SORPTION-INDUCED CHANGES IN DETECTION EFFICIENCY OF β-EMITTERS IN LIQUID SCINTILLATION SPECTROMETERS

R.TYKVA[a] and E.PODRACKÁ[b]

[a]*Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*
[b]*Institute for Clinical and Experimental Medicine, 146 22 Prague 4*

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Detailed examinations have been carried out in a liquid scintillation spectrometer on changes in detection efficiency of β-nuclides due to sorption of labelled substances to the inner surface of the vial. It has been shown that the influence of sorption on the counting conditions may assert itself already in the course of several hours after preparation of the sample. The difference between sorption effects in the case of a high-energy (^{32}P) and a low-energy (^{45}Ca) β-nuclide has been discussed on the basis of amplitude spectra. The stability of the sorption of labelled substances to the vial surface has been examined and various routes how to remove its influence on changes in detection efficiency have been studied.

The sorption of radionuclides and radionuclide-labelled substances is the object of numerous radiochemical papers. As it may be seen from the reviewed results[1,2], the experimental material does not permit an unambiguous interpretation in many respects. In tracing experiments with labelled substances, the undesirable sorption effects may lead to loss of activity during the storage, may change activity distribution in the experimental system or, last but not least, may change counting conditions. Examination of these effects and their removal thus appears as an important component of the methodical work with labelled substances. The relatively little attention paid to these problems might be ascribed to the complexity of relations concerning the sorption effects[1,2] and a great variety of samples.

The sorption of the sample in the vial of a liquid scintillation spectrometer brings about a change in the counting geometry[3,4]. The dependence between adsorption to the glass vial and molecular structure of a ^{14}C-labelled substance has been recently examined[5]. As shown in this Laboratory[6], the sorption leads to a long-termed shift of amplitude spectra in scintillation solutions on the basis of dioxane as well as toluene.

In the present paper, we have examined in detail the sorption effect of ^{32}P- or ^{45}Ca-labelled substances on counting conditions currently used in tracer experiments in the chemical and biological field. Various provisions for exclusion of the sorption effects have been discussed.

EXPERIMENTAL

Methods and Apparatus

Measurements were performed in the scintillation spectrometers Tri-Carb, Packard Instrum. Corp., Model 3375 (at 7°C) and Intertechnique, Model ABAC SL 40 (at 10°C). For the integral measurements in the Tri-Carb spectrometer, the following discrimination levels and amplifications were used: ^{3}H-channel, 110—1 000, 52%; ^{45}Ca-channel, 100—500, 20%; ^{32}P-channel, 50—1 000, 0·56%. Setting of channels in the Intertechnique spectrometer: ^{3}H, 0—450; ^{45}Ca, 0—750; ^{32}P, 0—900. The setting stability of counting channels was checked by sealed reference sources of [^{3}H]- or [^{14}C]-hexadecane which are being used for several years. The differential measurements were performed in the Intertechnique apparatus; channel width, 25 sections. Aqueous solutions of sodium dihydrogen [^{32}P]-phosphate (Swierk, Poland) and [^{45}Ca]-calcium chloride (Rotop, German Democratic Republic) were used as active samples. Unless stated otherwise, 0·1 ml of the sample was made up to the volume of 10 ml with the scintillator according to Bray[7] in a glass Packard vial. All the experimental data were corrected with respect to the disintegration halftime, always with respect to the preparation time of the corresponding sample.

RESULTS AND DISCUSSION

As it may be seen from differential measurements of ^{32}P (Figs 1*a* and 1*b*), the shift of amplitude spectra is relatively fast; consequently, the increase of counting rates

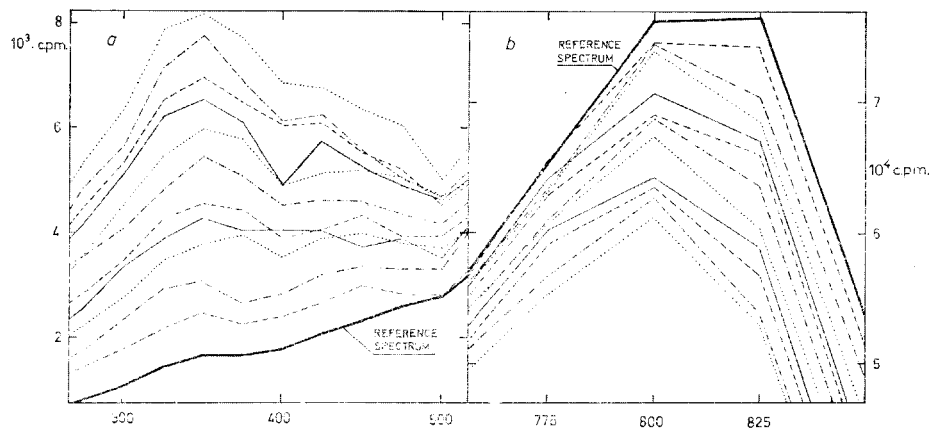

FIG. 1

Time Dependence of Changes in Two Parts of ^{32}P Amplitude Spectrum

a Part of the ^{3}H-channel; *b* ^{32}P peak. An aqueous solution of [^{32}P]-orthophosphate (0·1 ml) made up to the volume of 10 ml with the Bray scintillator, introduced into the spectrometer, and the measurement of the reference spectrum begun 1 h after the preparation; 0·4 min all measurements. Further measurements were begun in the following time intervals after the start of the reference spectrum measurement (direction, changing spectral courses), hours: 3 — — —; 6 — · —, 9 · · · ·; 12 ———; 15 — — —; 21 — · —; 27 · · · ·; 33 ———; 39 — — —; 45 — · —; 60 · · · · .

in the ^{3}H-channel and the decrease in the ^{32}P-channel assert themselves in relatively short intervals. In usual measurements, the relations mentioned thus result in a permanent decrease of the detection efficiency of ^{32}P in the ^{32}P-channel and simultaneously, to a permanent increase of the ^{32}P-detection efficiency in the ^{3}H-channel. This effect leads to a decreased detection accuracy of ^{32}P alone as well as to appreciable deviations in the case of the simultaneous detection of $^{32}P + {}^{3}H$ or other combinations of a high-energy and a low-energy β-nuclide.

In the corresponding differential measurements of ^{45}Ca, the change of the amplitude spectrum was observed in the ^{45}Ca-channel only (Fig. 2) while no statistically significant change could be encountered in the ^{3}H-channel. The whole amplitude spectrum of ^{45}Ca after the preparation of the sample is shown on Fig. 3. The time dependence of integral counting rates in the two channels at two different concentra-

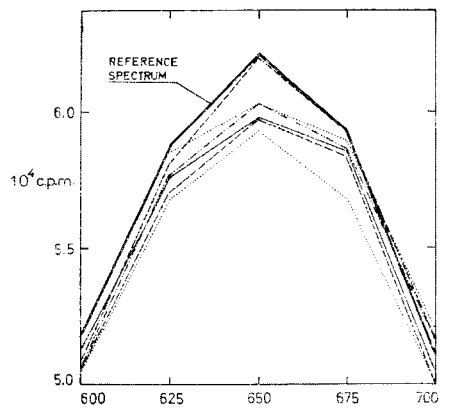

FIG. 2

Time Dependences of Changes in Peak Shapes of ^{45}Ca-Amplitude Spectrum

Aqueous solution of [^{45}Ca]-Calcium Chloride (0·1 ml) made to the volume of 10 ml with the Bray scintillator, introduced into the spectrometer, and the measurement of the reference spectrum begun 1 h after the preparation; 0·4 min all measurements. Further measurements were begun in the following time intervals after the start of the reference spectrum measurement (direction, the decreasing peak), hours: 2 ——; 4 · · · ·; 12 —·—·—; 24 ———; 48 ——; 70 · · · · ·.

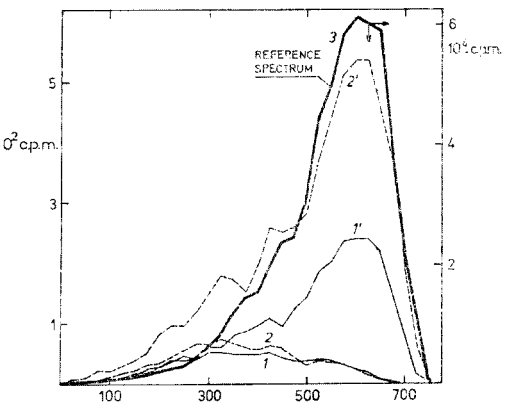

FIG. 3

Effect of the Vial Rinsing on the Course of Amplitude Spectrum and Comparison with Spectrum Measured One Hour after the Preparation of the Starting Sample

1, *2* Emptied and dried vials, measured for 10 min in each window. *1′*, *2′* vials refilled with 10 ml of the scintillator, measured for 4 min in each window: *1′* with washing, *2′* without washing. *3* Reference spectrum of the starting sample measured one hour after the preparation for 0·4 min in each window. The amplification of the Intertechnique spectrometer is different from that in Figs 1 and 2 (the discrimination levels are about 150 sections lower).

tions and at the same activity and mass of calcium chloride (Table I) exhibits a significant change in the upper counting window only, in accordance with the differential measurement. Thus in the case of ^{45}Ca, the sorption brings about a marked decrease in the detection efficiency in the ^{45}Ca-channel while the ^{3}H-channel is affected to a very small extent only. Results of the integral measurement shown in Table I also quantitatively express decrease of the ^{45}Ca-detection efficiency due to sorption of the corresponding sample.

A similar behaviour and similar conclusions to those with ^{45}Ca might be assumed in the case of other β-nuclides with a similar range of the energy spectrum (*e.g.*, in sorption of ^{14}C- or ^{35}S-labelled substances).

The counting rates in particular channels are affected by sample quenching which shifts the amplitude spectrum into the region of lower energy values[8]. In the case of scintillation solutions with quenching different from that of the used samples, the changes of the appropriate counting rates therefore differ from results obtained in the present measurements in spite of the identical channel settings. Furthermore, the course of the sorption depends on the vial and composition of the counting solution[1,2], structure and molarity of the active substance[1,5] and its specific activity[1,6]. The changes of detection efficiency due to sorption can be thus quantitatively expressed for a strictly defined experimental arrangement only.

The difference between the ^{32}P and ^{45}Ca samples is ascribed to different energy spectra of emitted electrons. In a great portion of the ^{32}P spectrum, the kinetic energy of β-particles is high enough to bring about by interaction with the vial walls the Čerenkov radiation[9], the peak of which lies in the ^{3}H-channel[10,11]. The sorption of the [^{32}P] sample thus results in the formation of a significant peak in the ^{3}H-channel and simultaneously, in decreased counting rates in the ^{32}P-channel owing to the changed sample geometry. On the other hand, the sorption of the low-energy ^{45}Ca nuclide manifests itself in decreased counting rates in the upper channel in view of the changed counting geometry and back scattering of emitted electrons from the vial. No peak, however, is formed in the ^{3}H-channel since the energy spectrum lies below the threshold energy necessary for the Čerenkov radiation[9,10,12]. The total counting rates in the ^{3}H-channel are influenced only negligibly since the increase due to a shift of higher parts of the amplitude spectrum into the ^{3}H-channel is simultaneously accompanied by a shift of a part of the spectrum from the ^{3}H-channel below the lower discrimination level into the noice region. With ^{32}P, this effect is not significant since the part of the amplitude spectrum in the ^{3}H-channel is very small.

In order to verify these conclusions and for detailed evaluation of the sorption effects, an aqueous solution of [^{45}Ca]-calcium chloride in the scintillator was kept for 5 days in a vial. The counting rates were measured in the ^{45}Ca-channel. After the preparation, the sample exhibited 554108 c.p.m. When the scintillator was poured out and the vial wiped with cellulose wool, there was obtained 1 590 $\pm$ 190 c.p.m. and, after drying at 20°C for 20 h, 682 $\pm$ 14 c.p.m.; the corresponding amplitude spectrum

TABLE I

Counting Rates in ^{3}H- and ^{45}Ca-Counting Channels at two Various Concentrations of Aqueous [^{45}Ca]-Calcium Chloride in the Scintillator

All data (in c.p.m.) are arithmetic means from three parallel samples, S.D. $< \pm 0{\cdot}5\%$ in all measurements. Tri-Carb apparatus. Line 1, ^{3}H-channel; line 2, ^{45}Ca-channel.

Aqueous solution ml	Elapse of time after the preparation of the sample, h													
	0·5	3	6	9	12	15	18	21	24	27	30	48	60	84
0·1	29 637	29 666	29 539	29 509	29 664	29 471	29 579	29 817	29 664	29 845	29 712	29 562	29 634	29 481
	98 755	98 044	97 576	97 162	97 031	96 840	96 603	96 503	96 110	96 304	95 997	95 390	95 568	94 995
1·7	49 125	48 956	48 772	48 756	48 819	48 664	48 802	49 276	48 837	49 192	48 975	48 456	48 430	48 512
	85 066	84 625	84 338	84 150	83 801	83 301	82 828	82 833	82 824	82 522	82 358	81 619	81 213	80 907

TABLE II

Acidification Effect of the Scintillation Solution on the Limitation of Sorption and on the Detection Efficiency of ^{32}P

All data are arithmetic means from three parallel samples, S.D. $< \pm 0{\cdot}8\%$ in all measurements. Tri-Carb apparatus. Line 1, ^{3}H-channel; line 2, ^{32}P-channel.

Sample	Elapse of time after the preparation of the sample, h					
	0,5	1	5	10	24	90
Without acidification c.p.m. (%)	3 147 (100·0)	3 136 (99·7)	3 446 (109·5)	3 794 (120·6)	4 670 (138·4)	5 982 (190·1)
	29 937 (100·0)	29 739 (99·3)	29 039 (97·0)	28 470 (95·1)	26 704 (89·2)	23 291 (77·8)
After acidification c.p.m. (%)	9 550 (100·0)	9 583 (100·0)	9 636 (100·9)	9 574 (100·3)	9 629 (100·8)	9 827 (102·9)
	5 940 (100·0)	5 932 (99·9)	5 979 (100·7)	6 002 (101·0)	5 903 (99·4)	5 677 (95·6)

is shown on Fig. 3. The vial was then three times rinsed with a mixture of dioxane and methanol (3 : 1, v/v) and dried to show a zero increase with respect to the background both in the ^{45}Ca- and ^{3}H-channel. After the addition of the scintillator (10 ml), there was recorded 2389 ± 24 c.p.m.; the character of the amplitude spectrum (Fig. 3) corresponded to the detection of ^{45}Ca after the preparation of the starting sample except for a shif into the region of lower energy values. With the use of three vials and identical procedures without rinsing with the dioxane–methanol mixture, the average value was 982 ± 18 c.p.m. (after a long-termed drying) and 5425 ± 27 c.p.m. (after the addition of the scintillator). Results of the corresponding differential measurements are shown on Fig. 3.

The values recorded prior to the rinsing process are ascribed to the residual scintillating substances on the inner surface of the vial, first along with traces of solvents[6] and then (after evaporation of solvents) to the mixture of 2,5-diphenyloxazole (PPO) and 1,4-bis(5-phenyl-2-oxazolyl)benzene (POPOP) from the Bray scintillator. When these substances are thoroughly washed out, there is recorded only the background since the energy of β-particles emitted by ^{45}Ca is not sufficient to evoke the Čerenkov radiation. When the washed vial is refilled with 10 ml of the scintillator, the record of counting rates is about half of that obtained with unrinsed vials. From this comparison, a relatively strong sorption of a portion of the sample to the vial may be assumed. As it may be seen from comparison of the corresponding differential spectra (Fig. 3), the rinsed vial exhibits a relatively lesser proportion of higher amplitudes. From this observation there may be assumed that only a part of the radionuclide is sorbed directly to the inner surface of the vial, otherwise in accordance with resistance of the remaining activity to rinsing. In comparison with the spectrum of the freshly prepared sample (Fig. 3), the vials containing the sorbed sample exhibit (due to a changed counting geometry) a relative increase of the amplitude spectrum in the lower energy region irrespective of the rinsing procedure.

Concerning the potential exclusion of the sorption effect by experimental procedures or empirical corrections, the following observations could be mentioned. The required properties of the scintillator and the vial, the character and composition of the sample, and the age of the solution considerably limit the prospect how to affect the sorption[1,2]. Treatment of the vial surface by passivation with a solution of an inactive substance did not meet with success in this Laboratory owing to a long-termed equilibration between the solution and the surface[6,13] and the isotope exchange between the passivating layer and the active sample. Of a limited applicability is the addition of a greater amount of a non-active carrier substance[3] which would lower the sorption of the actual active substance. The channel ratio correction method[4] was proposed for single-labelled samples. The time-dependent empirical corrections cannot be used because of the very different vial surfaces[14] and the dependence of sorption on the specific activity of the sample to be measured[1,6,13]. The sorption effect may be successfully excluded for a certain period of time by acidification[1,13]

as shown by long-termed storage of samples of water prior to analyses of trace elements[15,16] and radionuclides[17]. When a usual scintillation solution (1·8 ml of aqueous [^{32}P]-orthophosphate + 8·2 ml of the Bray scintillator) is compared with the same solution acidified with 0·5 ml of the analytical grade nitric acid (Table II) it may be seen that (at least up to 24 hours after the preparation of the sample) the sorption effect on the instability of the corresponding counting rates may be completely excluded but that the detection efficiency strongly decreases due to the quenching-induced shift of amplitude spectra[8]. Consequently, the acidification of the scintillation solution may be used with samples of a higher activity only. The values of the non-acidified sample also indicate a quantitative decrease in the detection efficiency of ^{32}P due to sorption of the present sample. Generally taken, the influence of sorption on the decreased detection efficiency of β-emitters in liquid scintillation counting can be advantageously excluded by the use of emulsions instead of solutions[18].

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