ACTA CHEMICA SCANDINAVICA 25 (1971) 1359–1369

Scintillation Counting of Aqueous Solutions of ^{3}H RNA

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The radioactivity in various aqueous solutions of ^{3}H RNA has been recorded in nine known and two new scintillation liquids by 1. direct addition to the scintillation liquids; 2. prior treatment with solubilizer; 3. prior plating on filters. By proper combination of method and scintillation liquid, stable high-efficiency measurements could be obtained.

Liquid scintillation counting is a method of increasing importance within the biological sciences. This is probably due to the accuracy, efficiency and convenience with which for example the isotopes ^{3}H, ^{14}C, and ^{32}P can be measured. Recent studies within scintillation spectrometry are mainly concerned with the properties of new scintillation liquids or with development of new methods or apparatuses. Only a few of the studies deal primarily with the practical aspects of liquid scintillation counting. Among the exceptions from this generalization are the studies by Rogers and Moran [1] on a comparison of various quench correction methods, and the recent work by Davies and Hall [2] on the comparison of the direct addition method with the glass-fiber disc method, with particular reference to the quench problems.

It is the purpose of this paper to describe the optimal conditions for recording the radioactivity in various aqueous solutions of ^{3}H RNA. This compound is polar and – as expected – only partly soluble in most of the eleven different scintillation liquids studied.

The results indicate that by proper choice of method and scintillation liquid, various sample volumes and various samples containing acids, bases and salts can be measured with optimal and stable efficiency. The information obtained from this study has also been used for development of a method for high-efficiency measurement of labelled substances in polyacrylamide gels.[3]

Abbreviations used. PPO, 2,5-diphenyloxazole; dimethyl-POPOP, 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene; α-NPO, 1,4-di-(2-(5-(1-naphthyl)oxazolyl))-benzene; butyl-PBD, 2-(4′-*t*-butylphenyl)-5-(4″-biphenyl)-1,3,4-oxadiazole; NCS, Nuclear Chicago Solubilizer = a mixture of quaternary ammonium compounds in toluene; RNA, ribonucleic acid (mixed types); ATP, adenosine triphosphate; TCA, trichloracetic acid; PCA, perchloric acid.

MATERIALS AND METHODS

Radioactive material. Most of the experiments were performed using ^{3}H labelled RNA. The specific activity was 25 300 cpm per μmol, estimated by direct addition of 10 μl sample to 10 ml DM scintillation liquid (counting within the period 5 – 10 min after).

Scintillation liquids. Reagents. PPO, dimethyl-POPOP, hyamine 10 – x hydroxide and diluene were purchased from Packard Instr. Co., Ill. Toluene and ethanol were of technical grade; use of these products resulted in the same counting efficiency as use of the products of analytical grade. All other chemicals were Merck products (analytical grade).

Compositions. The composition of 10 of the 11 scintillation liquids studied are shown in Table 1. The eleventh mixture is Insta-gel (Packard). DM is the mixture proposed

Table 1. The composition of 10 of the 11 scintillation liquids. For origin and purity of the chemicals, see Materials and methods. The 10 scintillation liquids divide into 4 classes, according to their composition. Insta-gel of unknown composition comprises a fifth class.

	Per liter	I	II		III	IV					
		T	TE	TC	TH	DM	XDE	TDM	XDC	TXDE	TDE
PPO	g	4	3	4	3.5	4	5	5	10	4.5	4
Dimethyl-POPOP	mg	50	70	50	70	200	100	100	500	125	150
Toluene	ml	1000	660	600	700			385		250	500
Xylene	ml						385		140	190	
Dioxane	ml					800	385	385	430	340	300
Methanol	ml					100		230			
Ethanol (99% v/v)	ml		330		300		230			215	200
Methyl-cellosolve	ml			400							
Ethyl-cellosolve	ml								430		
Hyamine hydroxide	ml				75						
Ethyleneglycol	ml					20					
Naphthalene	g					60	80	80	80	70	80

by Bray,[4] XDE is the mixture of Kinard [5] but with 0.01 % dimethyl-POPOP instead of 0.005 % α-NPO, TDM [6] is a modification of XDE, as toluene-dioxane-methanol is used instead of xylene-dioxane-ethanol. XDC [7] is a 1:3:3 mixture of xylene:dioxane:ethyl-cellosolve.

TXDE and TDE are two new inexpensive high-efficiency mixtures, made according to the following criteria: *1.* reduction of the percentage of dioxane and xylene in XDE, but only to such a degree that the counting efficiency when small volumes of aqueous solutions of ^{3}H RNA are added directly is still high; *2.* substitution of part of the dioxane and the xylene with toluene and ethanol; *3.* optimal proportion between toluene and ethanol; *4.* optimal concentration of naphthalene.

External standard ratio. This was determined on 10 ml aliquots of the various scintillation liquids in polyethylene vials by means of a liquid scintillation spectrometer (Beckman LS 133) with a gain setting of 200. The external standard ratio of a background standard and 10 ml TDE (glass vials) were 0.73 and 0.66, respectively.

Determination of radioactivity. The various aqueous solutions of ^{3}H RNA were treated in one of the following ways before scintillation counting was performed. *1. Direct addition.* The sample was added directly to 10 ml scintillation liquid and counted. Most of the results obtained with all three techniques are given as efficiencies relative to the counting efficiencies obtained when 10 μl ^{3}H RNA is added directly to DM and counted after 5 min. (When 5 ml scintillation liquid was used instead of 10 ml, the relative efficiencies were lowered by 11 %.) *2. Treatment with solubilizer.* The sample was treated in glass tubes

with NCS at 60°C for 5 min. After cooling to 25°C, the sample was transferred to polyethylene vials with 10 ml scintillation liquid. *3. Plating on discs.* The samples were plated on filter paper discs (Whatman No. 3, $d = 2.4$ cm) or glass-fiber discs (Whatman GF/C, $d = 2.5$ cm); dried at 37°C, and then the discs were placed in 5 – 10 ml scintillation liquid.

All samples were counted in a Packard model 3324 scintillation spectrometer at 8°C with a window of 50 – 1000, and a gain of 50 %.

Other chemicals. NCS was purchased from Amersham/Searle Corp., Ill.

RESULTS

Background counting rate, external standard ratio and price per volume. The background counting rates in the ^{3}H-channel were found to be 19 ± 2 cpm for all the scintillation liquids. The external standard ratios fall into 3 groups: one group with 0.65 – 0.69 (T, the 6 dioxane-based scintillation liquids and Insta-gel), one group with 0.46 – 0.47 (TE and TC), and finally TH with a ratio of 0.36. The relative price per volume is practically solely determined by

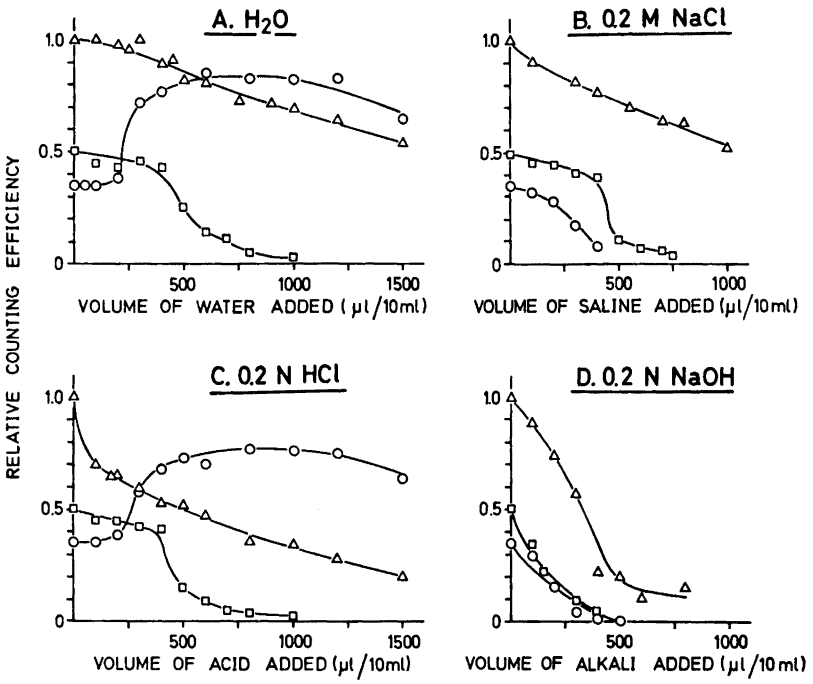

Fig. 1. Direct addition of increasing volumes of distilled water (a), 0.2 M sodium chloride (b), 0.2 N hydrochloric acid (c), and 0.2 N sodium hydroxide (d) to three of the scintillation liquids: DM(△), TH (○), and TE (□). 10 µl ^{3}H RNA was added directly to 10 ml scintillation liquid in polyethylene vials. The measurements were based on the number of counts registered in the period from 5 to 10 min after the additions. The results are based on 3 – 5 experiments and are expressed as relative counting efficiencies. The radioactivity of 10 µl aqueous solution of ^{3}H RNA in DM measured after 5 min is arbitrarily set to 1.00.

the prices of PPO, dioxane and hyamine. T, TE and TC are cheap mixtures with relative prices of 0.2, 0.2 and 0.3, whereas TH and Insta-gel are the expensive ones (both 1.0). Among the dioxane-based scintillation liquids (0.5 – 1.0), TDE is the cheapest mixture (0.5).

Direct addition of increasing sample volumes. DM and TE show decreasing counting efficiency with increasing volume of water added (Fig. 1a). The curve for TH shows a marked increase in the counting efficiency after addition of 200 – 400 μl of water. This coincides with the appearance of two phases which form after addition of 310 μl per 10 ml scintillation liquid (TE and DM form two phases after addition of 470 μl and 4550 μl of water, respectively).

Fig. 1b illustrates the effect of addition of 0.2 M sodium chloride. All three curves show decreasing counting efficiency with increasing volume added. DM has the highest counting efficiency. By comparison of Figs. 1a and 1b it can be seen that DM is only moderately affected by 0.2 M NaCl. Results (not shown) indicate that 0.02 M NaCl and water have the same effect on DM. TE is not more affected by 0.2 M NaCl than by water, but 1.0 M NaCl causes within the first 10 min after addition a marked decrease in the counting efficiency. TH is apparently the most sensitive to saline, since 0.2 M NaCl causes a rapid decrease in the efficiency.

Fig. 1c illustrates the effect of 0.2 N hydrochloric acid. It is evident that TH and TE are only moderately affected in comparison to the effect of water, whereas the counting efficiency of DM is markedly reduced.

Fig. 1d shows that 0.2 N sodium hydroxide causes decreases in the efficiencies of all three scintillation liquids. Furthermore, the time-dependent decreases are rapid, and lead in most cases to complete loss of efficiency of the scintillation liquids (XDE, TXDE and TDE in Table 2).

Time-dependent changes in efficiencies obtained by direct addition. The addition of 10 μl aqueous solution of ^{3}H RNA to the 11 scintillation liquids (Table 2) results in the following relative counting efficiencies: class IV and V 0.92 – 1.38; class II about 0.5; class III 0.35, and class I 0.0. However, time-dependent changes in the counting efficiency take place especially with TC, DM, TDM, and XDC, and to less extent with XDE, TXDE, TDE and Insta-gel. Measurements after 48 h (not shown) result in a slight further decrease of the counting efficiencies of all liquids, except TH.

In all liquids, except TH, addition of water results in an immediate and a time-dependent decrease in the counting efficiency. With TH, a constant counting efficiency during 24 h is observed when 100 μl is added, but a slight decrease is seen when 200 μl is added. With this volume of water, Insta-gel shows dramatic decrease due to the formation of a two-liquid phase system (at 150 μl/10 ml) (see also Fig. 2). The relative counting efficiency of TE and TC is reduced to about 0.35 (after 24 h when 100 μl of water is added, and did not change by further addition of 100 μl of water. Notice, that the counting efficiencies, of the dioxane-based scintillators are $2 - 2\frac{1}{2}$ times higher than those of TE, TC and TH, when 100 or 200 μl samples are added and measured after 24 h.

TE and TC are uninfluenced by 200 μl of 0.2 N sodium chloride (Table 2). The other liquids show somewhat more pronounced decreases than those observed with 200 μl of water. In the case of addition of 200 μl of 0.2 N hydro-

chloric acid, only TH is unaffected, whereas the others are less efficient than with water added.

The effect of 0.2 M sodium chloride and 0.2 N hydrochloric acid on the different classes of scintillation liquids may be summarized as follows. Class II and class III are more susceptible to 0.2 M NaCl than to 0.2 N HCl, whereas the opposite is the case for class IV. The effects of sodium chloride and hydrochloric acid are not additive, since a solution of 0.2 M NaCl in 0.2 N HCl resulted in counting efficiencies equal to those caused by the most quenching of the two agents, but not in lower counting efficiencies. The addition of 0.2 N sodium hydroxide leads to dramatic decreases in all of the mixtures.

Direct addition of various acidic samples. The effect of direct addition of acidic samples was further investigated by addition of 200 µl samples of 0.2 N, 1.0 N, and 5 N HCl, 0.2 N TCA, and 0.2 N PCA to 10 ml TE, TH, DM and TDE. This results in immediate and time-dependent changes in the counting efficiencies of TE, DM and TDE, whereas TH is unaffected, except when 5.0 N HCl is added. Formic acid (0.2 N) causes only a minor reduction in the efficiencies of TE, DM and TDE, whereas HCl and PCA causes pronounced decreases. The magnitude of the quenching effects of the acids depends naturally on the volume of scintillation liquid used.

Direct addition of large sample volumes to Insta-gel. Fig. 2 illustrates the results obtained when large volumes of aqueous ^{3}H RNA are added directly

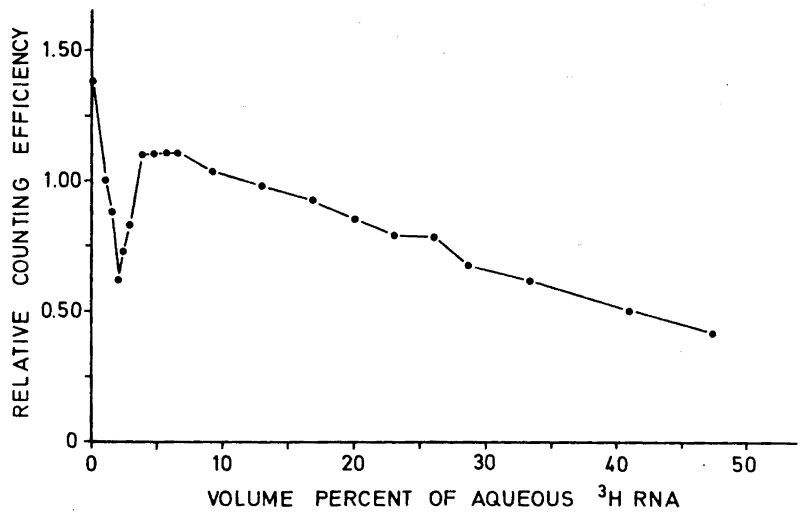

Fig. 2. Direct addition of large sample volumes to Insta-gel. 10 µl ^{3}H RNA plus varying amounts of water were added directly to 10 ml Insta-gel in polyethylene vials. Measurements were performed 5 min after the addition, and were based on a counting period of 10 min. Results are expressed as relative counting efficiencies, comparable to those of Fig. 1 and the tables.

to Insta-gel. The rapid decrease in the counting efficiency from 1.38 to 0.62 (at about 2 % (v/v) of aqueous ^{3}H RNA) is shown in Table 2. Up to about

Table 2. Time-dependency of the counting efficiencies after direct addition of water, saline, acid, and alkali. 10 µl ^{3}H RNA was added directly to 10 ml scintillation liquid. Measurements were performed at various periods after additions, and were based on a counting period of 10 min. Results are means of 2–5 experiments, and are expressed as relative counting efficiencies comparable to those of Fig. 1.

		I	II		III	IV						V
Further additions	Time of measurement after	T	TE	TC	TH	DM	XDE	TDM	XDC	TXDE	TDE	Insta-gel
	5 min	0.00	0.50	0.56	0.35	1.00	1.03	0.92	0.99	1.02	1.20	1.38
	24 h	0.00	0.48	0.40	0.36	0.80	0.95	0.79	0.88	0.98	1.11	1.29
+ 100 µl H_2O	5 min		0.48	0.50	0.39	0.95	0.94	0.89	0.81	0.89	0.90	1.20
	1 h		0.43	0.45	0.39	0.88	0.90	0.81	0.78	0.80	0.87	1.13
	24 h		0.35	0.36	0.39	0.76	0.80	0.71	0.71	0.77	0.78	0.98
+ 200 µl H_2O	5 min		0.39	0.44	0.39	0.91	0.88	0.81	0.77	0.87	0.92	0.62
	24 h		0.35	0.33	0.37	0.72	0.78	0.66	0.63	0.78	0.78	0.20
	48 h		0.32	0.31	0.34	0.68	0.75	0.62	0.60	0.73	0.75	0.15
+ 200 µl 0.2 M NaCl	5 min		0.43	0.44	0.34	0.85	0.86	0.79	0.76	0.85	0.82	0.60
	24 h		0.32	0.32	0.31	0.66	0.75	0.62	0.59	0.75	0.69	0.16
+ 200 µl 0.2 N HCl	5 min		0.36	0.41	0.38	0.60	0.69	0.61	0.58	0.71	0.71	0.59
	24 h		0.30	0.29	0.40	0.47	0.59	0.44	0.47	0.59	0.60	0.18
+ 200 µl 0.2 N NaOH	5 min			0.40	0.34	0.79		0.78	0.74			0.71
	24 h		0.06	0.30	0.29	0.58	0.18	0.60	0.56	0.16	0.10	0.27

4 % (v/v) of aqueous ^{3}H RNA, the counting efficiencies show time-dependent decreases (about 8 % to about 68 % decrease during the first 24 h). From about 4 % (v/v) to about 47 % (v/v), the counting efficiency decreases linearly. Under these conditions, the final samples are emulsions which show constant counting efficiencies. Furthermore, the counting efficiency is not influenced by 0.2 N PCA, HCOOH or HCl, 0.2 N NaCl, 0.2 N NaOH or 96 % (v/v) ethanol. The presence of acids or salts at a concentration of 2 N produces a significant decrease in counting efficiency.

Treatment with solubilizer. The time-dependent decreases in the counting efficiencies of the scintillation liquids (Table 2) can be markedly or completely abolished by treatment of the sample with Nuclear Chicago Solubilizer (NCS) for a few minutes at 60°C (Tables 3 and 4). Table 3 shows that the decrease

Table 3. Treatment with solubilizer, and counting in the TDE scintillation liquid. 10 µl ^{3}H RNA plus additions as indicated were treated with NCS. Counting was performed in 10 ml TDE. Measurements were performed after 5 min, 24 h, and 8 days, and were based on counting periods of 10 min. The results are means of 3 experiments, and are expressed as relative counting efficiencies, comparable to those in Table 2.

Further additions	Volume NCS µl	Relative counting efficiency after 5 min	after 24 h	after 8 days
–	–	1.20	1.11	1.01
15 µl H_2O	25	1.19	1.19	
100 µl H_2O	–	0.90	0.78	0.63
	25	0.87	0.87	
	100	0.82	0.80	0.78
200 µl H_2O	–	0.92	0.75	0.63
	25	0.92	0.92	
	50	0.86	0.87	0.84
	200	0.80	0.76	
200 µl 0.2 N PCA	–	0.66	0.53	0.47
	50	0.46	0.34	
	100	0.89	0.88	0.86
	200	0.87	0.86	
200 µl 0.2 N HCl	–	0.61	0.47	0.40
	50	0.83	0.72	
	100	0.90	0.85	0.85
	200	0.88	0.84	
200 µl 5 N HCl	–	0.16	0.13	0.14
	200	0.17	0.18	0.18
200 µl 0.2 N NaOH	25	0.36	0.25	
200 µl 0.2 M NaCl	25	0.36	0.23	
500 µl H_2O	–	0.71	0.13	0.12
	100	0.70	0.74	0.71
	150	0.62	0.61	0.59

in counting efficiency from 0.90 to 0.78, obtained when the sample volume in 110 µl, may be changed to a constant counting efficiency of 0.87 during 24 h by treatment of the sample with 25 µl NCS before transfer to TDE. Use of larger volumes of NCS is non-optimal. Essentially the same results are seen when the sample volume is 210 µl of water. When 200 µl 0.2 N PCA

or 0.2 N HCl is added instead of 200 μl of water, quenching effects are seen with samples which are not treated with NCS. Treatment of these samples with 100 μl of NCS fully restores the counting efficiency. The results obtained when 5 N HCl is added indicate that the NCS, which is 6 N with regard to quaternary ammonium compounds in toluene, must be added in excess of the acid present. Addition of 200 μl of 0.2 M NaCl or 0.2 N NaOH gives low counting efficiencies which decrease during the first 24 h. Addition of a large volume (500 μl) of water and treatment with 100 μl NCS give a rather high, stable efficiency (0.70).

Table 4 shows the counting efficiencies obtained when NCS-treated samples are counted in T. It is evident that in this case the counting efficiency is more

Table 4. Treatment with solubilizer, and counting in the T scintillation liquid. The experiments were essentially as those presented in Table 3, but counting was performed in T.

Further additions	Volume NCS μl	Relative counting efficiency after 5 min	after 24 h
–	–	0.00	
15 μl H_2O	50	0.46	0.21
	100	0.86	0.57
	200	1.56	1.48
	250	1.58	1.58
	300	1.46	1.46
	500	1.41	1.34
200 μl H_2O	750	0.63	0.59
	1000	1.18	1.17
	1200	1.18	1.19
	1500	1.07	1.07
	2000	0.98	0.95
200 μl 0.2 N HCl	1000	1.12	1.11
200 μl 0.2 N NaOH	1000	1.15	1.13
200 μl 0.2 M NaCl	1000	1.08	1.09

dependent on the amount of NCS added than in the case of TDE (Table 3). The optimal volume of NCS for counting of aqueous volumes of 25 μl and 210 μl is 250 μl and 1000 μl NCS, respectively. Use of higher or lower volumes than the optimal volumes results in time-dependent decreases in the efficiencies. Counting of NCS-treated samples in T (Table 4) gives higher counting efficiencies than counting in TDE (Table 3), as well with small (25 μl) as with large (210 μl) sample volumes. A great disadvantage by the NCS – T method is the need for 5 – 10 times higher volumes of the expensive reagent NCS than in the case of the NCS – TDE method.

The standard deviation of repeat measurements of different 25 μl aliquots treated with NCS was 2.4 % and 1.2 %, when measurement was performed in 10 ml of T and TDE, respectively.

The radioactivity of 200 μl samples of ^3H RNA in 0.2 N HCl, 0.2 N NaOH or 0.2 M NaCl may be determined by use of the NCS – T method. The resulting efficiencies are only slightly lower than those obtained with 200 μl of water.

When ^{3}H RNA samples (total volume < 50 μl) were treated with NCS and counted in the other scintillation liquids, the following optimal relative efficiencies were obtained: class II, 0.59; class III, 0.33; and class V, 1.41.

Counting efficiencies obtained by plating on discs. When the 10 μl ^{3}H RNA samples were plated on paper, and dried and counted in the various scintillation liquids, the T scintillation liquid was superior to the other liquids. Plating of 10 μl ^{3}H RNA on paper and counting in T gives an efficiency of 0.37 compared to direct addition in DM (1.00). When one filter paper was loaded with ^{3}H RNA and placed in different volumes of various scintillation liquids, it was found that the efficiency was constant during time and within a given scintillation liquid, independent of the volume within the region 4 – 15 ml.

The proportionality between counts/min and number of ^{3}H RNA loaded filter papers per vial was tested in the following way: 10 μl aqueous solution of ^{3}H RNA was added to each of a large number of filter papers ($d = 2.4$ cm). The filter papers were dried and transferred to two series of polyethylene vials, one containing 6 ml, the other 15 ml T per vial. A linear dependency between cpm and number of filter papers per vial was observed until about 7 filter papers per vial. Above this value, the samples containing 15 ml T still showed proportionality until 15 filter papers per vial, but the counting efficiency of the 6 ml samples was significantly reduced under these circumstances. The activity even of the heavily-loaded vials was constant during 24 h.

When the 10 μl ^{3}H RNA samples were plated on glassfiber-discs, dried and counted in T, the counting efficiency was 1.5 times as high (= 0.56) as when filter paper discs were used. The possibility of increasing the counting efficiency by elution and NCS-digestion of the discs was tested in the following way. A number of filterpaper and glassfiber discs loaded with ^{3}H RNA were placed in glass vials. Various volumes of water (50 – 300 μl) were added, and the vials were kept at 25°C for 1 h. Then various volumes of NCS (100 – 500 μl) were added, and the vials were placed at 65°C for 1 h. After cooling, 10 ml of the scintillation liquids T or TDE was added, and counting was performed after 2 h storage in the darkness. The results show that a procedure with 100 – 200 μl of water and 100 – 150 μl NCS with subsequent counting in TDE gives the highest efficiencies and the most reproducible results. With this procedure, the efficiency with filterpaper discs increased from 0.37 to 0.70, and with glassfiber discs it increased from 0.56 to 0.72.

Light-induced phosphorescence. A disadvantage especially of the dioxane-based scintillation liquids is their tendency to exhibit light-induced phosphorescence.[8] Exposure to daylight for 1 h induced in polyethylene vials with 10 ml scintillation liquids from a few cpm to millions of cpm, depending on the intensity of the light. Also with empty vials, counts may be registered after exposure to light. The effect is more pronounced in polyethylene vials than in glass vials, and is mainly recorded in the ^{3}H-channel of the spectrometer. The presence of water in the dioxane-based scintillation liquids increases the degree of the light-induced phosphorescence. A plot (not shown) of cpm *versus* time after illumination indicates a two-phase phenomenon with a very short phase with $t_{\frac{1}{2}}$ of about 1 min, followed by a long phase with $t_{\frac{1}{2}} = 2 - 4$ h. Direct sunlight for 1 h induced phosphorescence in DM, which had not disap-

peared after 2 days. The other dioxane-based scintillation liquids were less sensitive to light. Light-induced phosphorescence may also be seen with the other four classes of scintillation liquids, although they mainly showed short-lived scintillations which had disappeared completely within 2 h, even after exposure to direct sunlight for 1 h.

DISCUSSION

The time-dependent decrease in efficiencies (Table 2) is due to precipitation of ^{3}H RNA. It is observed to the same degree when polyethylene and glass vials are used, but it depends on the specific activity of the sample (the RNA precipitation is less pronounced at low RNA-concentrations). The phenomenon is also seen with ^{3}H ATP, but to a less degree with ^{14}C RNA and ^{32}P RNA. It is also observed when chemicals of "scintillation grade" are used. Use of these chemicals resulted in only slight changes in the efficiencies.

The 10 scintillation liquids with known composition are all based on PPO and dimethyl-POPOP (Table 1). In the case of T, TH, DM, TXDE, and TDE, it was tested that the concentrations of the primary and secondary solutes were optimal. The scintillator butyl-PBD developed by Ciba, and studied by Scales,[9] has also been used. The mixtures based on butyl-PBD show efficiencies similar to or less than the corresponding mixtures based on PPO-dimethyl-POPOP.

Three different methods for treatment of the sample have been used:

The main advantages with the direct addition method (Figs. 1 – 2 and Table 2) seem to be: 1. the methodological convenience. 2. the relative high efficiencies obtained, especially with the dioxane-based liquids and Insta-gel. The main disadvantages are the time-dependent decreases in the counting efficiencies observed with most of the scintillation liquids. These changes are due to precipitation of the labelled polar compound. An other disadvantage is the limited volume of the aqueous phase which can be used.

The NCS-procedure is especially useful for counting of large volume samples or samples containing acids, bases or salts (Tables 3 and 4). High and stable counting efficiencies are obtained if counting is performed in T, the dioxane-based liquids or diluene. The disadvantages are the time-consuming transfers of the "digested" samples, the risk of NCS-induced phosphorescence, and the rather high cost of NCS. It is worth-while to spend time with experimentation with individual samples to get optimal and stable counting efficiency (*cf.* Table 4).

The main advantages by the disc methods are: 1. counting efficiencies are stable during at least 24 h (the stability depends on the nature of the labelled compound and the type of scintillation liquid); 2. counting is performed at best in T, which is a cheap mixture; 3. the method is especially useful for counting of acid solutions; 4. the method may be used for large sample volumes, because a great amount of discs may be pooled in the vials. The main disadvantages are: 1. rather low counting efficiencies, especially for filter paper-discs (NCS-digestion of eluted discs increases the efficiency); 2. the method is rather elaborate (often the filtration step is a necessary part of the analysis).

The present investigation is concerned primarily with optimal counting of ^{3}H RNA. However, doubly labelled RNA ($^{3}H+^{14}C$ or $^{3}H+^{32}P$) may be treated in a similar way to get optimal ^{3}H counts. By variation of the gain, the ^{14}C or ^{32}P counts can easily be found (calculated).

All of the results are based on counting in glass or polyethylene vials. However, essentially the same results are obtained if the samples (treated according to any of the three methods) are counted in 1 – 4 ml scintillation liquid in small cylindrical glass-tubes, placed inside empty glass vials.

Acknowledgements. The expert technical assistance of Dorte Rosenfeldt is gratefully acknowledged.

The work was supported by *Novo's Foundation.*

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Received August 21, 1970.