EFFECT OF THE SCINTILLATOR ON THE INSTABILITY OF DETERMINATION OF ^{32}P AND $^{3}H+^{32}P$ IN LIQUID SCINTILLATION SPECTROMETER

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The time course of the changes of the counting rate caused by ^{32}P is determined in six commercially available scintillation mixtures applicable to aqueous solutions of the samples. The measurements were performed separately in the ^{3}H- and ^{32}P-measuring channels. Statistically significant changes were found during the first day after the sample preparation for Unisolve 100, Unisolve 1, and Aquasolve in both counting channels, and for Unisolve ELS in the ^{3}H-channel. Minisolve and Instagel exhibited steady values during 60 and 78 h, respectively, and after that period the changes proceeded very slowly. The stability of the counting rates for Minisolve and Instagel was checked also for ^{3}H alone and for combined ^{3}H + ^{32}P-labelled samples.

Substances labelled with the radionuclide ^{32}P (refs[1,2]), occasionally in conjunction with ^{3}H (ref.[3]), are widely applied to the study of living matter. The activity is being determined predominantly by autoradiography or liquid scintillation spectrometry. The results of the liquid scintillation counting are usually evaluated without respect to the time elapsed since the sample preparation, whereby, however, significant error may be introduced into the results, since, in fact, the counting rates observed during the determination of the ^{32}P or ^{32}P + ^{3}H mixture activity by liquid scintillation counting vary in dependence on time[4-6]. Thus, *e.g.*, during the measurement[6] of the ^{32}P activity using the frequently employed Bray scintillation solution[7] the counting rate in the ^{32}P-channel was found to decrease to approximately 80% within 48 h and that in the ^{3}H-channel to increase approximately to double. This phenomenon has been explained[4,5] in terms of the sample retention on the vial walls, observed before for ^{14}C-labelled samples[8-10]. In the case of low-energy β-emitters (^{3}H, ^{14}C, ^{45}Ca) the counting rate changes appear to a considerably lesser extent[11]. The instability of the measurements of ^{32}P and ^{3}H + ^{32}P mixtures could be partly suppressed by using Triton X-100 (ref.[6]), other procedures appeared to be less suitable or inapplicable[11].

In the present work the instability of the counting rates caused by the trapping of two model samples labelled with ^{32}P or simultaneously with ^{3}H and ^{32}P is examined in six commercially available scintillation mixtures. Taking into account the common practice of laboratories dealing with labelled substances we consider

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it desirable that the counting rates measured be steady for more than 48 h. Important for the mixtures regarded is also that a high detection efficiency be maintained and that the scintillator be applicable to aqueous solutions.

EXPERIMENTAL

The measurements were performed in a three-channel scintillation spectrometer Packard 3375 at the temperature of 280 K using the gain of the ^{3}H- and ^{32}P-channels 52 and 0·56%, respectively, discrimination levels 50—550 and 50—1000, respectively. The apparatus stability was checked periodically by means of reference radiation sources. 20 ml glass measuring vials 57 × 27 S Kavalier, Teplice were used. The prior unused vials were first washed with water containing a tenside (replaced after ten vials), rinsed repeatedly with distilled water, and dried. The vial sequence was maintained during the rinsing and sample preparation, for the effect of the surface impurities and of the different state of the glass surface to be minimized[12]. The following scintillation mixtures were used: Minisolve, Unisolve 1, Unisolve 100, Unisolve ELS 294 (Koch-Light Labs., England), Aquasolve (New England Nuclear Corp., USA), Instagel (Packard Instr. Comp., USA), scintillation gel SG-1 (Spolana), and the scintillation solution after Bray[7]. Furthermore, an isotonic solution of the carrier-free sodium [^{32}P]hydrogenphosphate (7·1 mg NaCl/ml, 1 mg P/ml, pH 7·1, $^{32}PO_4^{3-} > 98\%$) and an aqueous solution of 2,8-[^{3}H]adenine (14·8 kBq/ml; $2{\cdot}25 \,.\, 10^{-6}$ mmol/ml) were used. For the simultaneously ^{3}H- and ^{32}P-labelled samples the stock solution was prepared so that the specific activities of the two radionuclides were the same as those applied to the single labellings. Sets of ten identical samples were prepared for each of the scintillators. For selected scintillating media (Minisolve and Instagel), the stability of the measuring conditions was checked for series of ten samples either $^{3}H + ^{32}P$- or only ^{3}H-labelled. The samples were prepared by pipetting 1 ml of the aqueous solution in a vial containing 9 ml of the scintillation solution. Each sample was placed in the spectrometer shortly after the preparation, and the first measurement was performed one hour after the preparation. This time is taken as the zero. The further measurements were conducted in two-hour periods for twelve hours, then in six-hour periods for seven days. The values observed were corrected for the half-life and evaluted on a Hewlett–Packard computer 9830 A.

RESULTS AND DISCUSSION

Based on tentative measurements the scintillation gel SG-1 was rejected because of the poor reproducibility of measurement due to the formation of bubbles during the sample preparation.

The efficiency of the ^{32}P detection and the quenching for all the remaining scintillation mixtures examined were determined from the values observed in the time $T = 0$ using series of three parallel samples (Table I). As follows from these data, the highest efficiency of detection of the radionuclide ^{32}P was established for Instagel and Unisolve ELS 294, which also exhibits the lowest ratio of the counting rates in the ^{3}H- and ^{32}P-channels, hence the minimum quenching.

The time course of the counting rate changes brought about by the ^{32}P in the ^{32}P- and ^{3}H-channels, with the relative error of measurement not exceeding ±2%, is shown in Fig. 1. Unisolve 100 (Fig. 1*a*) and Aquasolve (Fig. 1*b*) are seen to display considerable counting rate changes, particularly during the first day after the sample preparation. In Unisolve 1 (Fig. 1*c*) the changes proceed appreciably more slowly, still after 24 h they are statistically significant. Unisolve ELS 294 (Fig. 1*d*) shows in the ^{3}H-channel a counting rate increment of per cent magnitude above the relative error of measurement in a time shorter than 24 h, and within 48 h this rise appears

TABLE I

Comparison of the Counting Rates Induced by ^{32}P in the Time $T = 0$ in Various Scintillation Mixtures

Scintillation mixture	^{32}P-Channel		^{3}H-Channel/^{32}P-Channel	
	cpm	% Bray	ratio	% Bray
Bray	87 101	100·0	0·102	100·0
Minisolve	77 859	89·4	0·116	113·7
Unisolve 1	92 481	106·2	0·098	96·1
Unisolve 100	56 971	65·4	0·240	235·2
Unisolve ELS 294	100 776	115·7	0·068	66·7
Aquasolve	95 378	109·5	0·080	78·4
Instagel	100 950	115·9	0·073	71·6

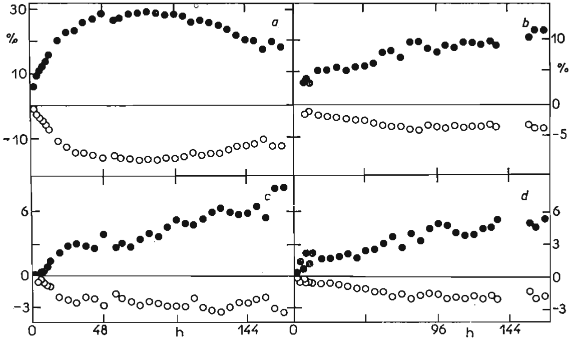

FIG. 1

Time Course of the Changes of the Counting Rate Induced by ^{32}P

Per cent with respect to the value for $T = 0$; channels: ● ^{3}H, ○ ^{32}P; *a* Unisolve 100; *b* Aquasolve; *c* Unisolve 1; *d* Unisolve ELS 294.

quite markedly, so that the possibility of simultaneous detection of ^{3}H and ^{32}P is limited. The most favourable results were obtained with Minisolve (Table II), where the counting rate measured was steady within the error of measurement for up to 60 h

TABLE II

Time Course of the Counting Rate Changes in Percentage Counting Rate Measured in the Time $T = 0$ for the Scintillation Solution Minisolve

Time h	^{32}P-Labelling		^{3}H-Labelling	$^{3}H + ^{32}P$-Labelling	
	^{3}H-channel	^{32}P-channel	^{3}H-channel	^{3}H-channel	^{32}P-channel
0[a]	10 210·33	76 031·00	21 874·33	33 049·25	75 723·0
0	100·0	100·0	100·0	100·0	100·0
2	99·9	101·5	102·1	100·6	102·1
4	98·1	101·1	102·5	101·0	101·1
6	99·7	100·8	101·5	100·7	101·1
8	99·3	100·4	101·2	100·4	101·2
10	99·8	100·4	100·7	100·4	100·5
12	99·8	100·6	100·1	100·5	100·3
18	100·7	100·5	100·1	100·0	100·1
24	100·2	100·6	99·4	99·5	100·1
30	100·1	100·7	100·1	100·1	100·4
36	100·6	99·5	99·6	100·1	99·6
42	101·2	99·5	99·5	100·2	99·5
48	101·7	99·5	98·5	99·1	99·7
54	101·5	98·3	98·7	99·5	98·7
60	102·5	98·9	97·8	99·8	98·9
66	103·0	97·8	97·0	98·9	98·2
72	103·6	98·2	96·7	99·0	98·6
78	104·7	97·2	96·5	98·1	97·4
84	105·2	97·0	95·8	98·2	97·5
90	102·5	97·7	97·0	98·1	98·8
96	103·8	98·2	96·8	98·9	97·4
102	106·3	97·3	96·7	99·0	97·8
108	104·1	97·1	96·9	98·8	97·4
114	103·9	97·7	97·7	98·6	98·0
120	105·4	97·4	96·7	99·0	98·1
126	104·6	97·8	97·4	98·5	97·8
132	105·2	97·7	97·3	98·9	97·7
136	104·3	97·7	97·0	98·4	97·9
158	102·5	99·3	98·4	99·3	98·6
162	104·4	97·8	97·8	99·4	97·8
168	105·1	98·0	97·0	98·8	98.3

[a] cpm at $T = 0$.

after the sample preparation, and with Instagel (Table III), which gave steady values for up to 78 h. From the data of Tables II and III it is also apparent that the counting rate changes for ^{32}P in the ^{32}P-channel itself did not for Instagel exceed the error

TABLE III

Time Course of the Counting Rate Changes With Respect to the Counting Rates Measured in the Time $T = 0$ for the Scintillation Solution Instagel

Time h	^{32}P-Labelling		^{3}H-Labelling	$^{3}H + ^{32}P$-Labelling	
	^{3}H-channel	^{32}P-channel	^{3}H-channel	^{3}H-channel	^{32}P-channel
0[a]	7 321·33	99 268·67	43 516·67	52 105·50	98 969·50
0	100·00	100·00	100·00	100·00	100·00
2	99·67	100·00	100·59	100·05	100·36
4	100·56	100·21	100·36	100·54	100·18
6	99·73	100·29	100·77	100·32	100·14
8	99·70	99·79	99·97	99·70	100·28
10	100·00	100·03	98·98	99·82	100·09
12	100·39	99·82	99·56	99·72	99·79
18	100·45	99·44	98·96	99·53	99·84
24	99·73	99·94	98·33	99·55	100·24
30	99·20	99·83	99·49	99·20	99·98
36	100·26	99·41	98·94	99·30	99·89
42	100·02	99·62	98·39	99·01	99·84
48	100·95	99·25	98·23	98·56	99·78
54	99·93	98·93	97·91	98·47	99·34
60	101·12	99·01	97·85	98·35	99·65
66	100·77	98·49	96·82	98·54	99·04
72	101·86	98·65	97·04	97·25	99·34
78	100·95	98·39	95·70	97·18	98·87
84	103·96	97·88	95·68	96·66	98·95
90	102·96	98·63	97·54	97·82	99·13
96	103·77	98·64	96·54	98·02	99·13
102	102·92	98·06	96·95	97·53	98·94
108	102·15	98·37	96·72	97·73	99·16
114	102·48	98·37	97·15	98·30	99·14
120	104·50	98·59	97·06	98·08	98·92
126	101·81	98·70	96·26	98·20	99·16
132	103·04	98·67	96·89	98·17	99·26
136	104·01	98·74	97·21	97·93	99·29
158	102·51	99·15	97·16	98·27	99·79
162	103·47	98·82	97·32	98·22	98·70
168	103·76	98·36	96·51	97·98	98·97

[a] cpm at $T = 0$.

of measurement during the whole seven-day measurement period (except a single, very low variation at the time of 84 h), with Minisolve these changes during the one-week measurement were very slight too (up to 1% above the error of measurement). For both mixtures the stability was found better than for the previously described[6] Triton X-100. For low activities we consider Instagel somewhat more suitable than Minisolve, owing to its counting rate stability and also to the higher efficiency of detection.

The suitability of Instagel and Minisolve was tested also for the simultaneous determination of ^{3}H and ^{32}P. During the measurements of simultaneously $^{3}H + ^{32}P$ labelled samples in the ^{3}H-channel, the counting rate on the one hand decreases owing to the retention of the tritium-labelled sample, and on the other hand increases due to the interaction of the electrons emitted by the radionuclide ^{32}P with the vial by the Cherenkov radiation mechanism[4,5]. The net change in the ^{3}H-channel resulting from these two processes depends upon the ratio of the activities of the ^{3}H and ^{32}P radionuclides and upon the course of their retention. We examined therefore in the two scintillation mixtures the stability of the counting rate brought about not only by the ^{32}P, but also by ^{3}H alone and by the $^{3}H + ^{32}P$ mixture (Tables II and III). The results obtained show that approximately the same values were measured for the effects examined in the two mixtures: in no case did the variations in the counting rate exceed the error of measurement within 48 h.

Although the course of the changes of the counting rates depends on the chosen counting conditions of the instrument employed[4] and on the nature of the sample measured[9], the results obtained indicate that an appropriate choice of the scintillation mixture can effect a considerable improvement of the measurement stability in the liquid scintillation spectrometer and a lowering of the objectionable instability resulting from the retention of the sample on the measuring vial wall.

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