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An Investigation of Various Wavelength-shifting Compounds for Improving Counting Efficiency when ^{32}P-Čerenkov Radiation is Measured in Aqueous Samples

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Various water-soluble wavelength-shifting compounds were investigated to assess their suitability for the improvement of counting efficiency when Čerenkov radiation from phosphorous-32 is measured in a liquid scintillation counter. Of these compounds esculin, β-methyl-umbelliferon and sodium salicylate led to the greatest improvement in counting efficiency. Especially esculin and β-methyl-umbelliferon are fairly stable under a variety of experimental conditions and improve counting efficiencies by a factor of about 1.3 and 1.2, respectively.

The use of ethanol as a water-miscible solvent combined with wavelength shifters soluble in both solvents does not improve counting efficiency.

1. Introduction

ČERENKOV radiation is emitted when a charged particle moves through a transparent dielectric medium (e.g. water) with a velocity exceeding that of light in the medium. The emitted light is directional and cone-shaped with a half-angle θ.[1,2] Cosine θ is defined as follows:

$$\cos\theta = \frac{1}{\beta n} \qquad (1)$$

where

β = velocity v of particle in the medium/speed of light in the medium, and

n = refractive index.

This means that for any medium there is a threshold velocity corresponding to a threshold energy. The threshold energy, E_{min}, for Čerenkov radiation can be computed from the relationship[3]

$$\beta n = 1 \qquad (2)$$

where E is related to β by the equation

$$\beta = \left\{1 - \left[\frac{1}{E(\text{keV})/511 + 1}\right]^2\right\}^{1/2} \qquad (3)$$

In water, which has a refractive index of 1.332, the minimum electron energy for Čerenkov radiation is 265 keV.

If it is assumed that the medium is nondispersive, a continuous spectrum of radiation is emitted, the greatest number of photons being produced in the ultraviolet; however, the spectrum extends into the visible but is negligible in the infrared[4] (see Fig. 1).

Liquid scintillation counters have been increasingly used to measure Čerenkov radiation of some β-particle emitting nuclides, e.g. ^{32}P.[5–14] The main advantage of this detection method over liquid scintillation counting is that no chemical quenching problems arise. Because about 85% of the emitted particles in the β-spectrum of ^{32}P have energies greater than 0.265 MeV, the highest possible detection efficiency in water is about 85%. However, due to the nature of the Čerenkov radiation and because of instrumental limitations, detection efficiency is generally much lower. Values of 19–27% have been reported.[5,6,10,11]

Detection efficiency can be affected by:

(a) the basic material of the counting vial e.g. polyethylene vials permit greater counting efficiency[5,6,15] than glass vials;[5,6,15]

(b) the liquid volume of the counting vial.[4,5,10,14,15]

When high efficiency photocathodes are used the detection efficiency can become as high as 43%[15] or 47%.[8] Detection efficiency can also be improved by the use of solvents with a higher refractive index and lower density; however, improvement is rather slight in the case of ^{32}P.[6]

Since a large proportion of the Čerenkov radiation occurs in the u.v. and u.v. light is easily absorbed by the solvent, the counting vial or the photomultiplier envelope, it would seem appropriate to use certain substances that have the property of absorbing short-wavelength light and re-emitting it isotropically in the

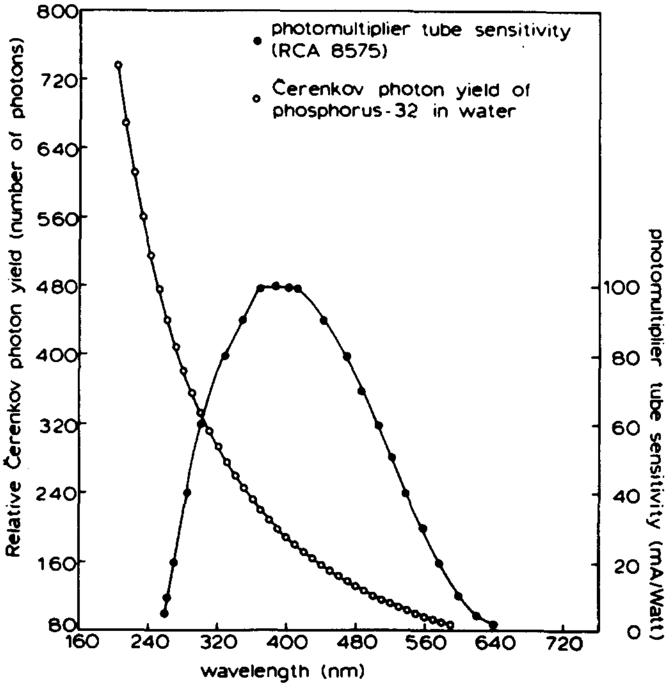

FIG. 1. Comparison of Čerenkov photon yield and spectral response of a typical photomultiplier tube used in a commercial liquid scintillation counter.

visible region. Thus the directional effect of the Čerenkov light and the effect of u.v. light absorption are reduced.[15,16] Wavelength shifting also decreases the mismatch between Čerenkov photon distribution and the spectral response of the photomultipliers normally used in liquid scintillation counters (see Fig. 1).

Although a significant increase in the counting efficiency can be obtained when one of these wavelength-shifting compounds is used, it should be remembered that in such a case the process is no longer a purely Čerenkov one. Thus, chemical quenching becomes possible.

Many investigators have already studied the characteristics and applications of different wavelength-shifting compounds.[3,6,14–20] From the different water soluble compounds tested best results were reported for 2-amino-6,8-naphtalene disulfonic acid, disodium salt,[18] 2-naphtylamine-6,8-disulfonic acid, sodium potassium salt,[6,21] 8-amino-1,3 naphtalene-disulfonic acid (ANDA)[16,17] and β-methyl umbelliferon.[16,19] Ross[16] has studied 13 different compounds and has found that at a concentration of about 100–200 mg/l β-methyl umbelliferon was fairly stable and relatively independent of pH.

In spite of the studies mentioned we could not get a clear picture of the parameter of counting when wavelength-shifters were used. So we undertook the present study with the intention of finding cheap, stable, efficient and commercially available compounds which are soluble in water and/or in solvents miscible with water. Wavelength shifters were selected on the basis of their absorption and fluorescence spectra.

2. Materials and Methods

All measurements were performed with a Packard 2425 liquid scintillation counter provided with RCA 8575 photomultiplier tubes. Counting was terminated when 50,000 counts were registered in the counting channel. Samples were counted at 16°C in glass or polyethylene vials.

All standard samples were prepared by pipetting into the counting vials an aqueous solution of $Na_2H^{32}PO_4$ of known absolute activity. The volumes pipetted into the counting vials were checked by weighing with a Mettler $H_{20}T$ analytical balance.

The ^{32}P source of known activity was bought from the Laboratoire Primaire de Métrologie des Rayonnements Ionisants, Gif-sur-Yvette, France. This ^{32}P source was calibrated with an uncertainty of 0.4%. Generally, the counting vials contained 10 or 12 ml of distilled water.

All wavelength shifters tested were commercially available and used without further purification.

3. Results

Table 1 shows our results relating to the improvement of counting efficiency with a number of wavelength shifters dissolved in distilled water. Optimum counting efficiency was obtained with β-methyl umbelliferon, esculin, quinine, thymine, sodium dihydrobenzoate and sodium salicylate.

Quinine and quinine sulfate can only be used in the pH range 0–3, which limits the applicability of these compounds.

Table 1. Effect of different wavelength shifters, dissolved in distilled water, on the counting of Čerenkov radiation from ^{32}P. Polyethylene counting vials; vial contents 10 ml; amplifier gain 30%; discriminator settings 20–1000

Compound	Used concentration in g/l	Counting efficiency (%)	Remarks
Water in glass vial		51.0	
Water is quartz vial		53.0	
Water in polyethylene vial		55.5	
Beta methyl umbelliferon	0.2	67.0	
Esculin	0.4	73.0	
Quinine sulfate	1.0	68.4	Can only be used from pH 0-3. Under these conditions rather stable
Quinine	1.0	66.2	
	10.0	76.0	
	25.0	77.0	
Thymine	0.5	65.9	
Alloxazin	0.1	55.1	
Pyrimidine-HCl	5.0	56.4	
Chinolinic acid	0.5	53.3	
Sodium dihydrobenzoate	1.0	66.3	
Pentobarbitol	1.0	57.3	
Phenobarbitol	1.0	57.0	
Lumichrome	0.001	57.2	
Sodium salicylate	0.5	66.8	
	1.0	68.0	
	100	76.0	Refractive index is increased
	1000	85.0	

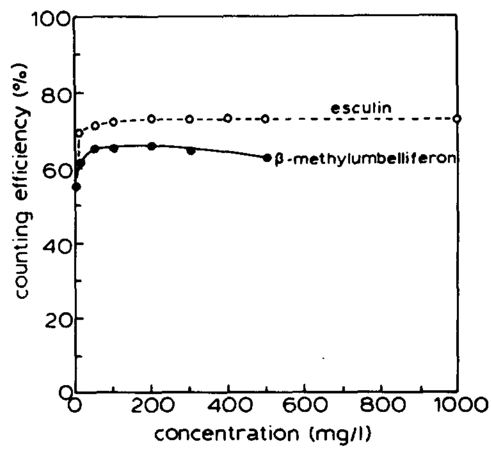

Fig. 2. Counting efficiency as a function of the concentration of wavelength shifters esculin and β-methyl umbelliferon. Polyethylene vials, content 10 ml; $Na_2H^{32}PO_4$ in distilled water at pH 7.0; amplifier gain 30%.

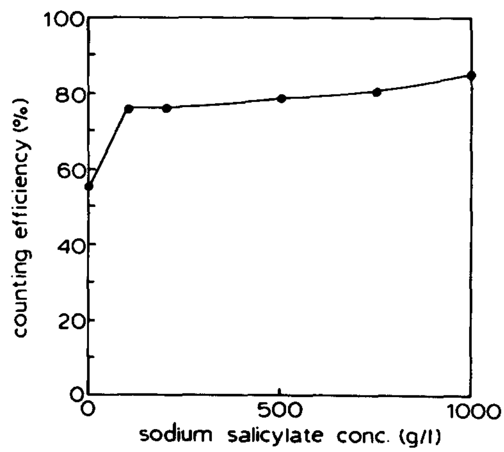

Fig. 3. Counting efficiency as a function of the concentration of the wavelength shifter sodium salicylate. Counting conditions as described in the caption to Fig. 2.

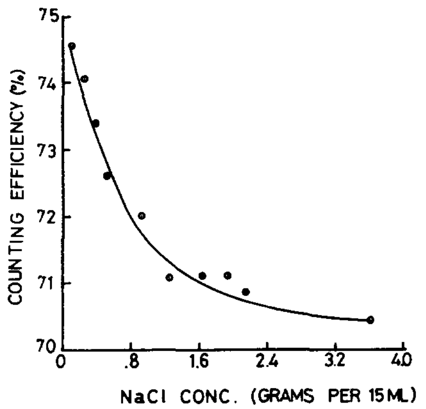

FIG. 4. The effect of salt on the function of the wavelength shifter sodium salicylate. Sodium salicylate concentration 200 g/l. Other counting conditions as described in the caption to Fig. 2.

Because the greatest counting efficiencies were obtained with esculin, β-methyl umbelliferon and sodium salicylate, these compounds were investigated further.

Figures 2 and 3 show the influence of compound concentrations on the counting efficiencies. Optimum concentration for β-methyl umbelliferon ranged from 100–300 mg/l, as has also been reported by Ross.[16] If higher concentrations are used, long term stability is affected and counting efficiency decreases, probably due to quenching of the Čerenkov light. This is not the case with esculin. The slow increase in counting efficiency with higher sodium salicylate concentrations, shown in Fig. 3, is most probably correlated with the increase in the refractive index.

After salt (NaCl) was added to the counting vials a slight increase in counting efficiency was observed in

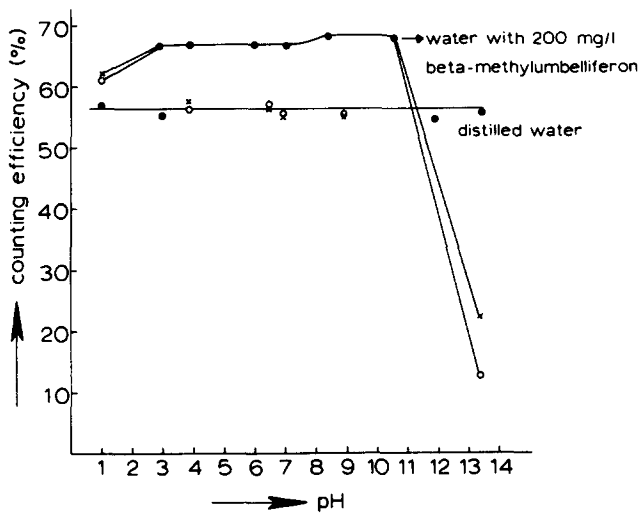

FIG. 5. The effect of pH on the function of β-methyl umbelliferon as wavelength shifter. (×) measured immediately after preparation of the samples; (O) measured upon two weeks storage of the samples. 200 mg/l β-methyl umbelliferon; other counting conditions as described in the caption to Fig. 2. For comparison the same measurements in distilled water only are given in this figure as well.

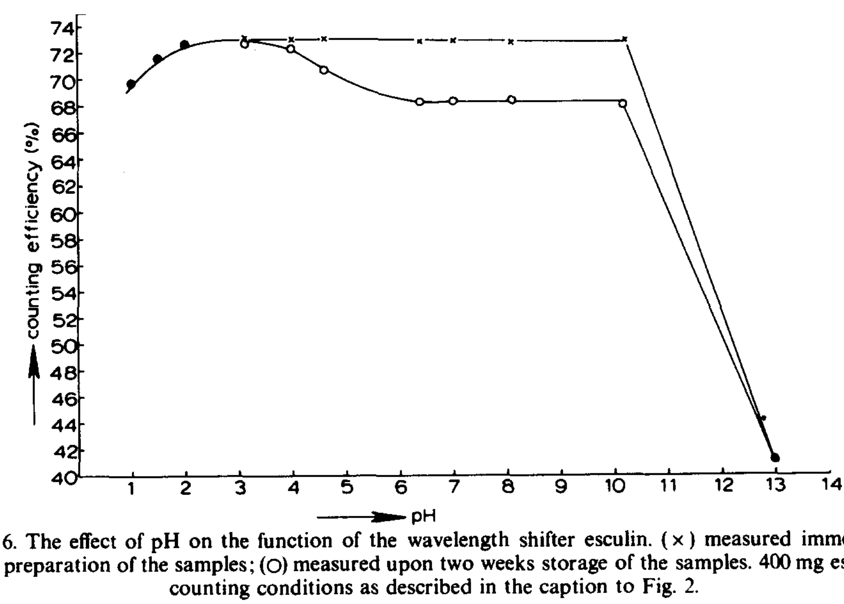

FIG. 6. The effect of pH on the function of the wavelength shifter esculin. (×) measured immediately after preparation of the samples; (O) measured upon two weeks storage of the samples. 400 mg esculin/l; counting conditions as described in the caption to Fig. 2.

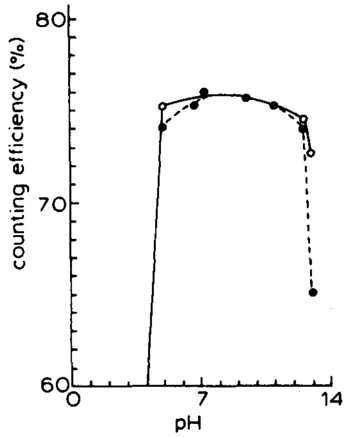

FIG. 7. The effect of pH on the function of the wavelength shifter sodium salicylate. (○) measured immediately after preparation of the samples; (●) measured upon two weeks storage of the samples. 200 g/l sodium salicylate; other counting conditions as described in the caption to Fig. 2.

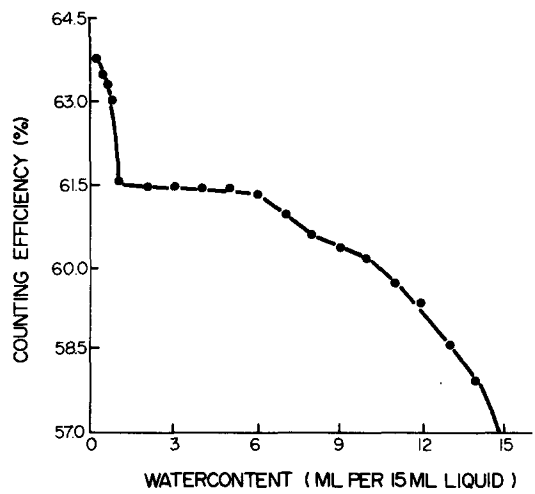

FIG. 8. Detection efficiency of ^{32}P-Čerenkov radiation in ethanol and the effect of increasing amounts of water.

the case of esculin and β-methyl umbelliferon; the increase was about 1%, even with salt concentrations of 125 g/l.

However, with sodium salicylate counting efficiency decreases upon the addition of salt; see Fig. 4.

Since the performance of wavelength shifters may also be affected by changes in pH, the influence of pH on counting efficiency was checked.

As shown in Figs 5 and 6, β-methyl umbelliferon and esculin are hardly affected by changes in the pH in the range 3–11. However, counting efficiency decreases at extreme pH values. Especially at higher pH (>11) a yellow colour develops, which causes severe quenching of the Čerenkov radiation. This yellow colour is probably caused by decomposition products of the wavelength shifters.

Sodium salicylate is more sensitive to pH changes, as shown in Fig. 7.

Since long term stability can be important in the case of a long series of experiments, we also investigated the effects of storing the samples at different pHs.

Storage was in the dark at 4–8°C. The results are given in Figs 5–7 as well.

The results show that β-methyl umbelliferon is not affected by storage for two weeks, whereas esculin shows approximately a 4% decrease in counting efficiency in the pH range 4–11. The performance of sodium salicylate is also affected by storage as shown in Fig. 7.

These data indicate that the wavelength shifters may decompose when stored for long periods. The colour quenching occurring under these conditions has to be corrected for by means of a quench correction curve according to the channels ratio method.[22]

Another way to improve the counting of Čerenkov radiation from aqueous samples containing ^{32}P, is to use organic solvents which mix well with water, e.g. ethanol or methanol. In the case of ethanol the counting efficiency varies depending on the ethanol/water ratio, see Fig. 8. So this ratio has to be kept constant when different samples are compared.

When water- and ethanol-soluble wavelength shifters are used in ethanol only, no real improvement in counting efficiency is observed (see Table 2).

Maximum counting efficiency was measured at the concentrations mentioned in this table. The only improvement is observed with β-methyl umbelliferon. However, the counting-efficiency-concentration curve shows a sharp maximum at 12 g/l (data not shown) and decreases when lower or higher concentrations are used. This is a disadvantage, especially when the solvent has to be mixed with water; in this medium β-methyl umbelliferon quenches Čerenkov radiation at concentrations higher than 400 mg/l (see Fig. 2).

4. Conclusions

It has been shown that a significant improvement in counting efficiency is obtained when esculin, β-methyl umbelliferon or sodium salicylate is used as wavelength shifter for ^{32}P Čerenkov radiation in water. Since esculin and β-methyl umbelliferon are both fairly stable under a variety of experimental conditions, they are the best choice. Both compounds are commercially available, reasonably priced and work well at low concentrations.

When the counting medium is very acidic (pH 0–3) quinine and quinine sulfate are also good wavelength shifters. With water-miscible organic solvents such as ethanol there is a slight improvement in the counting efficiency but the water/ethanol ratio must be known and kept constant when different samples have to be compared.

The use of wavelength shifters soluble in both solvents does not improve counting efficiency. Therefore, it is advisable to use either esculin or β-methyl umbel-

TABLE 2. A comparison of the counting efficiency of ^{32}P-Čerenkov radiation in different solvents and the effects of water- and ethanol-soluble wavelength shifters dissolved in ethanol. Polyethylene vials, vial content 15 ml; amplifier gain 30%

Compound	(g/l)	Counting efficiency (%)
Water		55.5
Methanol		60.7
Ethanol		64.5
Esculin	0.02	59.3
-methyl umbelliferon	12	74.6
Sodium salicylate	8	70.5
Thymine	0.15	59.3
Quinine sulfate	2.9	61.0

liferon as wavelength shifters when Čerenkov radiation of phosphorus in water is being measured.

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