

Chapter 11

Liquid Scintillation Counting of Calcium-45 in Biological Samples containing Environmental Strontium-90

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INTRODUCTION

The method described, basically a modification of that due to Carr and Parsons,¹ was developed to measure low specific activities of calcium-45 in human excreta containing strontium-90. Of the published methods for the liquid scintillation counting of calcium-45,¹⁻⁹ only those involving the suspension of a calcium salt in a scintillator^{3,4,9} had high enough calcium capacities for our purposes. Suspended sample methods are, however, prone to variations in counting efficiency that are not measurable by standard methods for quench correction when the calcium content exceeds 300 to 400 mg. The errors involved are small for calcium-45 alone but lead to serious inaccuracies when strontium-90 is present. Methods in which true solution of a calcium salt is obtained allow reliable quench correction¹⁰ but have only been used for samples containing up to about 500 mg of calcium.

In the present method calcium is separated from biological samples as the oxalate, converted to the chloride and dissolved in ethanol. A toluene-based scintillator solution is then added. Up to 1.4 g of calcium as chloride can be dissolved in 20 ml of scintillator system and the calcium-45 counted with an efficiency of 60 to 84%. Quench correction by the external standard method is shown to give reliable results both with calcium-45 alone and in the presence of strontium-90, a constituent of contemporary biological samples. Internal and external standard measurements of the calcium-45 counting efficiency of the samples are in good agreement. The detection limit of the method, i.e. when the calcium-45 rate = 10% of the background, is approximately 3.3 p Ci/g calcium.

EXPERIMENTAL

Reagents

Scintillator solution: butyl PBD (CIBA), 20 g/l in analar toluene.

Ethanol: absolute alcohol (BPC).

Buffered ammonium oxalate: pH 4.2. Add 4% w/v oxalic acid solution to 4% w/v ammonium oxalate solution (approximately 80 ml/l) until pH = 4.2.

Ammoniated water: approximately 1 to 250 dilution of '0.880' ammonia in distilled water.

Indicator: 1% w/v solution of bromocresol green in ethanol.

Sudan IV quencher solution: 0.005% w/v solution of Sudan IV (BDH) in scintillator solution.

Synthetic urine and faecal ash solutions: add the following components to approximately 4 l of distilled water, heat to 80 to 90°C and add concentrated HCl with stirring until a solution is obtained. Make up to a final volume of 5 l with water.

1. Faecal Ash		2. Urine Ash	
NaCl	15.2 g	NaCl	587 g
KCl	44.8 g	KCl	305 g
Saturated MgCl ₂	204 ml	Saturated MgCl ₂	94 ml
Fe ₂ O ₃	1.22 g	CaHPO ₄	43 g
H ₃ PO ₄ (88%)	92.0 g	H ₃ PO ₄	163 g
H ₂ SO ₄ (98%)	20.5 g		
CaCO ₃	80.0 g		

Procedure

After an initial concentration of calcium, if necessary the entire process may be carried out in a single centrifuge tube of suitable volume. $\frac{3}{4}$ in B24 stoppered test tubes (Quickfit and Quartz MF 25/2/6) are suitable when samples contain up to 100 mg of calcium, and 250 ml centrifuge bottles (MSE heat-resistant glass) may be used for the larger amounts.

1. Dry ash biological samples, dissolve in dilute hydrochloric acid, centrifuge to remove insoluble debris and transfer a suitable aliquot to the appropriate size of tube or bottle. Add calcium chloride solution if necessary to make the total calcium at least 20 mg in the smaller tubes or 200 mg in the 250 ml bottles.
2. Add a small excess of ammonium oxalate solution (a hot 10 to 15% solution can be used if the sample volume is large) and adjust the pH to approximately 4.2 with 0.880 ammonia solution (green to bromocresol green). Add buffered ammonium oxalate solution to roughly double the volume and stand for 30 min or until cooled to room temperature. Centrifuge at 1800 r.p.m. for 5 min, reject the supernatant and drain for 2 to 3 min.
3. Re-suspend the precipitate in buffered ammonium oxalate solution, centrifuge and drain as in (2).
4. Dissolve the precipitate in a minimum of 60% hydrochloric acid (heat if necessary) and repeat stages (2) and (3) above. (Buffered ammonium oxalate solution only should be added).
5. Wash the precipitate twice with ammoniated water, drain for 2 to 3 min.
6. Place the tubes in an oven at 160°C until thoroughly dry (2 to 3 h) and transfer to a muffle furnace at 525°C for at least 4 h but preferably overnight.
7. Dissolve the residue in a minimum of 50% hydrochloric acid and dry at 160°C.
8. Add ethanol (12.0 ml), stopper and shake at 40 to 50°C until solution is complete. (If rubber bungs are used they should be covered with a polythene sheet). Add the scintillator solution (8.0 ml), mix and transfer to counting vial. Black or reddish residues of carbon or iron are best removed by centrifugation after standing for 12 h. When strontium-90 is present allow 14 days for equilibration of strontium-90 with yttrium-90 before counting.

Preparation of quenched standards

Three sets of quenched standards were used each containing from 1.0 to 4.0 ml of Sudan IV quencher solution substituted for scintillator. One set was for background measurements and the others contained known activities of either calcium-45 or strontium-90.

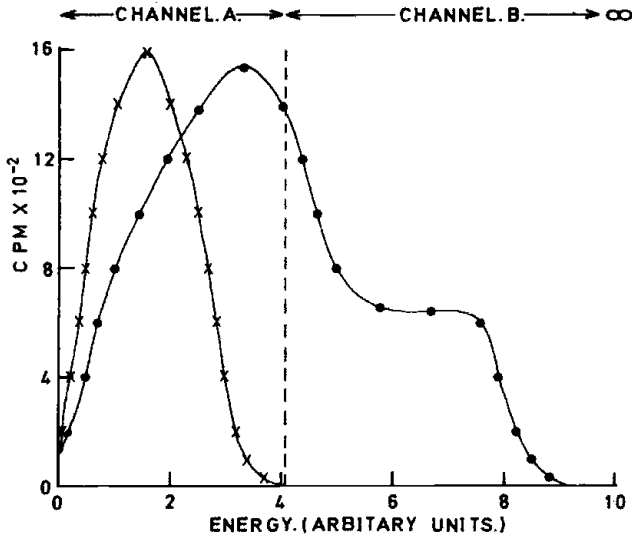


Fig. 1. Spectra of calcium-45 and strontium-90 (yttrium-90). X = calcium-45, ● = strontium-90 (yttrium-90).

Counting conditions and analysis of results

1. Instrument settings for external standard will depend on the counter used. On an early model 'Beckman Liquid Scintillation System' the gain was adjusted to give an external standard ratio of about 1.5 for the least quenched standard. Because of the statistical variation of the external standard ratio on this instrument each sample was counted three times and a mean ratio taken.
2. Adjust channel A to include all counts due to calcium-45 using the least quenched standard and channel B to contain events of a higher energy (see Fig. 1).
3. Count samples for a suitable time together with the quenched series appropriate to the expected calcium-45 and strontium-90 count rate of the samples (see Figs. 2 and 3).
4. From the data from the quenched series establish the following relationships:
 - (a) Efficiency of calcium-45 in channel A with external standard ratio.
 - (b) Efficiency of strontium-90 in channel A with external standard ratio.
 - (c) Efficiency of strontium-90 in channel B with external standard ratio.
 - (d) Background in channel A with external standard ratio.
 - (e) Background in channel B with external standard ratio.

In the work reported here the paper tape output from the counter was fed to an IBM 360 computer which fitted the above curves to three-term polynomials and calculated the d.p.m. of calcium-45 and strontium-90 in each sample together with the corresponding

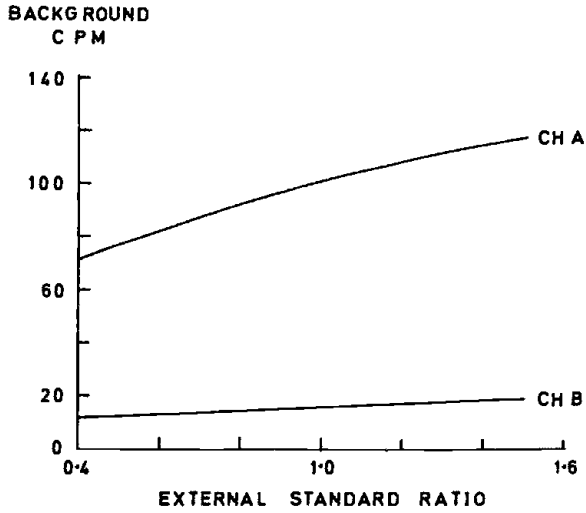


Fig. 2. Effect of quenching on background in channels A and B.

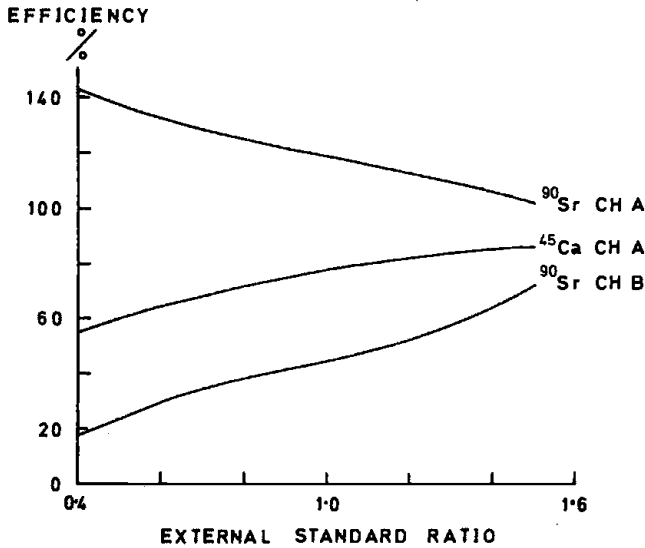


Fig. 3. Effect of quenching on calcium-45 and strontium-90 (yttrium-90) counting efficiencies.

standard errors and the standard error of the mean for duplicate samples. The statistical calculation assumed all counts to have a Poisson distribution and included errors in curve fitting.

RESULTS

Chemical recovery. No significant loss of calcium could be detected by flame photometric measurements but the recovery of strontium was sometimes reduced to about 85%. Since

the method was designed only to correct the calcium-45 counts for the contribution due to strontium-90 the reason for this was not thoroughly investigated. The solubility of strontium oxalate is significant over 30°C, therefore more careful temperature control during precipitation might ensure a quantitative recovery of strontium.

Effect of quenching on background and counting efficiencies. Figures 2 and 3 show the effect of quenching by Sudan IV on the background, calcium-45 and strontium-90 counting efficiencies in channels A and B. The decrease in background with increased quenching, especially in channel A is considerable and leads to significant corrections at low calcium-45 count rates.

Strontium-90 ($E_{max} = 0.516 \text{ MeV}$) is counted together with its daughter yttrium-90 ($E_{max} = 2.27 \text{ MeV}$) at equilibrium giving an efficiency of over 100% in terms of d.p.m. strontium-90. Quenching increases the contribution of strontium-90 (yttrium-90) in channel A. Contemporary biological samples e.g. food or faeces contain up to 16 pCi strontium-90/g calcium (36 d.p.m. strontium-90/g calcium).

Recovery of calcium-45 from urine and faecal samples. Table 1 shows the recovery of calcium-45 from a range of urine and faecal ashes containing different levels of strontium-90. The recovery of calcium-45 is seen to be good and essentially unaffected by the presence of strontium-90 at levels much higher than those experienced in experimental samples. Synthetic urine and faecal ashes were prepared using the figures given in *Documenta Geigy* for the average daily output in man of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Fe^{3+} , P and S.

Synthetic samples were used because of the difficulty of obtaining human samples in large quantities; they proved rather more difficult to process than experimental samples.

Table 1. Recovery of calcium-45 from urine and faeces containing strontium-90.

Sample	Ca content mg	d.p.m. ^{45}Ca		d.p.m. ^{90}Sr	Recovery %	
		Calculated	Added			
Synthetic	1	19	704 (49)	696	29	101
	2	19	717 (61)	696	290	103
Faecal Ash	3	640	670 (21)	696	29	96
	4	640	670 (20)	696	290	96
Human Faecal Ash	5	1288	-5.9 (5.2)	-	26	-
	6	1288	131 (6.7)	134	26	98
Synthetic Urine Ash	1	25	698 (63)	696	29	100
	2	25	722 (73)	696	290	104
	3	633	688 (30)	696	29	99
	4	633	700 (35)	696	290	101
	5	1265	670 (15)	696	29	96
	6	1265	671 (20)	696	290	96

Figures in parenthesis are standard errors of the mean for duplicate samples.

Stability of samples. Table 2 shows the observed c.p.m. and calculated d.p.m. of a pair of samples counted at various times after preparation. No significant variation of the calcula-

Table 2. Observed c.p.m. and calculated original d.p.m. of calcium-45 in two samples counted at intervals after preparation.

Days after preparation	c.p.m. calcium-45 observed		Original d.p.m. calcium-45 calculated	
	Sample 1	Sample 2	Sample 1	Sample 2
47	9434	9516	12977	13036
61	8966	8801	13070	12962
74	8469	8366	13130	13011
101	7475	7421	13022	12906
122	6845	6794	13063	12965
135	6553	6489	13080	13004
176	5441	5393	13017	12932
203	4096	4086	13087	13013
253	4062	4009	13020	12850

Table 3. d.p.m. calcium-45 measured in duplicate samples of urine and faecal ashes.

Sample	Calcium content mg	d.p.m. calcium-45 calculated	Standard error of the mean
Urine 1	890	681 707	58 ^a
Urine 2	900	642 627	25
Urine 3	1165	613 629	25
Faeces 1	1297	185 188	22
Faeces 2	1130	158 161	19
Faeces 3	1160	186 211	55 ^a

^a For these samples the difference between duplicates was just significant ($p = 0.05$).

ted original d.p.m. with time is observed. The uncorrected c.p.m. give a radioactive half-life for calcium-45 of 164.9 ± 1.0 days which is in excellent agreement with the published figures.

Reproducibility. Table 3 shows some typical experimental results from duplicate samples of human urine and faecal ashes. The standard errors of the mean shown were computed assuming that all errors were due to counting statistics and curve fitting errors. The results marked *a* are those in which the difference between duplicates was significant at the $p = 0.05$ level. The standard error of the mean is, in these cases, expanded by the appropriate heterogeneity factor. During the counting of about 300 samples the $p = 0.05$ level

Table 4. Comparison of calcium-45 counting efficiency in samples prepared from urine and faeces measured by the external and internal standard methods.

Sample	Calcium-45 counting efficiency	
	External standard	Internal standard
Urine Ash 1	71.0	70.6
2	71.1	71.5
3	68.8	69.1
4	69.1	69.2
Faecal Ash 1	61.9	63.4
Ash 2	64.9	63.4
3	65.4	66.4
4	66.6	66.4

was exceeded in about one in seven measurements indicating that additional sources of error were present. The absolute difference between duplicates was, however, mostly quite small and only four measurements needed repeating.

Calcium-45 measurements by internal standard. Table 4 shows the calcium-45 counting efficiency of some experimental samples as measured by the external and internal standard methods. The agreement between the two methods is seen to be good.

Possible interferences. *Chemical:* no samples of biological origin so far encountered have caused any serious difficulties with the separation of calcium or excessive quenching in the counting sample. *Radioactive:* any isotope significantly precipitable as the oxalate could of course cause errors and should therefore be removed prior to precipitation. No significant interferences have been encountered in contemporary biological samples.

DISCUSSION

The modifications to the Carr and Parsons method described above have considerably increased the experimental possibilities of the use of calcium-45 as a tracer particularly in the field of bone metabolism. The ability to allow for the effects of quenching on background and strontium-90 interference considerably increases the precision of measurements made on samples containing large amounts of calcium since even with careful preparation considerable variations in quenching occur with such samples. Measurements of calcium-45 in biological samples containing as little as 40 pCi calcium-45/g calcium can readily be made.

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DISCUSSION

J. F. Stoutjesdijk: Is it not possible to dissolve the ash immediately in hydrochloric acid? At ITAL, Wageningen, we ash several grams of plant material in a glass scintillation vial at about 400°C, dissolve the ash in hydrochloric acid and add a dioxane scintillation mixture. The hydrochloric acid produces considerable but constant quenching and corrections are not necessary.

J. Nolan: Yes, it is possible to do what you suggest for small biological samples, containing up to perhaps 20 mg calcium. It would, however, be impractical for the low specific activity samples with which I am concerned. It would be very difficult to evaporate 8 l of human urine and ash it in a counting vial! Also the colour of the dissolved residue would severely reduce counting efficiencies and thereby seriously affect our limits of detection.

B. Scales: Could you please give some indication of the long-term stability of your calcium chloride-containing samples. Is there any deterioration of the counting efficiency over a period of months?

J. Nolan: We could detect no change in counting efficiency or background in samples over a period of at least a year. The vials were closed with polythene inserts and capped with standard metal foil lined tops; they were stored at approximately 10°C in the dark. I think the explanation for the negligible amount of evaporation of the samples is that the polythene and cap material are less permeable to the 12:8 ethanol:toluene mixture than they are to more or less pure toluene.