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DIRECT DETERMINATION OF ^{32}P OR ^{36}Cl IN TISSUE SOLUBILIZER SOLUTIONS BY CHERENKOV-SCINTILLATION COUNTING

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Phosphorus-32 and chlorine-36 radioactivity was measured directly in commercially available tissue solubilizers using a liquid scintillation counter. Various wavelength-shifting compounds: β-naphthol, 4-methylumbelliferone, 7-amino-1,3-naphtalenedisulfonic acid, 2-hydroxy-3,6-naphthalenedisulfonic acid, anthranilic acid and salicylic acid were investigated to assess their suitability for the improvement of counting efficiency. Salicylic acid was selected which is fairly stable in alkaline solutions of tissue solubilizers and remarkably improves counting efficiency up to 90% for both nuclides. 0.1 g of soft tissues or blood can be solubilized with 1 cm^3 of tissue solubilizer containing 2 g/dm^3 of salicylic acid directly in 6 cm^3 scintillation minivials. The sample channels ratio method for colour quench correction was found to be satisfactory.

Introduction

Measurements of β-activity of such samples as biological tissues, proteins, nucleic acids or other macromolecules by means of liquid scintillation technique are performed after solubilization with commercial mixtures (Protosol, Lumasolve, NCS, BTS–450 and others) which constitute of xylene or toluene solutions of quaternary ammonium bases. However, the strongly alkaline components of these mixtures can react with the scintillator producing chemiluminescence thus distorting results of activity counting.[1,2]

For activity counting of hard β-emitters ($E_{max} > 0.5$ MeV) an alternative detection method is available, namely the monitoring of Cherenkov radiation which is generated in solutions by β-particles of velocity higher than the light velocity in a given medium.[3,4] The Cherenkov radiation may be easily measured in standard automatic scintillation counters. The threshold value of energy of β-particles above which emission of Cherenkov photons occurs depends on the refraction index of the medium, e. g. 0.263 MeV for water (n = 1.33) and 0.174 MeV for toluene (n=1.50). About 46% of β-particles emitted by ^{36}Cl and about 86% of those emitted by ^{32}P have

energy higher than the threshold value for water (0.263 MeV).[3] A further increase in the counting efficiency can be achieved by application of solvents of higher refraction index or by admixture of so-called wavelength-shifting compounds, since the spectrum of emitted photons corresponds mainly to the ultraviolet region, for which the sensitivity of photomultipliers is already low. Numerous compounds have been described as wavelength shifters to the range of highest photocathode sensitivity, i. e. about 400 nm.[4-9] Although the admixture of a wavelength-shifting compound results in a considerable increase of the counting efficiency, both Cherenkov photons and scintillation photons are emitted in the solution simultaneously and the system turns into a hybrid Cherenkov scintillation medium.[10] As a result the chemical quenching occurs in the solution, like in scintillation solutions, and quenching correction is necessary.

The effects of wavelength shifters on the counting efficiency of some nuclides have been described for aqueous solutions or, lately, Triton X–100.[8,9]

This paper was aimed at examination of effects of chosen wavelength shifters on the counting efficiency of ^{32}P and ^{36}Cl in solubilized biological samples.

Materials and method

Reagents

All chemicals applied in these experiments were commercial reagent grade and were used without further purification. The Beckman BTS–450 (Beckman Instruments Inc.), Protosol (New England Nuclear) and Lumasolve (Lumac) were chosen as common commercial tissue solubilizers.* Freshly prepared solutions of solubilizer with wavelength-shifting compounds were always used.

Sample preparation and activity measurement

0.1 g of soft tissue or 0.1 cm^3 of blood was placed in the MILLI-6 vials[11] (Lumac). To this sample 0.05 cm^3 of $Na^{36}Cl$ or $Na_2H^{32}PO_4$ solution, 1 cm^3 of solubilizer containing wavelength shifter and 0.1 cm^3 of 30% hydrogen peroxide solution were added. Then the vials were closed with teflon plugs and placed in heater for 2 hrs at 55 °C. Thereafter, solubilization samples were cooled to room temperature, placed into standard scintillation vials and the activities were determined in the SL–30

*Certain commercial materials are identified in this paper in order to adequately specify the experimental procedure. In no case such identification implies recommendation nor does it imply that the material identified is necessarily the best available for this purpose.

liquid scintillation counter (Intertechnique). The sample channels ratio method was applied for standardization.

Eventually, salicylic acid (SA) was chosen as the best wavelength shifter and was added to all solutions in concentration of 2 g/dm^3. The mixture (5 cm^3) of isopropanol-toluene (1:1 v/v) with salicylic acid was added before counting for dilution of solubilized tissue in order to suppress the remaining yellow colour.

Results and discussion

Effects of various wavelength-shifting compounds, such as 7-amino-1,3-naphthalenedisulfonic acid, 2-hydroxy-3,6-napthalenedisulfonic acid, 4-methylumbelliferone, anthranilic acid, β-naphthol and salicylic acid (SA), on the counting efficiency of ^{32}P and ^{36}Cl in a solubilizer solution were studied (Table 1). Salicylic acid, which

Table 1
The influence of some wavelength shifters on the detection efficiency of ^{32}P and ^{36}Cl nuclides in the Beckman Tissue Solubilizer BTS–450

Compound	Concentration, g/dm^3	Detection efficiency*		Remarks
		^{32}P	^{36}Cl	
BTS–450 pure only	–	0.666	0.201	–
7-amino-1,3-naphthalene-disulfonic acid	2	0.710	0.296	moderately stable**
2-hydroxy-3,6-naphthalenedisulfonic acid	0.5	0.910	0.697	
	1.0	0.924	0.735	unstable***
	2.0	0.935	0.772	
4-methylumbelliferone	2.0	0.925	0.684	unstable***
anthranilic acid	0.5	0.869	0.657	moderately stable**
	1.0	0.880	0.758	
	2.0	0.896	0.763	
β-naphtol	0.5	0.895	0.807	moderately stable**
	1.0	0.932	0.819	
	2.0	0.964	0.892	
salicylic acid	0.5	0.932	0.850	
	1.0	0.963	0.912	stable
	2.0	0.990	0.933	

*Related to the detection efficiency determined by liquid scintillation countig in Aquasol (10 cm^3) solution.
**Yellow colour after a few days.
***Yellow colour after 24 hrs.

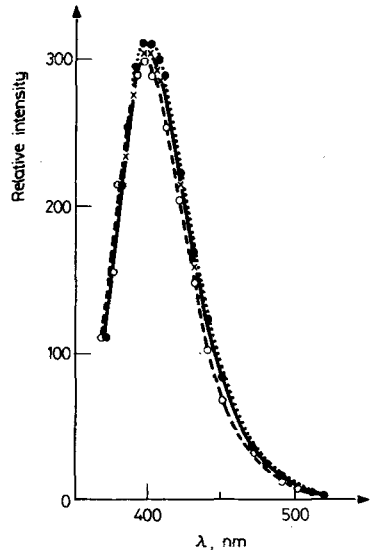

Fig. 1. The fluorescent spectra of the salicylic acid solutions (2 g/dm^3) in three commercial solubilizers. curves: (x) BTS–450, (o)-Lumasolve, (●)-Protosol

shows strong fluorescence[12] in alkaline solutions with λ_{max} = 395 nm (Fig. 1) and considerably (up to above 90%) enhances the counting efficiency for both nuclides in solutions of standard solubilizers (Table 2), was found to be the best wavelength shifter.

Solutions of solubilized tissues and blood show a strong yellow colour which cannot be removed by bleaching with hydrogen peroxide. In order to decrease the colour intensity of these solutions, the samples were added with 1–5 cm^3 of an isopropanol-toluene mixture. It has been checked (Table 2) that addition of this mixture does not diminish the counting efficiency of either of the nuclides. The results are shown in Table 3. A considerable enhancement of the counting efficiency was observed already after addition of 1 cm^3 of the mixture but even maximal amount of the mixture used (5 cm^3) did not eliminate the yellowish colour completely thus resulting in the necessity of proper standardization of the samples. Previously[13] we have found that the automatic external standardization method, based on activity measurements of a ^{137}Cs source in two channels of an SL–30 counter, cannot be used for Cherenkov radiation. Instead, the measurements of the activity ratio of the sample in two selected channels appeared suitable for our determinations. Two channels: 0–800 (A) and 0–450 (B) were selected on the basis of pulse height spectra presented in Fig. 2. An exemplary dependence of the counting efficiency E for ^{36}Cl on the sample channels ratio R in two solubilizers is shown in Fig. 3. It is noteworthy that the experimental points on the E = f(R) plot conform with the same

Table 2
The lack of influence of the mixture (Mix)* isopropanol-toluene on the detection efficiency in the solubilizer – SA solutions**

Solution	Detection efficiency	
	^{32}P	^{36}Cl
BTS–450 1 cm^3	0.990	0.935
BTS–450 1 cm^3 + Mix 2 cm^3	0.980	0.930
BTS–450 1 cm^3 + Mix 4 cm^3	0.985	0.925
Lumasolve 1 cm^3	0.985	0.925
Lumasolve 1 cm^3 + Mix 2 cm^3	0.980	0.930
Lumasolve 1 cm^3 + Mix 4 cm^3	0.985	0.930
Protosol 1 cm^3	0.990	0.930
Protosol 1 cm^3 + Mix 2 cm^3	0.985	0.935
Protosol 1 cm^3 + Mix 4 cm^3	0.990	0.935

*The mixture (Mix) isopropanol-toluene (1:1 v/v) containing 2 g SA/dm^3.
**Solutions containing 2 g SA/dm^3.

Table 3
The influence of the mixture (Mix)* isopropanol-toluene on the ^{36}Cl detection efficiency in the solubilized tissues

Solution	Detection efficiency		
	Kidney	Liver	Blood
Protosol** 1 cm^3	0.480	0.480	0.050
Protosol** 1 cm^3 + Mix 1 cm^3	0.700	0.675	0.130
Protosol** 1 cm^3 + Mix 2 cm^3	0.765	0.750	0.190
Protosol** 1 cm^3 + Mix 3 cm^3	0.805	0.785	0.245
Protosol** 1 cm^3 + Mix 5 cm^3	0.818	0.815	0.345

*Mix containing 2 g SA/dm^3.
**Protosol containing 2 g SA/dm^3.

calibration curve for both solubilizers and for their solutions diluted with the isopropanol – toluene mixture.

Table 4 presents the recovery of activities introduced into various tissues. The recovery values are close to the theoretical ones and demonstrate the possibility of direct counting of ^{32}P and ^{36}Cl in solubilized tissues.

The high counting efficiencies for both ^{32}P and ^{36}Cl in the solubilizer–SA solutions, above 90% of that achieved in the L. S. C. system, seemed to be in excess of these expected for a pure Cherenkov system. Since the solubilizer – SA solutions

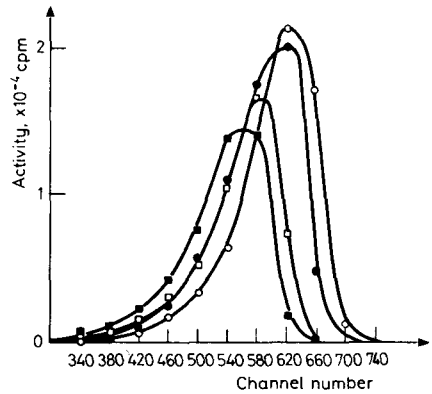

Fig. 2. Pulse height spectra of ^{32}P and ^{36}Cl in the salicylic acid (2 g/dm^3) solubilizer solutions curves: (o)-^{32}P and (□)-^{36}Cl in Protosol, (●)-^{32}P and (■)-^{36}Cl in Lumasolve

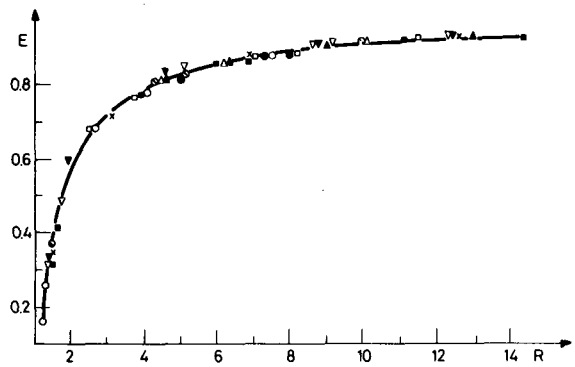

Fig. 3. The relation between counting efficiency E of ^{36}Cl and the sample channels ratio R in two commercial solubilizers (2 g SA/dm^3) solutions: (●)-Protosol 1 cm^3 (■)-Protosol 1 cm^3 + isopropanol-toluene mixture (Mix) 1 cm^3, (▼)-Protosol 1 cm^3 + Mix 2 cm^3, (▲)-Protosol 1 cm^3 + Mix 3 cm^3, (x)-Protosol 1 cm^3 + Mix 5 cm^3; (o)-Lumasolve 1 cm^3, (□)-Lumasolve 1 cm^3 + Mix 1 cm^3, (▽)-Lumasolve 1 cm^3 + Mix 2 cm^3, (△)-Lumasolve 1 cm^3 + Mix 3 cm^3, (○)-Lumasolve 1 cm^3+Mix 5 cm^3

contain substituted aromatic groups this system was checked with ^{14}C, the β-radiation of which is too weak to cause Cherenkov photons; a rather high ^{14}C counting efficiency of about 69% was obtained (Table 5). Thus it seemed probable that for hard β-emitters this system can generate both Cherenkov and liquid scintillation photons and the system turns into hybrid Cherenkov scintillation medium.[10] To prove further this suggestion we determined the effect of the addition (0.1 cm^3) of carbon tetrachloride, a strong chemical quencher, on the counting efficiency (Table

Table 4
The recovery of ^{32}P and ^{36}Cl activities in labelled tissue samples

Solution	Recovery, %					
	Kidney		Liver		Blood	
	^{32}P	^{36}Cl	^{32}P	^{36}Cl	^{32}P	^{36}Cl
Protosol* 1 cm^3	98	105	103	102	91	91
Protosol* 1 cm^3 + Mix** 1 cm^3	104	99	100	98	110	93
Protosol* 1 cm^3 + Mix** 2 cm^3	105	102	100	101	96	110
Protosol* 1 cm^3 + Mix** 5 cm^3	103	99	103	99	94	103
Lumasolve* 1 cm^3	94	101	94	104	107	107
Lumasolve* 1 cm^3 + Mix** 1 cm^3	96	102	99	103	106	101
Lumasolve* 1 cm^3 + Mix** 2 cm^3	98	102	98	103	106	105
Lumasolve* 1 cm^3 + Mix** 5 cm^3	103	100	103	99	94	95

*Protosol and Lumasolve containing 2 g SA/dm^3.
**Mix: isopropanol-toluene (1:1 v/v) mixture contining 2 SA/dm^3.

Table 5
The influence of carbon tetrachloride on the counting efficiencies in the solubilizer-salicylic acid (SA) solutions

Solution	^{14}C E^*_{max} = 0.154	^{36}Cl E^*_{max} = 0.714	^{32}P E^*_{max} = 1.71
Protosol	0	0.20	0.66
Protosol + 0.1 cm^3 CCl_4	0	0.10	0.49
Protosol (2 g SA/dm^3)	0.69	0.94	0.98
Protosol (2 g SA/dm^3)+0.1 cm^3 CCl_4	0	0.09	0.47

*E_{max} in MeV.

5). It is seen that carbon tetrachloride suppresses the liquid scintillation process completely (result for ^{14}C). Counting efficiencies for ^{32}P and ^{36}Cl, although remarkably diminished, did not vanish completely because the pure Cherenkov counting is not susceptible to chemical quenching.[10] Surprisingly, it was found that also in pure commercial solubilizer the counting efficiencies for ^{32}P and ^{36}Cl are sensitive to chemical quenching; e. g. in this case deviation from pure Cherenkov system are probably caused by some unknown additives.

It is evident from the obtained results that the above method is convenient for activity measurements of biological samples labelled with ^{32}P, ^{36}Cl or other β-emitting nuclides with E_{max} higher than the threshold energy for generation of Cherenkov

radiation in a solubilizer solution. The advantages of the proposed method are: great speed and simplicity of sample preparation, small amount of materials required, low price and good detection efficiency for high energy β-emitters.

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