

Chapter 5

Determination of P-32 in Biological Samples by Cerenkov Counting

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INTRODUCTION

The use of Cerenkov counting techniques for high-energy β -emitters offers several advantages compared with liquid scintillation counting. These include lower costs, increased sample volume, and absence of chemical quenching leading to less stringent restrictions on sample preparation procedures. The ability to recover the sample, uncontaminated with scintillator, for subsequent processing or analysis may also be an advantage. The inherent lower counting efficiency is more than offset by the ability to increase sample volume, but a potential improvement in efficiency is possible as a result of the inclusion of a wavelength shifter.¹⁻²

The high-energy β -emitter of greatest interest in biological research is P-32 and there have been several applications of Cerenkov counting within this field of study.³⁻⁶ However, although wavelength shifters offer considerable potential gain in counting efficiency their inclusion has not been widespread. We have been concerned with the determination of P-32 in faeces, urine and plasma from sheep and have investigated the suitability of 4-methylumbelliferone (MU), previously recommended as the compound of choice,¹ as wavelength shifter for these samples.

SAMPLE PREPARATION

It was necessary to ash both faeces and urine before determination of P-32 activity. Faeces were dry ashed in a muffle furnace overnight at 650 °C using silica crucibles. The ash was dissolved in 2 N hydrochloric acid and diluted to volume with water. Alternatively, a wet ashing procedure used also for urine, can be carried out directly in the counting vial. To 1 g faeces or 5 ml urine concentrated nitric acid was added in small quantities. The container was carefully heated in an aluminium block, and addition of further nitric acid continued until no further reduction in colour was apparent. Further decolorization was achieved by dropwise addition of hydrogen peroxide (100 volumes). The final solution was only slightly coloured but correction for colour quenching was necessary. Plasma samples could also be ashed similarly if total phosphorus was of interest. In our case the protein was precipitated with trichloroacetic acid (final concentration 8% w/v) and P-32 activity estimated in the supernatant which was colourless and free from colour quenching.

EFFECT OF ME ON P-32 COUNTING

Standards

The effect of MU at a concentration of 100 g l⁻¹ on the Cerenkov spectrum obtained on our Nuclear Enterprises 8310 spectrometer using P-32 in aqueous solution in

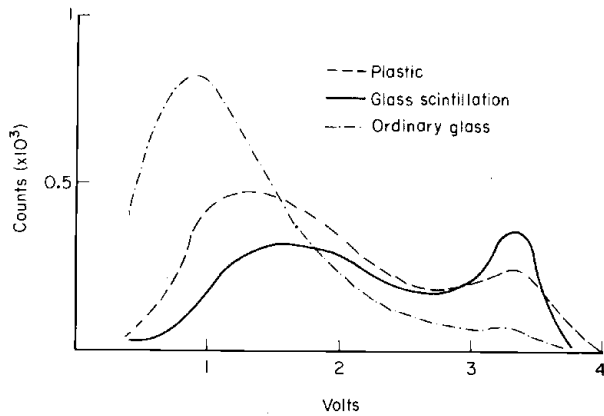


Fig. 1. Effect of counting vial material on Cerenkov spectrum of P-32 with MU.

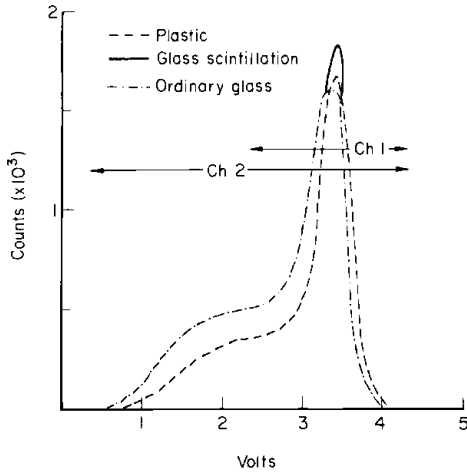


Fig. 2

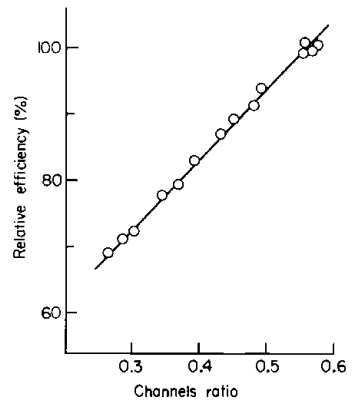


Fig. 3

Fig. 2. Effect of counting vial material on Cerenkov spectrum of P-32 without MU.

Fig. 3. Calibration curve for colour quench correction in counting P-32 with MU using counting channels ratio.

vials made of different materials is shown in Figs 1 and 2. The spectra obtained with plastic and glass scintillation vials were fairly similar without MU and almost identical when MU was added. The ordinary glass vials, however, have in the absence of MU, a spectrum which was quite different from those obtained with scintillation vials, but the difference was less marked when MU was present. In spite of these findings we favoured the use of ordinary glass vials because of their robustness and consequent suitability for ashing in the counting vial. The overall efficiencies in these vials were 34% without and 56% with MU. Using methyl orange as an artificial quenching agent it was found possible to correct for colour quenching in the presence of MU using channels ratio technique. The channels were set as indicated in Fig. 2 and the quench curve obtained was as shown in Fig. 3.

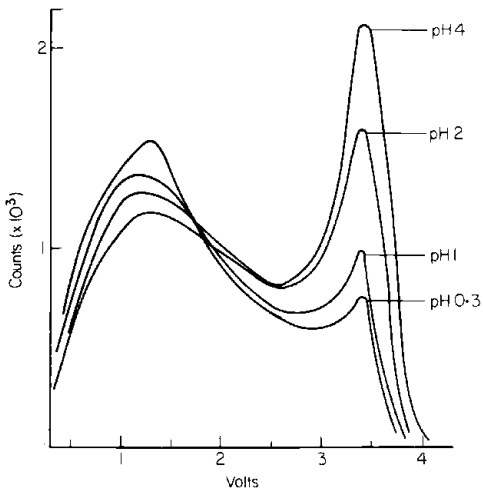


Fig. 4

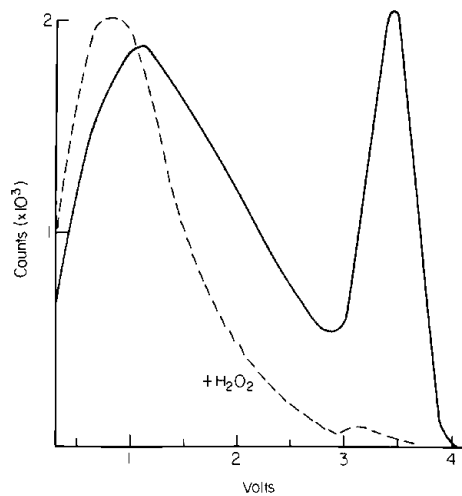


Fig. 5

Fig. 4. Effect of pH on spectrum of P-32 with MU in ordinary glass vials.

Fig. 5. Effect of hydrogen peroxide on spectrum of P-32 with MU in ordinary glass vials.

Biological samples

Faeces samples which had been dry ashed and dissolved in hydrochloric acid before addition of MU and P-32 gave channels ratios outside the range determined with artificially quenched standards in aqueous solution, and it was clear that the spectrum was dramatically changed, presumably due to the acid present. The effect of pH was therefore examined. P-32 was added to solutions of 2 N hydrochloric acid titrated to various pH's, ranging from 0.3 to 7.0, with saturated sodium bicarbonate, before addition of MU. From pH 4 to pH 7 the spectra were identical, but below pH 4 the effect of increasing acidity was as shown in Fig. 4. It is clear that below pH 4 the effect of MU is progressively reduced. This does not confirm Ross's earlier report that MU is stable from pH 2 to 12.¹ It was felt, however, at this stage that the problem could be overcome by titrating all samples to pH 4 with an automatic titrator. However, it was soon discovered that samples that had been wet ashed and subjected to the final adjustment of pH to 4 also counted anomalously. Further examination showed that the addition of hydrogen peroxide to an aqueous solution of P-32 containing Mu also produced changes in the spectrum as shown in Fig. 5. It was apparent, therefore, that effective use of MU placed considerable restrictions on sample preparation. In addition, because of the nature of the wet ashing process in which nitric acid and hydrogen peroxide were added dropwise, the final chemical composition of the ashed samples would necessarily be variable and might be expected to result in variable counting efficiencies. It was therefore decided to abandon the use of the wavelength shifter.

QUENCH CORRECTION

Since our liquid scintillation counter is equipped with a Cs-137 external standard source which is not suitable for use in the Cerenkov technique, we used the sample channels ratio for quench correction. The channels used were from 0.3 to 1.1 and from 0.3 to 4.0 volts. Calibration curves were constructed using increased quantities of methyl orange, ashed faeces and ashed urine. The lines obtained are shown in Fig. 6. A linear regression gave a good fit in each case but each quencher produced a different regression. The regression data for each sample type were stored in the computer program which was used for calculation of the results. Plasma trichloroacetic acid filtrates were not quenched.

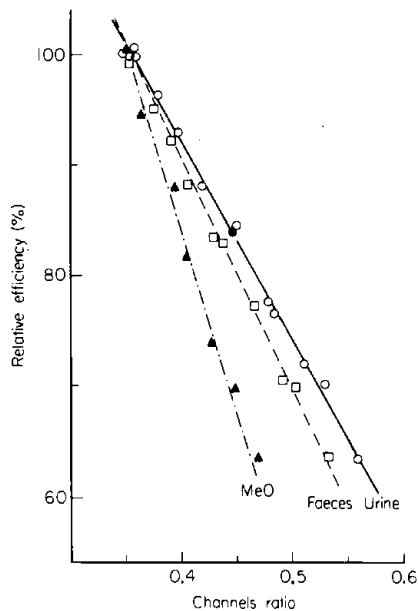


Fig. 6. Calibration curves for colour quench correction in counting P-32 without MU using counting channels ratio.

CONCLUSION

The inclusion of MU as wavelength shifter in Cerenkov counting has two disadvantages which must be offset against the considerable potential increase in counting efficiency. Firstly, because its efficacy is negated by some commonly used (and probably many other) solubilization techniques its use puts severe restrictions on sample preparation and removes one of the major advantages of the technique. Secondly, where recovery of the sample is important, the use of MU introduces contamination which may interfere with the subsequent processing. In the absence of a wavelength shifter the process still gives acceptable efficiencies for most users and permits all the advantages of Cerenkov counting to be enjoyed. For most biological samples, therefore, it would probably be wise to omit the use of wavelength shifter.

Although the use of an external standard for quench correction would be preferred on the grounds of statistical counting accuracy, adequate quench correction by a sample channels ratio technique can be obtained with samples of reasonable activity.

REFERENCES

1. H.H. Ross, in Organic Scintillators and Liquid Scintillation Counting (D.L. Horrocks and C.T. Peng, Eds), Academic Press, New York and London, 1971, p.757.
2. R.P. Parker and R.H. Elrick, Int. J. Appl. Radiat. Isot. 17, 361 (1966).
3. M.K. Johnson, Anal. Biochem. 29, 348 (1969).
4. J. Plesums and W.H. Bunch, Anal. Biochem. 42, 360 (1971).
5. R.P. White and B.G. Ellis, Soil Sci. Am. Proc. 32, 740 (1968).
6. H. Braunsberg and A. Guyver, Anal. Biochem. 10, 86 (1965).

DISCUSSION

E.D. BRANSOME: Is it not likely that, by adding a wavelength shifter to your Cerenkov samples, you have in fact converted them to liquid scintillation samples, now subject to impurity quenching as well as colour quenching (i.e. you have added an organic scintillator)? Ross* has suggested a way out of this dilemma by constructing a vial with wavelength shifter in a sealed external compartment surrounding the sample.

Finally, were your comments about external standard channels ratios founded upon observations with or without 4-methyl umbelliferone?

B.S.W. SMITH: That may be the explanation, although I do not know to what extent the effects we observed were due to quenching or to chemical instability of the methyl umbelliferone (MU). We were concerned to establish whether the use of MU offered practical advantages in our particular situation. Literature reviews on Cerenkov counting indicate the potential advantages of the use of wavelength shifters. On the other hand, as I have said, they have not often been used in Cerenkov counting of biological samples. Our conclusion is that the advantages are less than the disadvantages.

In answer to your last question, I should have made it clear that my comments related to the situation without wavelength shifter.

* H.H. Ross, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.79.