·238			
Whatman No 3; 9·9 μCi/cm²			Whatman No 3; 51 μCi/cm²		
0	0·168		0	0·376	
2	0·229	1·363	2	0·570	1·515
5	0·380	2·262	5	0·880	2·340
20	0·872	5·190	20	1·702	4·530

[a] Light flow through unexposed (ϕ_0) and exposed (ϕ) film; $S = \log \phi_0/\phi$; [b] S_0 blackening without PPO effect.

PPO was selected as the scintillator in view of the sensitivity of the photographic emulsion for photons in the emitted energy spectrum[9], its relatively high luminescence effect[12], good solubility, accessibility in radiochemical laboratories, and low cost. Contrary to the original Wilson procedure[6] exposition is carried out after evaporation of the solvent from the chromatographic paper, which brought about a simplification in manipulation with the paper and the film while retaining the advantages of fluorography. The blackening grows with increasing PPO concentration (Table I); for the 51 $\mu Ci/cm^2$ radioactivity the growth is distinctly slower in view of the larger density of the blackened particles. At a 20 g/l concentration the ratio of the corresponding blackening and the blackening achieved without the use of the scintillator is larger for 9·9 $\mu Ci/cm^2$ than for 51 $\mu Ci/cm^2$, for both sorts of chromatographic papers used. For 2 g/l the increase in blackening is relatively low and for concentrations above 50 g/l it is irregular. We consider that for the evaluation of the systems with different radioactivity values of tritiated spots the utilisation of concentrations in the 5 to 20 g/l range is suitable. For the determination of low radioactivity within a short time interval concentration up to 50 g/l is desirable.

In view of absorption of photons emitted by the scintillator the effect of the reflector will be less pronounced with papers with a higher mass per unit area (Table II). This is the reason why Wilson and Spedding[11], who used paper Whatman No 4, could observe only a weak effect of the reflector. Another cause of the decrease in the reflector effect may consist in its imperfect contact with the paper, causing the absorption of light quanta in the air layer between the paper and the reflector. The effect of POPOP as a wave-length shifter is also much lower (Table II) at the concentration used, in comparison with the reflector. However, in contrast to Randerath's observation[9] it is well provable. In analogy to the character of the effect of PPO on blacken-

TABLE II

Effect of the Reflector and the Wave-Length Shifter on the Blackening[a]

Paper[b]	Reflector	POPOP	Blackening[c]	S_i/S_0[d]
Whatman No 1	−	−	0·542	
	+	−	0·689	1·272
	−	+	0·570	1·052
Whatman No 3	−	−	0·380	
	+	−	0·448	1·179
	−	+	0·402	1·058

[a] Concentration, g/l toluene: PPO 5; POPOP 0·25; [b] radioactivity 9·9 $\mu Ci/cm^2$; [c] defined in Table I; [d] S_0 blackening without the reflector and POPOP.

ing, the effect of POPOP also depends on the area unit mass of the chromatographic paper: when both sorts of paper are immersed in a solution with an equal concentration of POPOP the blackening of Whatman No 1 paper is stronger, but the ratio of this blackening to that brought about without the wave-length shifter is larger in the case of Whatman No 3 paper (Table II).

On the basis of the observed dependences, a unified documentation may be introduced for radiochemical purity during the production and storage of tritiated substances. After chromatographic separation of a sample aliquot on a narrow strip of paper Whatman No 1 a solution of 50 g PPO/1 of toluene and a reflector were used. Unless after a 24 hours exposition and development no blackening is observable on the corresponding area of the film, the sample radioactivity is less than 60 $\pm$ 10 nCi/cm^2. The limit of the sensitivity of the radioactivity record of tritium per area unit of the chromatogram could be increased by an exposition at lowered temperature[13,9], by using an intensification process[14] and prolongation of exposition time, or also by the prolongation of development time. The increase in sensitivity might be desirable especially in applications of tritiated substances in tracer work. For the common measurements of radiochemical purity during the production and storage the value attained is fully sufficient.

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