Notes

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Hisashi Miyazaki, Yasuo Minaki, and Yoshio Yoshimura : An Improved Method for Liquid Scintillation Counting of Biological Materials.

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For the determination of radioactivity by liquid scintillation, the samples to be counted must be dissolved in the solvent systems that generally consist of xylene, toluene or dioxane. Since most biological materials such as animal tissue or protein are directly insoluble in those organic solvent systems, the solubilization of these materials is routinely required in the biochemical and pharmacological tracer experiments using soft-beta-emitters. In the case of preparing a large number of counting samples of biological materials, it is most popular to solubilize them by Hyamine hydroxide.[1,2] This method, however, needs the relatively expensive reagent of Hyamine hydroxide and sometimes phase separation of water from organic solvent occurs at low temperature especially for the samples containing much amount of water, *e.g.* blood sample and a large volume of tissue sample. For the aqueous biological samples ethanol[3] or cellosolve[4] has been widely used to obtain one phase system as blending agents in the scintillator solution systems as well as Hyamine hydroxide[5] or its salts.[6] Another system of ethanolic potassium hydroxyde solution for the solubilizer reported by Herberg[1] requires 24 hrs. to solubilize the sample and furthermore, it was found difficult to obtain homogeneous system.

This paper describes an improved method with a simplest procedure that was developed from the ethanolic potassium hydroxide system and has been usually employed in our laboratory for the liquid scintillation counting of the biological materials. The method involves the digestion of the sample by caustic alkali and solubilization in Bray's dioxane scintillator solution[7] by the addition of ethyleneglycol after decoloring with hydrogen peroxide. Since all preparation procedure can be performed in the counting vial, this method is most suitable for determining the radioactivity of a large number of samples. Of greater importance is the fact that considerably large quantity of biological materials (upto 200 mg. of animal tissues) can be easily dissolved and counted with a satisfactory efficiency. No separation of the liquid phases is recognized at low temperature. Therefore, an accurate determination of the radioactivity is possible for the samples with low activity by increasing the amount of the material. A similar method has been recently published by Petroff *et al.*[8] for upto 40 mg. of tissues employing methanolic 2*N* potassium hydroxide solution and toluene scintillator solution containing ethyleneglycol-monobutyl ether.

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1) R. J. Herberg : Anal. Chem., **32**, 42 (1960).
2) W. O. Brown, H. G. Badman : Biochem. J., **78**, 571 (1961).
3) G. T. Okita, J. Spratt, G. V. LeRoy : Nucleonics, **14**, 76 (1956).
4) G. A. Bruno, J. E. Christian : Anal. Chem., **33**, 1216 (1961).
5) H. Takahashi, T. Hattori, B. Maruo : *Ibid.*, **35**, 1980 (1963).
6) R. C. Meade, R. A. Stiglitz : Intern. J. Appl. Radiation Isotopes, **13**, 11 (1962).
7) G. A. Bray : Anal. Biochem., **1**, 279 (1960).
8) L. P. Petroff, H. H. Patt, P. P. Nair : Intern. J. Appl. Radiation Isotopes, **16**, 599 (1965).

Preparation Procedures

Digestion of the Materials : The digestion of the materials is carried out in the tinfoil-lined screw-cap vial with appropriate caustic alkali in an incubator or in a shallow water bath at 80~100° until the solvents in the vial are almost completely evaporated and brownish mucous residue is remained. Dried proteins and fresh animal tissues are digested by 0.5*M* potassium hydroxide ethanolic solution, of which 2 ml. portion is sufficient to digest 50~200 mg. of animal tissue, 0.1 ml. of blood and 30~80 mg. of protein. For the protein solution potassium hydroxide is added to make the concentration 0.5*M* per the material within above mentioned ranges. Over these ranges the solubilization is difficult in the present system and below those the digesting agent should be decreased to make 0.4 ml. of the agent per 10 mg. tissue or protein. Other caustic alkali, sodium hydroxide can be used in a similar way though the precipitate of sodium chloride is yielded in the final counting sample. The digestion takes about 10 min. in ethanolic solvent and about 30 min. in aqueous solvent.

Counting Sample : The digested residue is dissolved in 0.5 ml. of water (a turbid solution is occasionally obtained) and the solution was decolored with 1~5 drops of 30% hydrogen peroxide with sufficient time (over 15 min.). Then 3 ml. ethyleneglycole is added to the decolored solution. Mixture of the resultant clear solution and 10 ml. of dioxane scintillator solution[7] containing 6% (wt./vol.) naphthalene, 0.4% (wt./vol.) 2,5-diphenyloxazole, 0.02% (wt./vol.) 1,4-bis[2-(5-phenyloxazolyl)]benzene, 5% (vol./vol.) methanol and 1% (vol./vol.) ethyleneglycol, is acidified with 3 drops of conc. hydrochloric acid (quantity should be decreased in proportion to starting alkali) and provided for counting. Small amount of precipitate of potassium chloride at the bottom of the vial is rarely observed at low temperature although the counting efficiency is not disturbed at all.

The results obtained from our biological tracer experiments are given in Table I, where counting efficiencies, sources of biological sample and the nuclides administered as various labelled compounds to the animals are listed. The counting efficiencies were determined by channel ratio method.[9,10] TEN Liquid Scintillation Counter MODEL-GSL-161 (Kobe Kogyo Corp.) was used for counting.

Table I. Counting Efficiencies of Various Biological Samples

Biological samples	Quantities	Counting efficiencies (%)		Added 30% H_2O_2 (drops)
		^{14}C	^{35}S	
Liver	50 mg.	38	42	1
	100 mg.	37	34	1
	200 mg.	36	32	2
Kidney	50 mg.	35	37	1
	100 mg.	35	33	1
Small intestine	60 mg.	37	46	1
	150 mg.	39	46	1
Spleen	50 mg.	35	43	2
Blood	0.1 ml.	29	36	5
Protein[a]	40 mg.	37	—	3
	80 mg.	29	—	3

All samples were obtained from mice.
a) Liver protein residue after extractions by the procedure of Tyner *et al.*[11]

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9) L. A. Baillie : Intern. J. Appl. Radiation Isotopes, **8**, 1 (1960).
10) T. Iwakura, Y. Kashida, H. Morisaki : Radioisotopes, **14**, 132 (1965).
11) E. P. Tyner, C. Heidelberger, G. A. LePage : Cancer Research, **13**, 186 (1953).