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BY LIQUID SCINTILLATION COUNTING.**

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THE RADIOASSAY OF CALCIUM-45 AND STRONTIUM-89
FROM BIOLOGICAL SYSTEMS BY LIQUID
SCINTILLATION COUNTING

by
James E. ^{Edward} Hardcastle

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I hereby recommend that this dissertation prepared under my direction by James Edward Hardcastle entitled The Radioassay of Calcium-45 and Strontium-89 from Biological Systems by Liquid Scintillation Counting. be accepted as fulfilling the dissertation requirement of the degree of Ph.D.

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SIGNED: James E. Hardcastle

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ABSTRACT

This dissertation reports the development of methods for the radioassay of Ca^{45} , Sr^{89} , and $\text{Ca}^{45}\text{-Sr}^{89}$ double labelled samples by liquid scintillation spectrometry. The samples, obtained from such natural sources as plants and soils, are first digested and converted to the chloride form. Then the chloride salts of the isotopes are dissolved in a di-n-propyl phosphate-toluene-fluor scintillation mixture, and counted in a liquid scintillation spectrometer. A method is described for calculating the individual isotope activities from a $\text{Ca}^{45}\text{-Sr}^{89}$ double-labelled sample. The factors affecting this radioassay technique were studied. Counting efficiencies of 85 per cent for Ca^{45} and 65 per cent for Sr^{89} were obtained using this procedure. The procedure is simple, fairly rapid, and capable of high precision.

INTRODUCTION

Due to the concern afforded strontium as a long-lived radio-contaminant in the biosphere, and because of the chemical and physiological similarity of strontium and calcium, a reliable method for studying the interaction of these elements in the same sample is needed. The use of radioisotopes provides the most practical method for tracing elements in biological systems. The radioisotopes Ca^{45} and Sr^{89} , both beta emitters, are well suited for tracer studies of these elements.

When using the conventional Geiger-Muller or proportional counting techniques for the radioassay of Ca^{45} in massive salt matrices, two major problems are encountered. First, there is a reduction in precision due to mass absorption and matrix geometry errors. This problem is equally prevalent when counting Ca^{45} in soil extracts and soil materials. Second, if an infinitely thick sample is prepared to avoid the necessity of mass absorption corrections then the detection efficiency is reduced to two per

cent or less. These same problems are met when assaying Sr^{89} samples, though to a lesser extent.

There are two techniques commonly used for the radioassay of Ca^{45} - Sr^{89} double labelled samples. First, the two elements may be separated, and the individual isotopes assayed separately by Geiger-Muller or proportional counting. Among the methods that are currently being used for the separation of these elements are ion exchange, paper chromatography, electrophoresis, and differential precipitation. These methods can be time consuming, and are subject to losses due to incomplete recovery. The second technique for the radioassay of Ca^{45} - Sr^{89} mixtures is differential absorption counting. Here the total count rate of the sample is measured with a Geiger-Muller or proportional counter, then the sample is recounted with a filter in place to absorb all the emanations from the lower energy isotope Ca^{45} , and allowing a portion of the Sr^{89} emanation to reach the detector to be counted. The Ca^{45} count rate is obtained by subtracting the Sr^{89} count rate from the total sample count rate after the proper absorption corrections have been made. Both of the above described techniques require two counts to determine Ca^{45} and Sr^{89} in the same sample, and these counts

are subject to the disadvantages already mentioned in connection with Geiger-Muller and proportional counting.

One of the most efficient and convenient methods for counting beta emitters, and for employing the double-labelling technique is found in liquid scintillation spectrometry. With a two channel liquid scintillation spectrometer, it is possible to separate the beta energy spectra of Ca^{45} and Sr^{89} , thus providing a means of assaying for these isotopes simultaneously in the same sample. Because of the intimate association between the radioactive atoms and the fluor molecules in the liquid scintillation media, problems due to self-absorption are largely avoided; and thus, the beta detection efficiency is greatly improved.

The main problem encountered is the solubilization of the polar calcium and strontium salts (the common forms of these elements) in the nonpolar organic solvents used in the preparation of samples for liquid scintillation counting. In the method to be discussed, the highly polar calcium and strontium ions are incorporated into complex organic molecules with their resultant solubility in toluene, the most common

liquid scintillation solvent. The complexing agent used was di-n-propyl acid phosphate. This reagent was found to be a highly effective carrier of calcium and strontium salts, and also was found to have properties suitable for efficient liquid scintillation spectrometry.

The development of methods for the radioassay of Ca^{45} , Sr^{89} , and $\text{Ca}^{45}\text{-Sr}^{89}$ double labelled samples by liquid scintillation spectrometry are described.

LITERATURE SURVEY

There are many references in the literature to techniques for preparing radioisotopes of metallic ions for liquid scintillation counting. These techniques have been reviewed by several authors (11, 14, 15, 19) and may be categorized as follows:

1. Solubilization of an aqueous solution of an inorganic salt or the dry salt itself in a solvent system suitable for liquid scintillation counting. Such solvent systems are usually composed of dioxane or ethanol-toluene mixtures.

2. Conversion of the metal ion to a salt of an organic acid which is then soluble in toluene or other suitable liquid scintillation solvents. Napthenoates, octanoates, and 2-ethylhexanoates are common organic salts in this respect.

3. Extraction of metallic complex, soluble in organic media, into the scintillation solution. Esters of orthophosphoric acid, such as dibutyl phosphate, or dioctyl phosphate, are among the most efficient complexing reagents used to extract metal ions into organic scintillation solutions.

4. Suspension of the sample, either as an aqueous solution or dry salt, in a gel-scintillation solution. This can be done by adding a thixotropic agent to the scintillation solution. A variation of this technique is the use of plastic scintillator beads or anthracene crystals as fluors suspended in aqueous solutions.

Several authors (3, 12, 16, 17, 18, 20, 23, 25) describe the use of the above techniques for the radioassay of Ca^{45} samples. Lutwak (17) reports two methods; the first involved the solubilization of calcium salts in a 2-ethyl-hexanoic acid-toluene mixture, and in the second, calcium oxalate was dissolved in a tertiary mixture of toluene, ethanol and HCl. The second system was the better of the two, both in calcium salt holding capacity and Ca^{45} counting efficiency. This solvent system dissolved up to 50 mg of calcium in 10 ml of solvent with a maximum detection efficiency of 65 per cent. Carr and Parsons (3) increased the solubility of calcium in Lutwak's tertiary system by converting the oxalate to the chloride. This system was capable of dissolving 100 mg of calcium (as the chloride) in 12 ml of solvent with a maximum counting efficiency of 55 per cent. By increasing the

volume of solvent to 36 ml, 300 mg of calcium could be dissolved, but the counting efficiency was reduced to 45 per cent.

Sarnat and Jeffay (20) extracted up to 115 mg of calcium, as $\text{Ca}(\text{NO}_3)_2$, in a scintillation mixture of ethylene glycol-toluene-ethanol. The maximum Ca^{45} counting efficiency was 53 per cent. Carrier $\text{Ca}(\text{NO}_3)_2$ caused a reduction in counting efficiency of only 1.1 per cent per 100 mg.

Humphreys (12) developed two methods in which calcium perchlorate and calcium chloride could be dissolved in either an ethanol-toluene or tributyl phosphate-toluene scintillation solvent system. The quenching effect of carrier calcium in both these systems was 0.1 per cent per mg of calcium. Humphreys recommended the tributyl phosphate system, because of its much greater Ca^{45} counting efficiency. The maximum counting efficiency was 66 per cent, and up to 50 mg of calcium could be dissolved in 12 ml of the solvent mixture.

Steinberg (23) obtained a maximum Ca^{45} counting efficiency of 45 per cent in suspended systems, using both anthracene crystals and plastic scintillation beads in aqueous solutions. Myers and Brush (18)

obtained a counting efficiency of 78 per cent for Ca^{45} in a suspended scintillator system. These authors did not report how much calcium could be accommodated in the suspended scintillation systems that they described.

Lerch and Cosandey (16) tested several techniques for preparing Ca^{45} and P^{32} double labelled samples for radioassay by liquid scintillation counting. Their most efficient technique was one in which up to 200 mg of $\text{Ca}_3(\text{PO}_4)_2$ was deposited on fluted filter paper which, in turn, was immersed in a toluene-fluor solution for counting. The maximum Ca^{45} counting efficiency of this method was 48 per cent. A gel suspension method was recommended when quantities of the dry salt from 0.2 gm to 4.0 gm were used, but the Ca^{45} counting efficiency of this system was only 29 per cent.

There have been a few reports (5, 7, 8, 18, 24) concerning the preparation of samples of strontium salts in various media for the liquid scintillation assay of Sr^{90} . All of these methods made use of the suspended scintillator technique.

Uyesugi and Greenberg (25) dissolved up to 60 mg of strontium carbonate and strontium oxalate in acetic acid, evaporated this to dryness, dissolved the resulting

salt residue in one ml of 2-ethylhexanoic acid, and then added 15 ml of toluene-fluor solution. This solvent system is not satisfactory for dissolving calcium salts (17).

There has been only one (27) reported use of liquid scintillation counting for the radioassay of Sr^{89} , and this is a technique for the simultaneous radioassay of Ca^{45} and Sr^{89} in unicellular algae. The algae cells were first decolorized, then suspended in a scintillation solvent system. This system can hold up to 630 mg of algal cells with little change in Ca^{45} detection efficiency or quenching. However, the system already is highly quenched as indicated by the relatively high voltage tap settings. The authors do not give the actual counting efficiency of Sr^{89} and Ca^{45} . Because a single channel liquid scintillation counter was used, and two counts of each sample, at different instrument settings, were necessary to resolve the activities of the two radioisotopes, this technique was not truly a "simultaneous" determination of Ca^{45} and Sr^{89} . Lastly, this technique has a limited application to unicellular algae samples only.

The above described techniques of bringing cations into liquid scintillation systems do not

always form stable counting samples (6, 13, 16), and many times difficulty arises in trying to obtain homogeneous samples for counting. Many of these methods do not allow for yield recovery corrections. These limitations are particularly true of the gel suspension techniques.

Smith (22) has developed a unique method for resolving mixtures of the beta emitters Ca^{45} , Sr^{89} , Sr^{90} - Y^{90} in plant and animal samples. This was not a liquid scintillation technique, but made use of an anthracene crystal beta scintillation detector. There are two disadvantages of this method, the first of which is the low counting efficiencies of only 4.3 per cent for Ca^{45} , 16.3 per cent for Sr^{89} , and 18.8 per cent for Sr^{90} - Y^{90} . The second is the necessity of making two counts of each sample, at different instrument settings, to resolve the activities of the various isotopes. This is time consuming in routine analyses. The preparation of the plant and animal tissues for counting was not described.

EXPERIMENTAL

A. Scintillation Solutions and Fluor Systems

The scintillation solutions used to solubilize calcium and strontium salts, and to count Ca^{45} , Sr^{89} , and $\text{Ca}^{45}\text{-Sr}^{89}$ samples were prepared by first dissolving the primary and secondary fluors in toluene, and then adding the appropriate amount of the dialkyl phosphate. The standard scintillation solution used in this study consisted of 5.0 g/l 2,5-diphenyloxazole (PPO) and 0.5 g/l α -naphthylphenyloxazole (NPO) in di-n-propyl phosphate-toluene (1:3, v/v) solutions. Five other fluor systems were prepared in the di-n-propyl phosphate-toluene (1:3, v/v) solution, and were tested for their efficiency in Ca^{45} and Sr^{89} counting. These fluor systems consisted of (1), 2.5 g/l p-terphenyl (PT), (2) 5.0 g/l PPO, (3), 2.5 g/l PT and 0.5 g/l NPO, (4), 5.0 g/l PPO and 0.5 g/l 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (designated DMPOPOP), (5), 4.0 g/l 2,5-bis-2-(5-t-butyl-benzoxazolyl-thiophene) (designated BBOT). The fluors used in this study were scintillation grade, and were obtained from Packard Instrument Co., Inc., Downers Grove, Illinois.

The dialkyl phosphates used in this study were dibutyl phosphate (DBP), diisopropyl phosphate (DIP), di-n-propyl phosphate (DNP), diisoamyl phosphate (DIAP), diethyl phosphate (DEP), diphenyl phosphate (DPHP), and bis (2-ethyl-hexyl) hydrogen phosphate (BEHP). The first five dialkyl phosphates listed above are clear, colorless liquids, the BEHP is a brown colored liquid, and the DPHP is a grey colored solid. The DBP and BEHP were obtained from Distillation Products, Incorporated, Rochester, N.Y., the DNP from Hooker Chemical Co., Niagara, N. Y., and the DIP, DIAP, DEP, DPHP, were obtained from K and K Laboratories, Plainview, N.Y. The manufacturers supplied the dialkyl phosphates as approximately 50-50 mixtures of the mono and dialkyl phosphates, and they were used in this form. These dialkyl phosphates were technical grade, and were not purified before use.

B. Scintillation Counter and Counting Techniques

The "Tri-Carb", Model 314 EX-2 (Packard Instrument Co., Downers Grove, Illinois) liquid scintillation spectrometer was used in this study. The temperature of the deep-freeze assembly housing the sample chamber, photomultiplier tubes, and the preamplifiers was maintained at -5° C. The optimum spectrometer settings for

Ca⁴⁵ and Sr⁸⁹ counting were obtained using the "balance point" procedures outlined in the "Tri-Carb" Operations Manual.

Balance point operation is desirable for two reasons. The first is that this yields the maximum counting efficiency possible for the particular discriminator settings, and the second is that this condition results in more stable instrument operation. To obtain the balance point for individual isotopes, the high voltage (HVT) is adjusted until the beta peak is centered between the upper and lower discriminators. For maximum Ca⁴⁵ counting efficiency, the upper and lower discriminators were set at 0090 and 0500, respectively, and the amplifier gain was set 100 per cent. For maximum Sr⁸⁹ counting efficiency, the upper and lower discriminators were set at 0090 and 1000, respectively, while the amplifier gain was set at 10 per cent. The HVT for balance point counting of these isotopes then depends upon the makeup of the sample solution, that is, the concentration of DNP, the fluor system used, and the quantity of carrier salt present.

When a double labelled sample is to be assayed the instrument counting conditions are set so that

maximum separation of the two beta energy spectra is obtained. These conditions are found by counting the two isotopes separately. First, the instrument settings are adjusted to maximize the Ca^{45} counting efficiency in one channel, while reducing it to a negligible amount in the second channel. The choice of discriminator settings in the first channel is rather arbitrary, the main objective being to maximize the Ca^{45} count rate while reducing the Sr^{89} count as much as possible in this channel. The Sr^{89} is then counted in the second channel with little or no interference from Ca^{45} . Thus, to achieve balance point operation, the HVT was adjusted until the Ca^{45} beta peak was centered between the upper and lower discriminators of channel I, which were set at 0090 and 0500, respectively. The amplifier gain was set at 100 per cent. With this HVT setting, the gain on channel II was adjusted until the Sr^{89} beta peak was centered between the upper and lower discriminators of channel II, which were set at 0350 and 1000, respectively. These instrument operating conditions allow for maximum Ca^{45} counting efficiency, reduce the Ca^{45} count in channel II to a negligible amount, and still yield at high Sr^{89} counting efficiency in channel II.

C. Procedure

The samples containing the isotope (and salt carrier) were evaporated to dryness at 90-100° C in glass counting vials (20 ml volume with 22 mm screw caps, lined with polyethylene discs, Packard Instrument Co., Downers Grove, Illinois). After the samples had cooled, 15 ml of the alkyl phosphate-toluene-fluor solution was added, and the samples were shaken until all the salt residue dissolved. Samples containing less than 200 mg of carrier salt usually went into solution within 3 hours; samples with higher salt content required more time, but in no case was more than 8 hours of shaking necessary. Then the samples were counted after they had come to temperature equilibrium in the "Tri-Carb" deep freeze.

When developing analytical methods, it is necessary to investigate the pertinent factors that may be involved. In this respect the following items were investigated: (a) scintillation fluor systems and concentrations; (b) ratio of complexing agent to organic solvent; (c) the solvent system's capacity for strontium and calcium salts; (d) interferences from other cations and anions; (e) precision and

counting efficiency; (f) reagent and sample stability; (g) optimum sample volume; and (h) linearity of counting. Furthermore, it was necessary to develop a method for preparing biological samples (containing Ca^{45} and Sr^{89}) for liquid scintillation counting, and to determine the recovery efficiency of such a method.

RESULTS AND DISCUSSION

A. Preliminary Survey of Solubilizing Agents for SrCl₂ and CaCl₂

The wet digestion technique (to be fully discussed later) used to prepare the biological samples for liquid scintillation counting of Ca⁴⁵ and Sr⁸⁹, converted these isotopes to the chloride forms. Thus, it was necessary to find a reagent that would bring these two salts into solution in a suitable liquid scintillation medium. Several dialkyl phosphates were tested for their ability to serve as CaCl₂ and SrCl₂ carriers in a toluene-fluor matrix.

The results of this study are recorded in Table 1. The dibutyl phosphate and diisoamyl phosphate gave the highest counting efficiencies for Ca⁴⁵, the efficiencies being about 80 percent for each. Both were capable of solubilizing CaCl₂. However, these reagents were not adequate carriers of strontium chloride, whereas diisopropyl phosphate was capable of solubilizing both CaCl₂ and SrCl₂. The

Table 1. The Efficiency of Ca^{45} Detection Using Various Alkyl Phosphate Carriers in a Toluene-Fluor System

Alkyl Phosphate Added*	Salt Added	Ca^{45} Detection Efficiency	Observations
	mg	%	
Diisopropyl phosphate (50% mono-50% di)	0.5 CaCl_2	75.5	clear solution
	5.0 CaCl_2	75.0	clear solution
	5.0 CaCl_2 &		
	5.0 SrCl_2	75.5	clear solution
Dibutyl phosphate (45% mono-55% di)	0.5 CaCl_2	79.4	clear solution
	5.0 CaCl_2	79.8	clear solution
	5.0 CaCl_2 &		
	5.0 SrCl_2	78.4	trace precipitate

Table 1--Continued

Alkyl Phosphate Added*	Salt Added	Ca ⁴⁵ Detection Efficiency	Observations
	mg	%	
Diisoamyl phosphate (50% mono-50% di)	0.5 CaCl ₂	78.9	clear solution
	5.0 CaCl ₂	79.2	clear solution
	5.0 CaCl ₂ &		
	5.0 SrCl ₂	79.7	trace precipitate
Diphenylcresyl phosphate	0.5 CaCl ₂	55.4	clear solution
	5.0 CaCl ₂	13.3	precipitate
	5.0 CaCl ₂ &		
	5.0 SrCl ₂	12.0	precipitate

*All samples were duplicated and corrected for the appropriate background: 41 cpm for diisopropyl phosphate, 29 cpm for dibutyl phosphate, 36 cpm for diisoamyl phosphate, and 74 cpm for diphenylcresyl phosphate.

efficiency of Ca^{45} detection in the diisopropyl system was somewhat less, but was still quite good.

Diphenylcresyl phosphate was capable of dissolving only trace amounts of CaCl_2 , and the Ca^{45} detection efficiency was much poorer in this reagent than in the other reagents. Diethyl phosphate and diphenyl phosphate were insoluble in toluene at the 1:3 (v/v) ratio. Bis (2-ethylhexyl) hydrogen phosphate reacted with the fluor system, producing a brownish discoloration of the solution which resulted in serious quenching.

The ratio of dialkyl phosphate to toluene was 1:3 (v/v), and the fluor system was PPO-DMPOPOP. Benzene and xylene were tested, and found to offer no advantages over toluene as solvents for the dialkyl phosphate - CaCl_2 - SrCl_2 complex.

After having started this study, the original suppliers of diisopropyl phosphate stopped making this reagent. Di-n-propyl phosphate (DNP) was found to be an excellent substitute, and was used for the remainder of this study. Two lots of DNP, obtained one year apart, were tested and found to be essentially the same in Ca^{45} -detection efficiency (Table 2), and salt-holding capacity.

Table 2. Comparison of Two Lots of
Di-n-propyl Phosphate

Samples	Lot 1965	Lot 1966
1	8229 counts	8254 counts
2	8282	8262
3	8225	8201
Avg.	8245	8239
S.D.*	45	47

*Level for significant difference.

B. Selection of Fluor for Optimum Counting of Ca⁴⁵-Sr⁸⁹
Double Labelled, Ca⁴⁵ and Sr⁸⁹ Samples in the DNP-
Toluene Matrix

For liquid scintillation to be a sensitive and efficient process, the fluorescent emission spectrum of the excited fluor molecules must correspond to the sensitive spectrum of the photocathode. Fluor systems usually consist of a primary and a secondary solute. The function of the primary solute is to receive the excitation energy from the solvent molecules, and either emit it as photons, or transfer it on to the secondary solute. The most efficient primary solutes, PPO and PT, have fluorescent emission peaks at wave lengths below

the sensitive spectrum region of the photocathode used in the Packard "Tri-Carb". Thus, secondary solutes are used to shift the wave length of the light emitted by the primary solute to a region of greater photocathode sensitivity. The commonly used secondary solutes cannot function as primary solutes because they are usually too insoluble, too expensive, or have too great a degree of self-quenching.

The purpose of the work described in this section was to select a fluor system for optimum counting of Ca^{45} and Sr^{89} samples in the DNP-toluene system.

Comparison of Four Fluor Mixtures. The fluors used in the preliminary study, PPO and DMPOPOP, seemed to react with the dialkyl phosphates, causing a yellow-green color. This color formed immediately upon mixing of the reagents, and was then stable for several months. However, this color appeared to be a source of "color quenching", and thus, other fluor systems were tested.

The results of a comparative study of four fluor systems are given in Table 3. The PPO-DMPOPOP and the BBOT (which also had a light yellow color in the DNP-toluene reagent) were shown to be less efficient

Table 3. Comparison of Fluor Systems for Ca⁴⁵ and Sr⁸⁹ Counting in Di-n-propyl Phosphate:Toluene Solution (1:3, v/v)

Discriminator Setting in Each Channel	Items of Measurement	Fluor Systems			
		PPO-NPO	PT-NPO	PPO-DMPOPOP	BBOT
Ca ⁴⁵ counting in Channel I with discriminator settings, 0090-0500	HVT	3.40	3.25	3.55	3.80
	Gain, %	100	100	100	100
	Efficiency, %	84	85	72	73
	Background, cpm	27	28	25	26
Sr ⁸⁹ counting in Channel II with discriminator settings, 0350-1000	HVT	3.40	3.25	----	----
	Gain, %	30	31	----	----
	Efficiency, %	62	62	----	----
	Background, cpm	22	18	----	----

for Ca^{45} counting than were the PPO-NPO and PT-NPO systems. Thus, the DMPOPOP and BBOT systems were not examined for Sr^{89} counting efficiency. The other two systems were closely comparable in both Ca^{45} and Sr^{89} detection efficiencies. These two systems, containing NPO, were clear and colorless, indicating that it was the DMPOPOP which caused the yellow color in the original fluor matrix.

In general, the more the sample is quenched, the greater is the high voltage (HVT) required to attain the balance point operating condition. Thus, the high HVT setting, and the lower Ca^{45} detection efficiency showed that BBOT was the most quenched system. The least quenched fluor system for Ca^{45} detection was the PT-NPO system. The increased gain necessary for attaining the Sr^{89} balance point indicated that the Sr^{89} count was more quenched in PT-NPO than in PPO-NPO. However, a general comparison of these two showed little advantage of one over the other at this point in the discussion. These two fluor systems were subjected to further testing.

Primary Fluor Concentration. The concentration of fluors used in the above experiments were similar to

those found in current liquid scintillation literature. However, these concentrations are not necessarily the optimal fluor concentrations, and, as Bush and Hansen (2) point out, the most practical comparison of different fluors in a given sample is based on counting efficiencies at the optimal concentrations of the respective fluors. These authors have also shown the need for increased amounts of the primary fluor in quenched samples, and preliminary work showed that quenching occurred in DNP-toluene samples containing large quantities of CaCl_2 . Thus, it was necessary to determine the optimal concentrations of PT and PPO.

Two series of fluor solutions were prepared in DNP-toluene (1:3, v/v). One series contained PPO in concentrations of 1.0, 2.0, 3.0, 4.0, and 5.0 g/l. The other series contained PT in concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 g/l. All the above fluor solutions contained 0.5 g/l NPO. Duplicate samples were prepared for each of the above fluor solutions from a stock solution of Ca^{45} containing only trace quantities of CaCl_2 . Figure 1 shows how the Ca^{45} count rate varied with fluor concentration. The change in the slope of the line between 4.0 and 5.0 g/l PPO is only 0.85 per cent (which is about equal to the

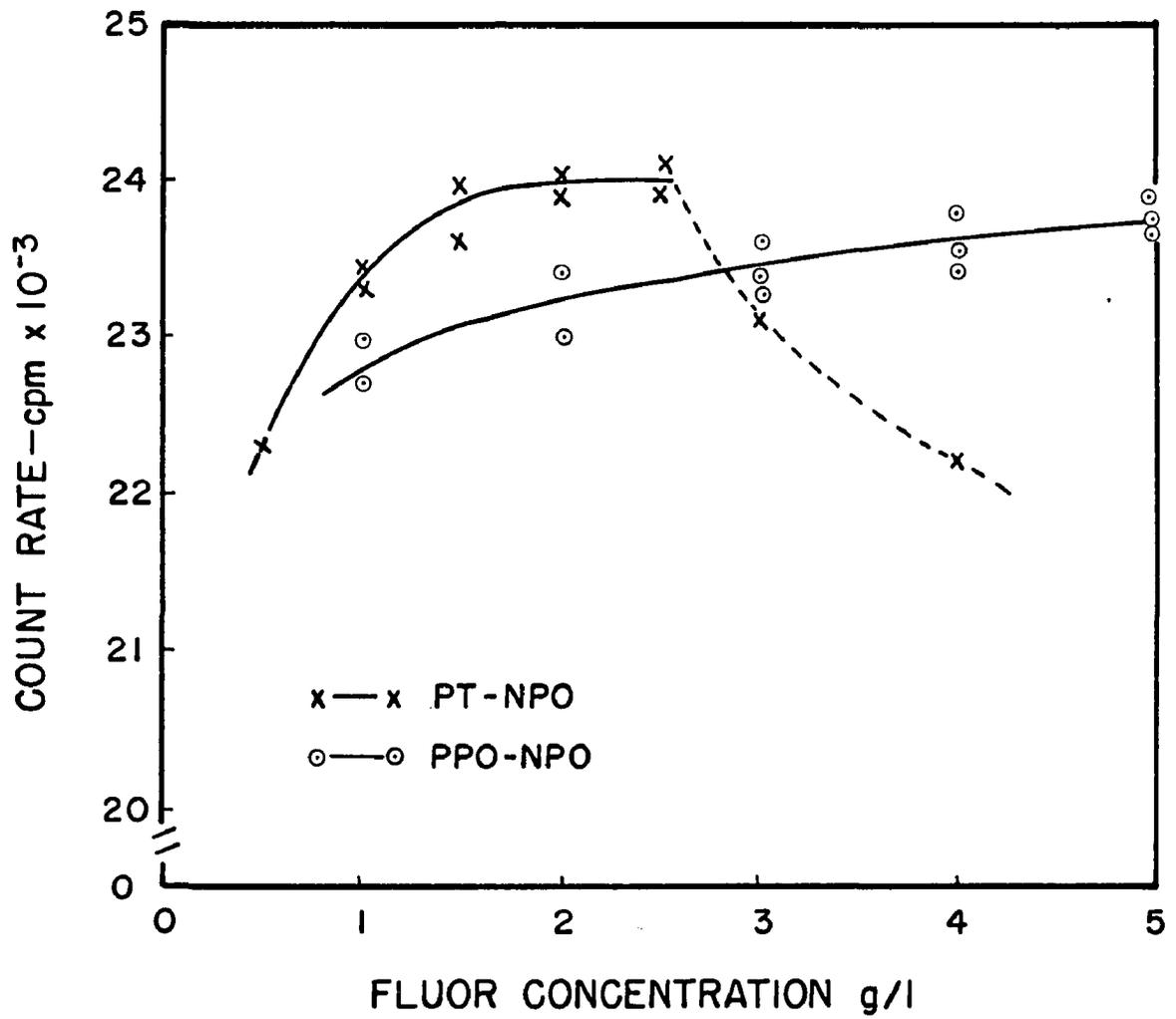


Figure 1. Effect of Fluor Concentration on Ca^{45} Count Rate.

precision of the method), indicating that 5.0 g/l was nearly optimal for PPO. However, the maximum Ca^{45} count rate in PT is between 2.0 and 2.5 g/l, and at higher PT concentrations the count rate decreased markedly. The reason for this decrease is that PT is not completely soluble in quantities greater than 2.5 g/l at -5°C .

The effect of fluor concentration on the Sr^{89} count rate was studied in the same manner as described above for Ca^{45} . Figure 2 shows that at 5.0 g/l PPO, the Sr^{89} count rate begins to level off, and though this may not be the optimal concentration for Sr^{89} , it is a practical concentration for Ca^{45} - Sr^{89} double-labelled sample counting. However, the Sr^{89} count rate in the PT-NPO fluor system showed no levelling-off effect up to 2.5 g/l, and quantities greater than 2.5 g/l PT were not completely soluble. In fact, at the higher concentrations, a reduction in Sr^{89} count rate was observed, and this probably was due to excess solute quenching.

Secondary Solute. A study was performed to determine the necessity of using a secondary solute to increase the counting efficiency of the PT and PPO

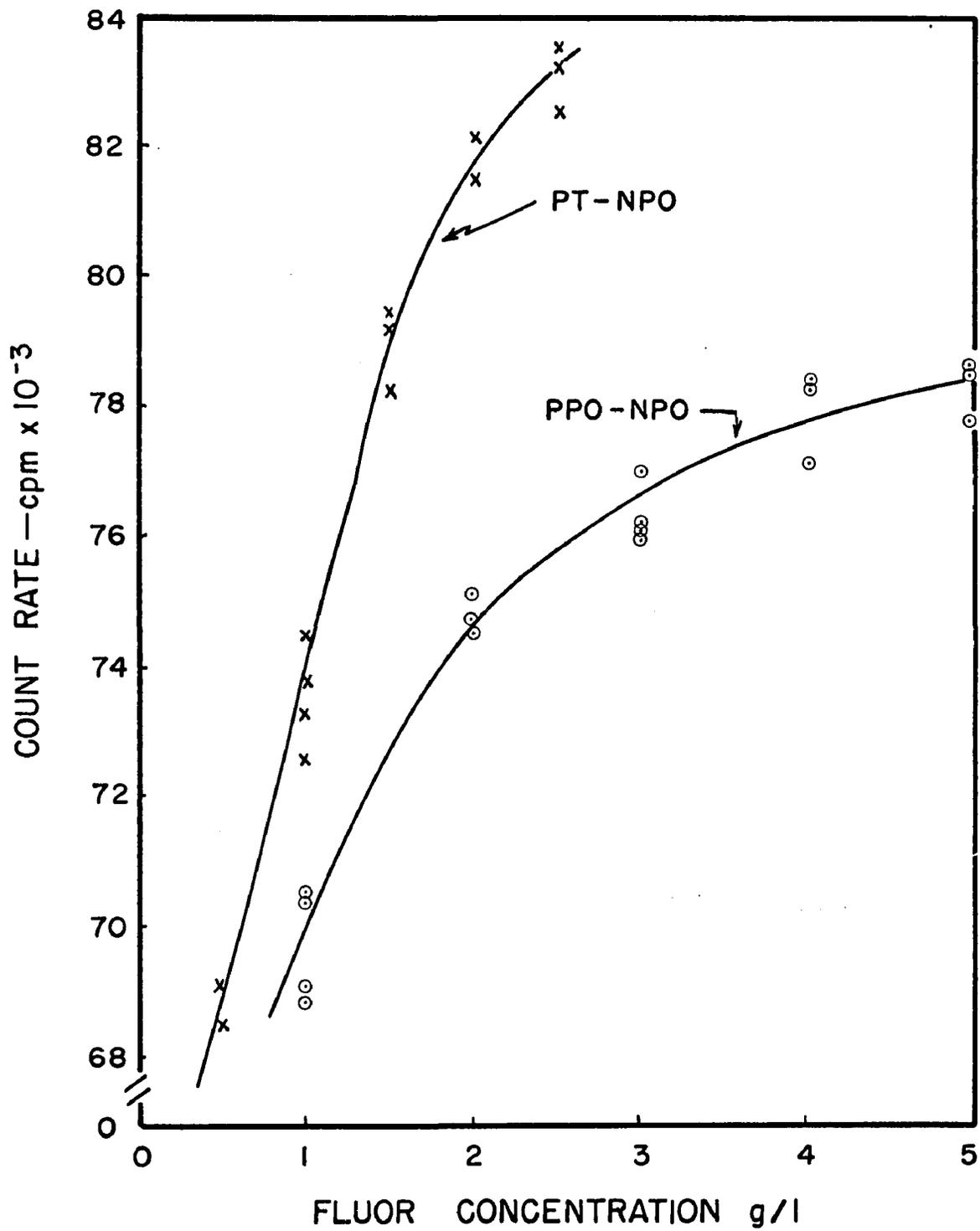


Figure 2. Effect of Fluor Concentration on Sr^{89} Count Rate.

systems. Four fluor solutions were prepared, one contained 2.5 g/l PT; the second contained 2.5 g/l PT and 0.5 g/l NPO; the third contained 5.0 g/l PPO; and the fourth contained 5.0 g/l PPO and 0.5 g/l NPO. Duplicate Ca^{45} and Sr^{89} samples (containing trace levels of salts) were prepared in accordance with the given procedure using the above fluor systems. These samples were counted using balance point settings determined for both isotopes in each fluor solution.

The addition of NPO to both PT and PPO (Table 4) caused slight, but significant, decreases in both the Ca^{45} and Sr^{89} count rates. The reason for these decreases in count rates was probably due to solute quenching. Some investigators (15) suggest using the ratio of the square of Ca^{45} efficiency to Sr^{89} efficiency ($e_{\text{Ca}}^2/e_{\text{Sr}}$), in the lower energy channel, as a figure of merit to compare different counting systems. Such a comparison (Table 4) showed that the systems without secondary solutes were better than those with secondary solutes. This work, then, indicated that there was no particular advantage to using a secondary solute, and there may be some disadvantage to using any at all.

Table 4. Effect of Secondary Solute on Ca⁴⁵ and Sr⁸⁹ Count Rates

Discriminator Settings in each Channel	Items of Measurement	Fluor Systems			
		PT	PT-NPO	PPO	PPO-NPO
Ca ⁴⁵ counting in Channel I with discriminator set- tings, 0090 to 0500.	HVT	3.10	3.10	3.20	3.32
	Gain, %	100	100	100	100
	CPM	8280	8130	8161	8086
Sr ⁸⁹ counting in Channel I with dis- criminator settings, 0090 to 0500.	CPM	1824	1776	1756	1782
	e^2_{Ca}/e_{Sr}	1.88	1.86	1.90	1.84
Sr ⁸⁹ counting in Channel II with dis- criminator settings 0350 to 1000.	HVT	3.10	3.10	3.20	3.32
	Gain, %	17.5	17.5	15.0	17.5
	CPM	11131	10780	12008	11711
The background was 24 cpm for each fluor system, in both channels.					

From the above studies either PT (2.5 g/l) or PPO (5.0 g/l) with no secondary solute, would be suitable fluors for $\text{Ca}^{45}\text{-Sr}^{89}$ counting. However, in highly quenched systems, secondary fluors may be required. This was discovered when Ca^{45} samples containing up to 1000 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were prepared and counted in a DNP-toluene (1:1, v/v) mixture containing only PT as the fluor. Figure 3 shows that there was a 50 per cent reduction in the Ca^{45} count rate when only 100 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was dissolved in the above solution. When 1000 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was dissolved in this system, the reduction in Ca^{45} count rate was over 80 per cent. The addition of NPO (0.5 g/l) to the individual samples increased the Ca^{45} count rate, but the quenching effect was still greater than desirable. The addition of PPO (5.0 g/l) to each of the samples, already containing PT and NPO, increased the count rate very markedly. In fact, the count rate decreased linearly as the quantity of dissolved $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ increased, and 1000 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ caused only a 15 per cent decrease in the Ca^{45} count. An experiment to be reported later in this dissertation will show that in the presence of a secondary solute (NPO), the quenching effect of increased amounts of DNP was relatively small. Then the

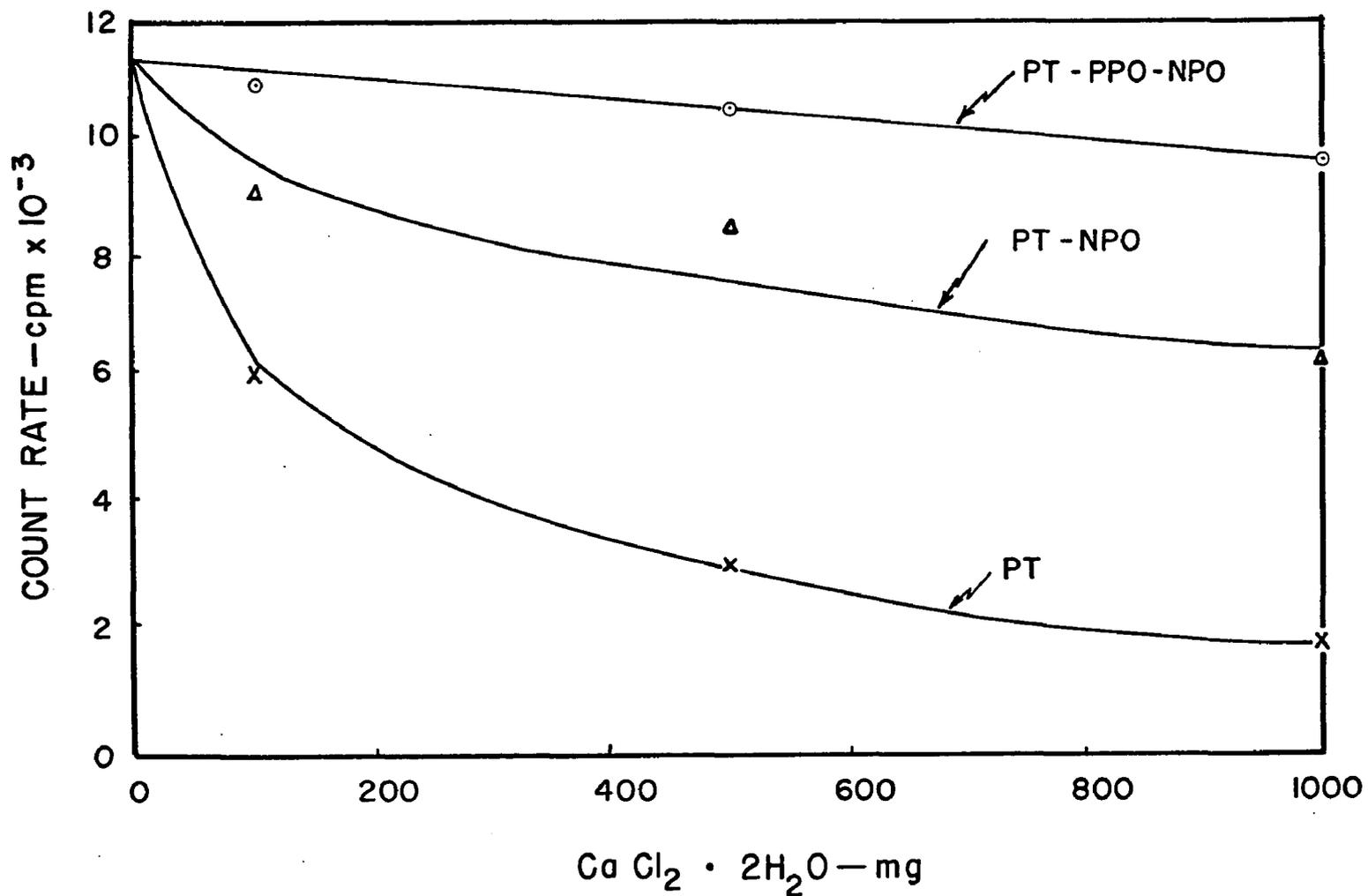


Figure 3. Salt Quenching Effect in Three Different Fluor Systems in DNP:Toluene (1:1) Solution.

great quenching effect observed in the above experiment was due to the combined factors of poor fluor system, increased amount of DNP, and increased amounts of solubilized $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$.

The results of these studies then indicate that the best fluor system for the radioassay of Ca^{45} - Sr^{89} samples by liquid scintillation counting would be PPO-NPO (5.0-0.5 g/l). Such a fluor system would give good counting characteristics over a wide range of quenching conditions. No secondary solute would be required where the samples were known to contain only trace levels of CaCl_2 or SrCl_2 . However, it should be kept in mind, as this study indicates, and as Bush and Hansen (2) noted, highly quenched systems may require increased amounts of solutes, and, in fact, could require an altogether different fluor system.

C. Radioassay of Ca^{45} , and Ca^{45} - Sr^{89} Double Labelled Samples

Calculation of Activity of Ca^{45} Samples. Two techniques were used to calculate Ca^{45} activity in samples containing only this isotope; one, the "channels-ratio" technique, and the other, the "internal standard"

technique. Generally speaking, the "internal standard" technique provides the more accurate and precise way for determining quenching in a given sample. However, Herberg (10) has recently published an excellent discussion of the "channels-ratio" technique in which he states that with sufficiently long counting times, the accuracy and precision attainable with the "channels-ratio" technique can be as good as that attainable by use of "internal standards". Also, Herberg pointed out that samples of widely different efficiencies may require different "channels-ratio" quench correction curves. Another advantage of the "channels-ratio" technique is that it is less time consuming, requiring only one count of each sample. Thus, the "channels-ratio" technique is recommended for more convenient routine radioassay of large numbers of samples.

The "channels-ratio" technique requires that one channel be used at balance point (analyzer monitor), while the other is used at the far end of the beta spectrum. In this work with Ca^{45} in the DNP-toluene system, the ratio of analyzer monitor count rate to the quench monitor count rate was arbitrarily set at 10:1 for the least quenched sample. The spectrometer discriminator settings were 0090 and 0500 for

channel I, and 0336 and 1000 for channel II. The gain was 100 per cent for both channels. Samples with 0 to 500 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were counted, observing the quench ratio. This ratio (R) is calculated and plotted (Figure 4) as a function of the ratio of the absolute count (dpm) to the observed count (cpm). This ratio of absolute count to observed count is called the correction factor. The data for Figure 4, the quench correction curve, is given in Table 5. The R-value can be calculated for any unknown sample, and then the corresponding correction factor (CF) determined graphically. Multiplication of the observed count rate by CF results in the absolute count of the sample in question. This is comparatively simple and obviates the necessity of using an internal standard or any further time-consuming experimental manipulations.

Calculation of Sr^{89} and Ca^{45} Activity from Double Labelled Samples. Counting a double labelled sample with the "Tri-Carb" liquid scintillation spectrometer yields a different count rate in each of the two channels; one corresponding mainly to the low energy isotope, and one the high energy isotope. Ideally, one would attempt to adjust the instrument settings so that only the counts from one isotope appear in one channel and only the

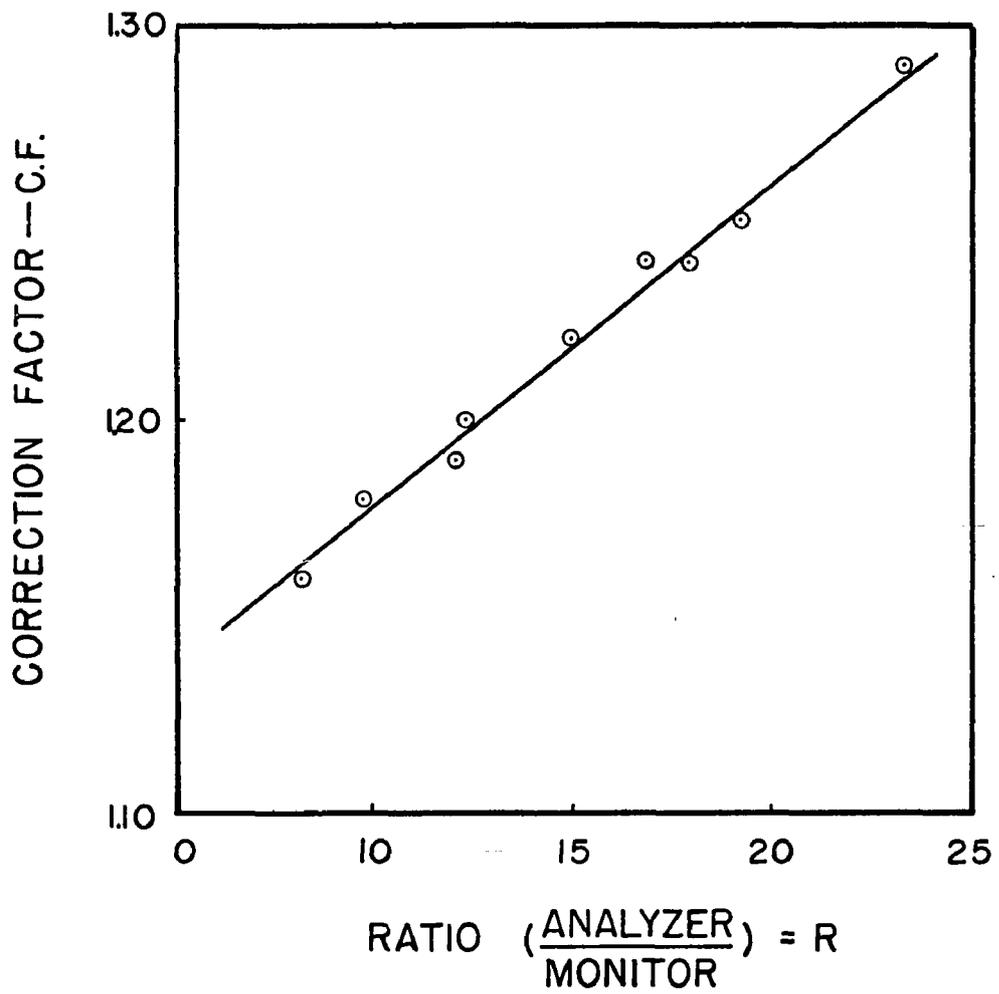


Figure 4. Channels Ratio Method for Quench Correction.

Table 5. Method for Quench Correction of Ca^{45}
Count Rate

Quench Agent (CaCl_2)	Count Rate		Ratio (R)	Correction Factor (C.F.)
	Channel I (Analyzer)	Channel II (Quench)		
mg	cpm	cpm		
0	30650	3120	9.80	1.18
0	31158	3791	8.22	1.16
100	30229	2458	12.30	1.20
100	30241	2492	12.10	1.19
200	29667	1979	15.00	1.22
300	29171	1726	16.90	1.24
400	29032	1595	18.15	1.24
500	28006	1204	23.40	1.29
500	28899	1508	19.15	1.25

counts from the other isotope appear in the second channel. It is not possible to effect a complete separation of the two beta energy spectrums. Though it is possible to contain essentially all the counts from the lower energy isotope in one channel, the counts from the higher energy isotope will appear in both channels. Thus, it is necessary to set up and solve two simultaneous algebraic equations to calculate the activity of each isotope being counted.

In the present case the low energy isotope is Ca^{45} (0.25 Mev) and the high energy isotope is Sr^{89} (1.47 Mev). It should be pointed out, here, that this double labelled sample counting technique does not yield significant results where the ratio of energies of the two isotopes is less than four. The lower energy channel is labelled Channel I and the higher energy channel is labelled Channel II. In the following sections the necessary equations are derived and solved.

For ease of derivation and future use in a FORTRAN Program, the following designations are used:

- A - total count rate in channel I
- B - total count rate in channel II
- C - count rate of $\text{Sr}^{89} + \text{Sr}^{90}$ in channel I
- D - count rate of $\text{Sr}^{89} + \text{Sr}^{90}$ in channel II
- E - count rate of Ca^{45} internal standard in channel I
- F - activity (in dpm) of Ca^{45} internal standard
- G - activity (in dpm) of Ca^{45} sample to be determined
- H - activity (in dpm) of Sr^{89} internal standard
- K - activity (in dpm) of Sr^{89} sample to be determined.

Subscripts m and p are used to designate internal standards and unknown samples, respectively.

Calculation of the Ca⁴⁵ Activity from Double Labelled Samples. The necessary equations for calculating Ca⁴⁵ activity are:

$$A = (G)(E/F) + C_p$$

$$B = (G) (\text{Ca}^{45} \text{ efficiency in channel II}) + D_p$$

The counting conditions were set so that the Ca⁴⁵ efficiency in channel II was essentially zero. So the solution of these two equations for the Ca⁴⁵ activity, G, is:

$$D_p A - D_p C_p = G(E/F) D_p$$

$$B C_p - D_p C_p = 0$$

and
$$D_p A - B C_p = G(E/F) D_p$$

so
$$G = \frac{A - B(C_p/D_p)}{(E/F)}$$

Now
$$\frac{C_p}{D_p} = \frac{C_m}{D_m}, \text{ so that } G = \frac{A - B(C_m/D_m)}{(E/F)}$$

$$= \text{Ca}^{45} \text{ activity (in dpm).}$$

In this formula, the factor $B(C_m/D_m)$ corrects channel I count for any Sr^{89} (contaminated with Sr^{90}) that is being counted in channel I, and E/F is merely the counting efficiency of Ca^{45} in channel I.

Calculation of the Sr^{89} Activity from Double Labelled Samples. The Sr^{89} activity could be obtained by solution of equations similar to the ones used for Ca^{45} , if Sr^{90} were not present or if the efficiency of Sr^{90} could be determined. However, Sr^{90} is present in commercial Sr^{89} samples, and the determination of Sr^{90} counting efficiency would require the cumbersome and inconvenient use of three internal standards, one each for Ca^{45} , Sr^{89} , and Sr^{90} . Thus, the following method for calculating Sr^{89} activity was developed.

For any Sr^{89} secondary standard, containing Sr^{90} , that is to be used as an internal standard:

$$D_1 = X_1 + R_1$$

and

$$D_2 = X_2 + R_2$$

where D is the $\text{Sr}^{89} + \text{Sr}^{90}$ count rate, X is the count rate of Sr^{89} , and R the count rate of Sr^{90} . The subscripts, "1" and "2", refer to different counting dates.

For this work, R_1 equals R_2 , since essentially no decay of the long-lived Sr^{90} occurred in the relatively short duration of these experiments. Now, $X_2 = X_1 e^{-\lambda t}$, where " λ " is the decay constant of Sr^{89} , and " t " is the time elapsed between counting dates.

Substituting $X_1 e^{-\lambda t}$ for X_2 in the second equation and subtracting equation 2 from equation 1 yields:

$$D_1 - D_2 = X_1 - X_1 e^{-\lambda t}$$

Solving for X_1 yields:

$$X_1 = (D_1 - D_2) / (1 - e^{-\lambda t})$$

Now the activity of the Sr^{89} (H) in the internal standard can be calculated from the equation:

$$H = \frac{X_1}{\text{Efficiency of Sr}^{89}}$$

The efficiency of Sr^{89} is determined by counting a sample of Sr^{89} containing essentially no Sr^{90} .

The Sr^{90} to Sr^{89} ratio is the same in both the unknown sample and the internal standard. This ratio is designated L, so that:

$$\frac{R_p}{X_p} = \frac{R_m}{X_m} = L.$$

$$\text{Further, } X_p + R_p = X_p + X_p L$$

and this is the total count rate of Sr^{89} plus Sr^{90} for any unknown sample. Thus,

$$X_p + X_p L = B.$$

Solving for X_p yields:

$$X_p = \frac{B}{(1 + L)} = \frac{B X_m}{(X_m + R_m)}, \text{ where}$$

$$(X_m + R_m) = D_1 = D_m$$

Now the activity of the Sr^{89} in the unknown sample may be determined by dividing X_p by the counting efficiency of Sr^{89} in the unknown sample. This efficiency is obtained by counting an Sr^{89} internal standard in the unknown sample, and using the ratio X_m/H .

$$\text{So, } K = X_p / \frac{X_m}{H} = \frac{B X_m}{(X_m + R_m)} / \frac{X_m}{H}$$

$$\text{and } K = \frac{BH}{X_m + R_m} = \frac{BH}{D_m}$$

The formulas for calculating Ca^{45} and Sr^{89} activities (i.e., G and K) can be written into a

FORTTRAN Program, and thus, the calculations of large volumes of data were simplified. Herberg (9) has discussed the statistical aspects of double isotope liquid scintillation counting.

Optimum DNP-Toluene Ratio. The more alkyl phosphate that can be used, the more CaCl_2 or SrCl_2 that can be solubilized. However, an increase in the DNP to toluene ratio would increase the quenching due to the DNP (though alkyl phosphates can be good energy transfer agents).

To determine the quenching effect of DNP, carrier-free samples of Ca^{45} and Sr^{89} were prepared in 1:4, 1:3, 1:2, and 1:1 DNP-toluene scintillation solutions. The 1:4 mixture (Figure 5) was found to be the most efficient and the 1:1 the least efficient for both Ca^{45} and Sr^{89} counting. Even so, the 1:1 mixture resulted in a counting efficiency 90 per cent that of the 1:4 mixture.

Optimum Sample Volume. Since, even in liquid scintillation methods, sample geometry is a factor, it was deemed important to examine the influence of sample volume on Ca^{45} and Sr^{89} detectability. Thus, carrier-free samples of Ca^{45} and Sr^{89} were prepared

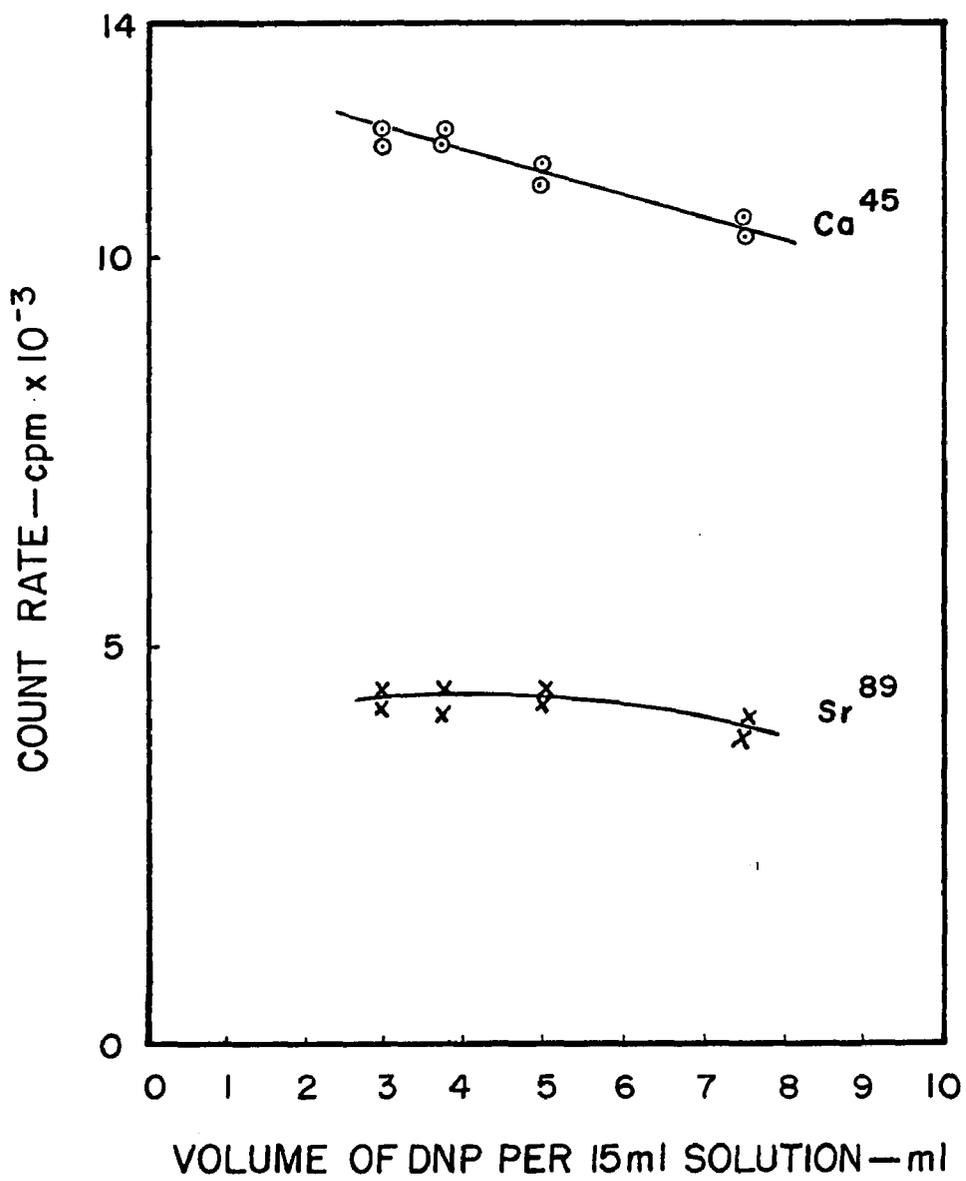


Figure 5. Effect of DNP on Ca^{45} and Sr^{89} Count Rates.

in various volumes (from 10 to 20 ml) of DNP:toluene (1:3, v/v). Figure 6 shows that the optimum Ca^{45} and Sr^{89} detection occurred between 10 and 16 ml of the DNP reagent. Since the volume used is critical with respect to the amounts of calcium or strontium which can be solubilized, a volume of 15 ml was chosen. This represented a compromise between counting efficiency, CaCl_2 and SrCl_2 capacity, and insensitivity of the count rate to slight volume changes (15 ml was still on the flat portion of the curve).

Salt-Holding Capacity of the DNP-Toluene Matrix.

This experiment was designed to determine the maximum quantities of CaCl_2 and SrCl_2 that could be solubilized, and also to determine the magnitude of the quenching effect of these salts on the Ca^{45} and Sr^{89} count rates. Three series of samples were prepared as follows:

- (1) one ml of a stock Ca^{45} solution was added to each of a series of samples containing quantities of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ranging from 0 mg to 1000 mg;
- (2) one ml of a stock Sr^{89} solution was added to each of a series of samples containing $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in the same range as above;
- (3) to the third series of samples, containing the same quantities of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ as in the preceding

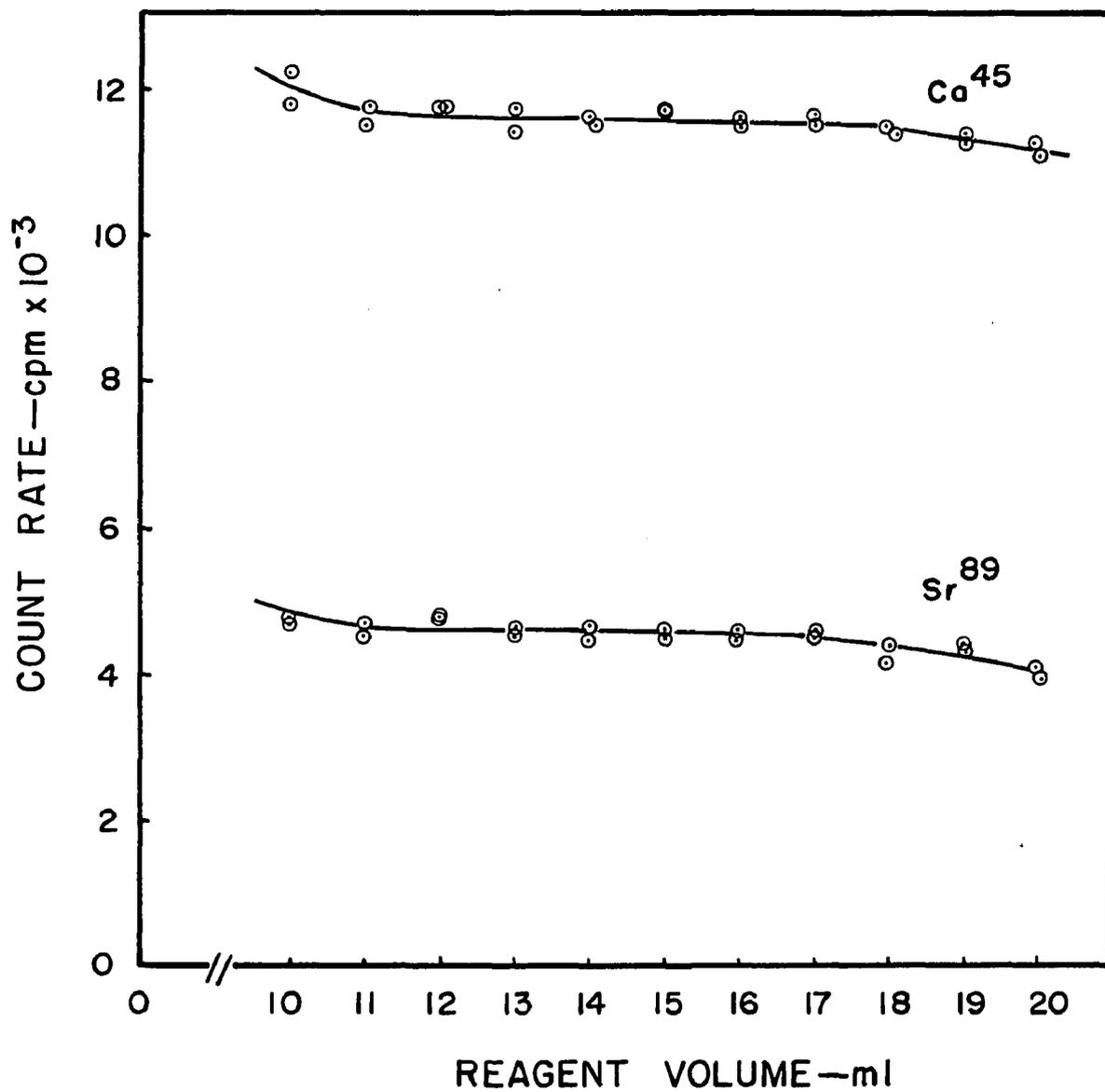


Figure 6. Effect of Changes in Reagent Volume on Ca⁴⁵ and Sr⁸⁹ Count Rate in DNP-Toluene (1:3, v/v).

two series was added one ml each of the stock Ca^{45} and Sr^{89} solutions. This last represented a double labelled sample. These samples were evaporated to dryness at $90-100^\circ \text{C}$, and dissolved in DNP-toluene (1:3, v/v).

The above experiment was repeated, increasing the quantities of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ up to 2000 mg, and using the 1:1 (v/v), DNP-toluene reagent. The samples were counted and the result plotted as counting efficiency versus weight of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Figure 7).

Increased concentrations of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ caused only a slight quenching of the Ca^{45} counting efficiency in the 1:3 reagent. This quenching effect resulted in a loss of counts at the rate of 0.01 per cent per mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. A 100 mg difference in $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ content between samples would have only 1.0 per cent relative error, and this is about the same as the statistical counting errors. The slope of the line for the 1:1 reagent was the same as that for the 1:3 reagent, and thus, the rate of loss of counts would be the same. These systems have high capacities for $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in that 15 ml of the 1:3 mixture will solubilize 500 mg and the 1:1 mixture will solubilize 1000 mg of the salt. This provides an ample working range for most experiments that will be encountered. Even if certain

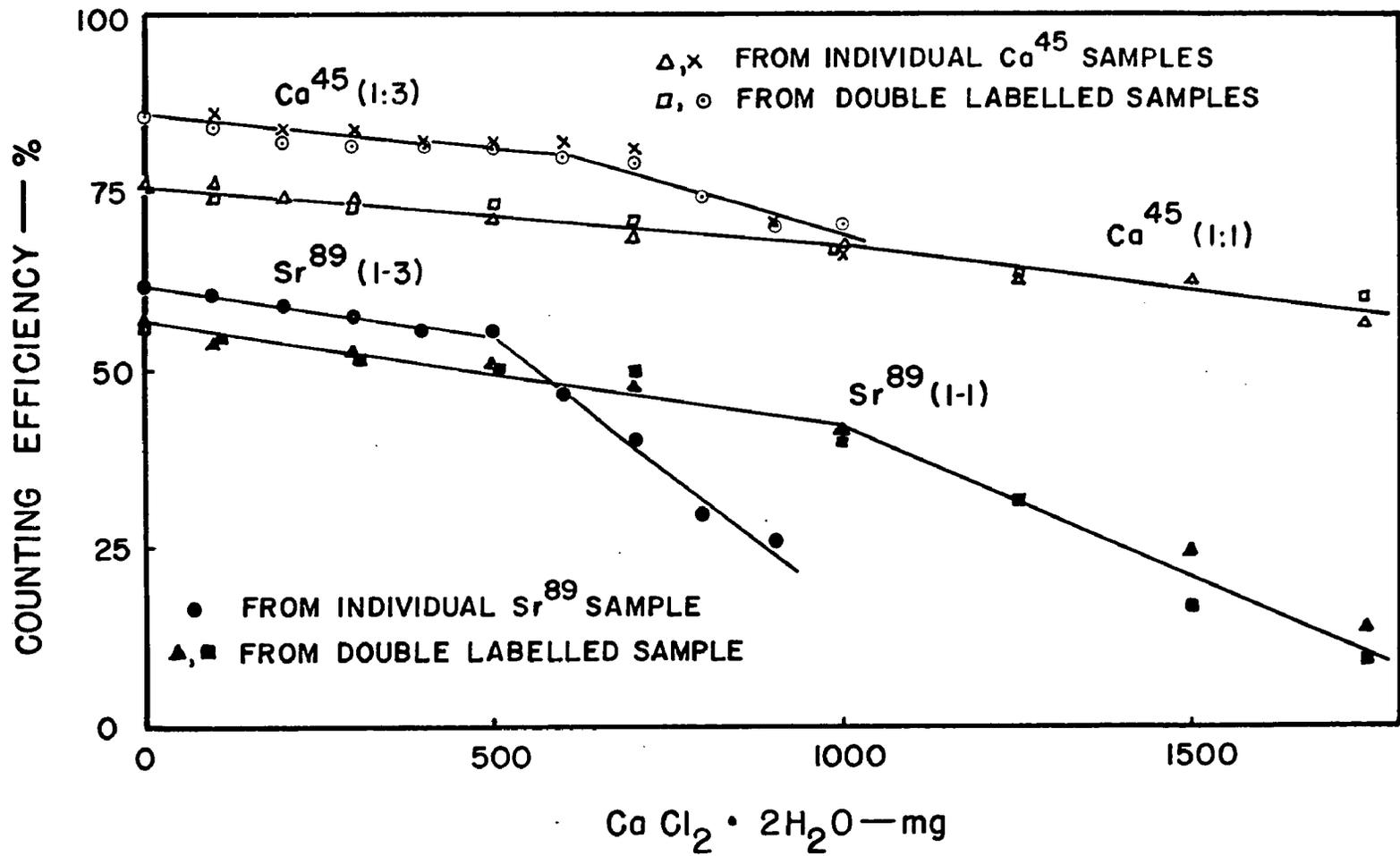


Figure 7. Quenching Effect of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ on Ca^{45} and Sr^{89} Counting Efficiencies.

samples, containing up to 1000 mg CaCl_2 , required the use of the 1:1 mixture, the Ca^{45} detection efficiency would be reduced only to about 67 per cent.

Dissolved $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ caused a somewhat greater quenching effect on Sr^{89} counting efficiency than it did on Ca^{45} counting efficiency. The loss of counts, however, was only 0.015 per cent per mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ for both the 1:3 and 1:1 reagents.

The quantities of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ that could be dissolved in each mixture could be judged visually, as well as observing where the "break" came in the efficiency versus weight of salt plots. This is a common technique for determining solubilities. Visually, it was observed that the 1:3 sample solutions were clear with no trace of precipitated salt up to 500 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. The 600 mg samples were cloudy, and those with larger amounts of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ had visible residues. The "break" in the Ca^{45} (1:3) curve came at 600 mg, and the one for Sr^{89} (1:3) came at 500 mg. In the 1:1 reagent there was no visible residue up to 1000 mg, and the "breaks" in the Sr^{89} and Ca^{45} plots came at 1000 mg.

In the above experiments, the double labelled samples were counted, and resolved into the individual isotope count rates. The efficiencies were calculated,

and plotted against weight of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Figure 7). There was no interference between the two isotopes in a double labelled sample, and the efficiencies of the isotopes were the same in a double labelled sample as they were in samples of the individual isotopes.

The capacity for these reagents to solubilize $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ was determined in the same manner as above. The quantities of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ used in these experiments ranged from 0 to 100 mg. Strontium chloride was much less soluble in the DNP-toluene reagent than was calcium chloride, the 1:3 mixture being able to solubilize only 50 mg, and the 1:1 only 100 mg. Yet this should not be a great limitation, since the calcium to strontium ratio in most biological samples is very high, usually exceeding 100 to one. There was no change (Figure 8) in the Ca^{45} and Sr^{89} counting efficiencies in the salt solubility range. Thus, SrCl_2 in the quantities that are encountered in biological samples should cause no interference in this procedure.

Precision and Efficiency. The precision of Ca^{45} and Sr^{89} counting in the DNP-toluene reagent was determined from counts of the individual isotopes, and

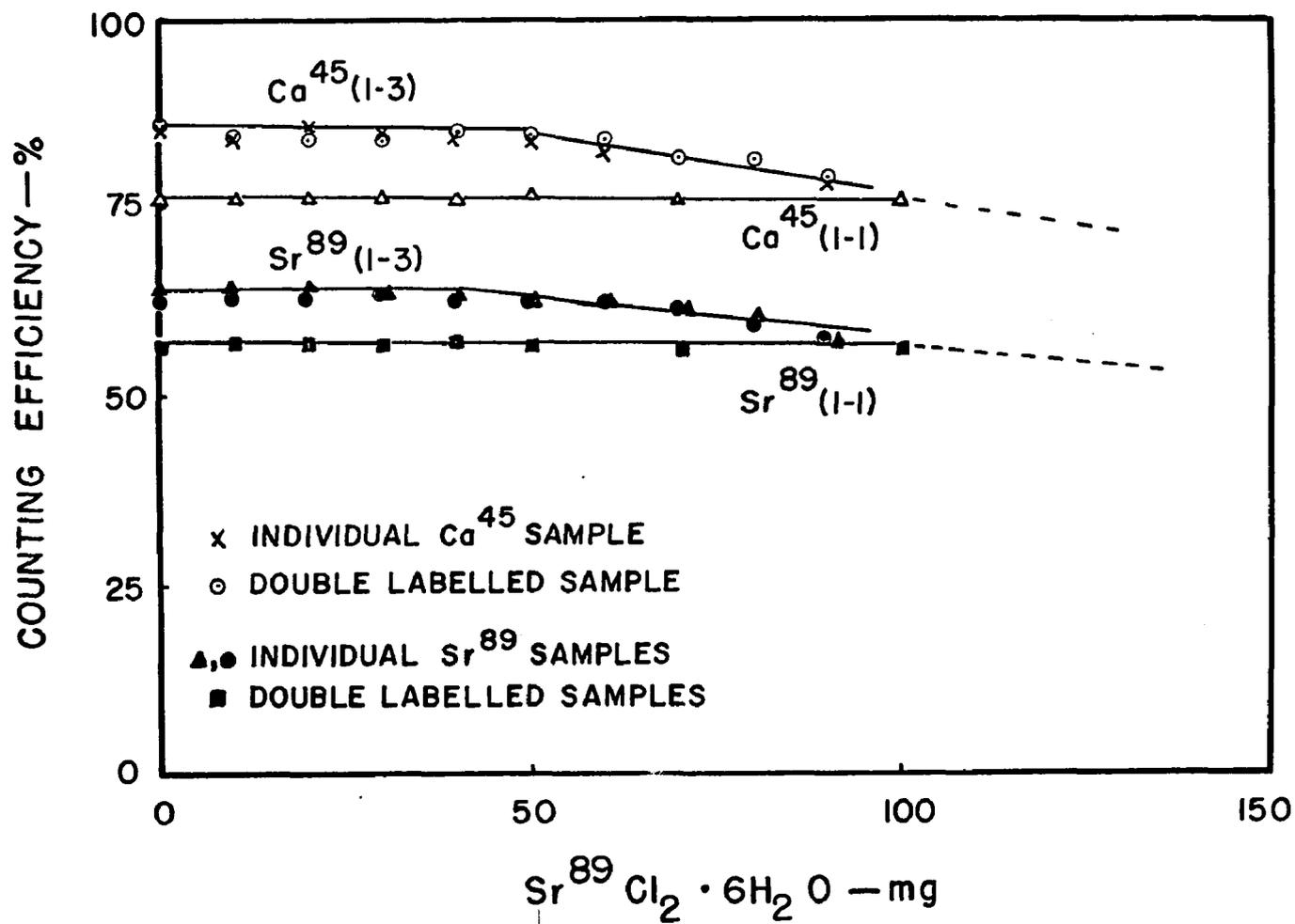


Figure 8. Quenching Effect of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ on Ca^{45} and Sr^{89} Counting Efficiencies.

from double labelled samples. The precision was also determined in the presence of trace levels of the salts $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, as well as in the presence of measurable quantities of these salts. Also, the precision was determined for several different counting rates.

The results listed in Tables 6 and 7 show that a precision of better than one per cent is possible for samples with moderate counting rates, where it is possible to collect 10,000 counts in a reasonable time. At lower counting rates, the precision was reduced to 3 to 4 per cent. Most of this loss in precision can be attributed to poorer counting statistics, and the precision could be improved by increasing the counting time. Dissolved salts seemed to have little or no effect on the precision of the method. The errors in the method can be attributed to: (1) normal laboratory technique errors, such as in pipetting and weighing, and (2) counting statistics.

Ca^{45} and Sr^{89} standards were obtained from the National Bureau of Standards. Samples of these, containing only trace levels of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, were prepared in the DNP:toluene (1:3,v/v) reagent. A Ca^{45} sample obtained from Oak Ridge National Laboratory

Table 6. Precision of Ca⁴⁵ and Sr⁸⁹ Counting

Isotope*	Number of Samples	Average Observed Count Rate	Relative Standard Deviation
		cpm	%
Ca ⁴⁵	8	9008	1.11
Ca ⁴⁵	10	88.8	4.26
Sr ⁸⁹	7	10591	1.08
Sr ⁸⁹	10	103.7	3.30
Background			
Channel I	4	25.9	1.7
Channel II	4	15.8	1.3
*Samples contain only one isotope. The samples were prepared in DNP-toluene (1:3,v/v), and were counted for 10 minutes			

Table 7. Precision of Ca⁴⁵-Sr⁸⁹ Double Labelled
Sample Counting in DNP-Toluene
(1:3, v/v)

Identification		Number of Samples	Average Observed Count Rate*	Relative Standard Deviation
			cpm	%
1.	Ca ⁴⁵	8	9054	0.67
	Sr ⁸⁹	8	10383	0.82
2.	Ca ⁴⁵	10	86.2	3.38
	Sr ⁸⁹	10	105.0	2.88
3.	Ca ⁴⁵	10	12455	0.32
	Sr ⁸⁹	10	4683	0.91
1.	Counting rates of Ca ⁴⁵ and Sr ⁸⁹ resolved from double labelled samples containing trace levels of CaCl ₂ ·2H ₂ O and SrCl ₂ ·6H ₂ O.			
2.	Counting rates of Ca ⁴⁵ and Sr ⁸⁹ resolved from double labelled samples of low activity, and containing only trace levels of SrCl ₂ ·6H ₂ O and CaCl ₂ ·2H ₂ O.			
3.	Counting rates of Ca ⁴⁵ and Sr ⁸⁹ resolved from double labelled samples containing 500 mg. CaCl ₂ ·2H ₂ O and 10 mg. SrCl ₂ ·6H ₂ O.			
* All samples were counted for 10 minutes.				

was also prepared. The Sr^{89} samples obtained from ORNL usually contained too much Sr^{90} to be used as counting standards.

The NBS Ca^{45} standard had an activity of 6000 dpm/ml on February 1, 1962. This sample was counted on January 12, 1965, and found to have a count rate of 3847 cpm/ml. Using a half life of 152 days for Ca^{45} (this half life for Ca^{45} was determined a number of times during this study), the NBS standard had an activity of 4442 dpm/ml on January 12, 1965. Thus, the counting efficiency was calculated to be 86.6 per cent. The ORNL Ca^{45} sample gave an efficiency of 86.1 per cent.

The NBS Sr^{89} standard had an activity of 5.346×10^4 dpm/ml on September 1, 1964. Using the half life of 50.4 days for Sr^{89} , this NBS standard was found to have an activity of 5532 dpm/ml on February 12, 1965. This sample was counted on February 12, 1965, and found to give a count rate of 3590 cpm/ml. The counting efficiency then was calculated to be 64.9 per cent.

Linearity of Counting. A method such as this should be valid for samples with greatly different

counting rates, and specific activities. The counting rate should be linear over a wide range, and the counting efficiency should not vary with the counting rate, as long as the salt (CaCl_2 and SrCl_2) content, and other quenching factors, remain constant. To test the linearity of counting Ca^{45} and Sr^{89} , samples, containing only trace levels of salts, and varying widely in activities, were prepared in the 1:3 reagent. The samples were counted, and the observed counting rate plotted against the actual disintegration rate (Figure 9). Both the Sr^{89} and Ca^{45} count rates were quite linear over a great range of activities, the Ca^{45} being linear from 260 to 110,000 cpm, and the Sr^{89} from 100 to 40,000 cpm. The isotopes were not tested outside these ranges.

Double labelled samples varying widely in counting rates, and containing 500 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 10 mg $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, were prepared in the 1:3 reagent. These samples were counted, the individual isotope counting rates resolved, and the observed counting rates plotted against the actual disintegration rates (Figure 9). The linearity of counting of each isotope, resolved from a double labelled sample count, was maintained over a wide range of specific activities. The

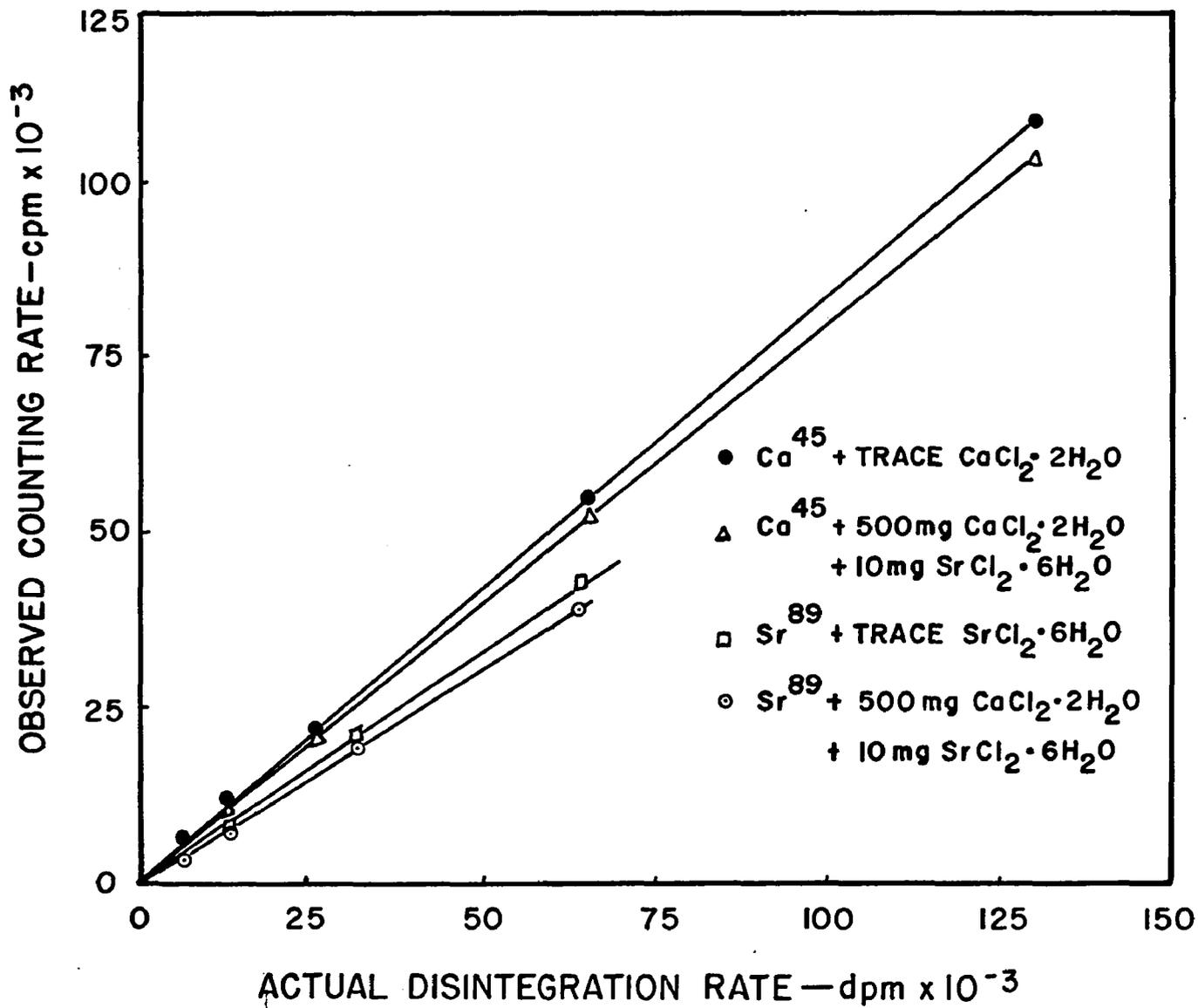


Figure 9. Linearity of Ca^{45} and Sr^{89} Counting.

Ca^{45} counting rate was linear over a range of specific activities of 0.5 to 220 cpm per mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and the Sr^{89} over a range of 10 to 4000 cpm per mg $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$.

It should be noted that the slopes of the lines in these plots (observed counting rate/actual disintegration rate) are a measure of the counting efficiency of the system.

Cation Interferences. Ions such as Fe^{+++} , K^+ , Na^+ , NH_4^+ , Mg^{++} , Mn^{++} , Ba^{++} , Al^{+++} , and Zn^{++} could be present in plant and soil extract samples, and thus, it was necessary to determine whether they had a quenching effect on Ca^{45} and Sr^{89} counting. Ten and 50 milligrams of the chlorides of these cations were added to standard amounts of Ca^{45} and Sr^{89} . The samples were then prepared in the prescribed manner using the 1:3 scintillation solution. After 24 hours of shaking, only the Mn^{++} , Zn^{++} and Al^{+++} salts were completely solubilized. There was only a slight residue in the Mg^{++} samples. The samples were counted at this time.

The data in Table 8 shows the effect of the various cations on the Ca^{45} and Sr^{89} detection efficiencies. For the purpose of comparison, an assignment of 100 per cent was made to Ca^{45} and Sr^{89} samples

Table 8. Effect of Various Cations on Ca^{45} and Sr^{89} Counting Efficiencies

Cation	Ca^{45} Efficiency*		Sr^{89} Efficiency*	
	10 mg	50 mg	10 mg	50 mg
	%	%	%	%
Al^{+++}	100.5	79.5	92.4	66.4
Mn^{++}	101.8	86.8	92.2	73.8
Zn^{++}	102.8	100.2	96.9	92.7
Mg^{++}	101.9	91.3	99.0	84.0
K^+	101.4	98.0	87.7	87.4
Na^+	96.5	94.7	87.8	86.0
NH_4^+	102.9	99.6	91.7	91.9
Ba^{++}	93.9	55.9	91.2	75.0
Fe^{+++}	82.2	16.3	76.7	10.3

*Percentages listed are based on an arbitrary assignment of 100% to Ca^{45} and Sr^{89} samples containing only trace quantities of CaCl_2 and SrCl_2 , but no other interfering cations.

containing only trace quantities of CaCl_2 and SrCl_2 , and containing no interfering cations. There was no loss in Ca^{45} counting efficiency due to the salts of Mn^{++} , Al^{+++} , Zn^{++} , and Mg^{++} at the 10 mg level, and only moderate losses due to salts of these ions at the 50 mg level. Even though the NH_4Cl and KCl did not go into solution completely, there was no decrease in Ca^{45} count rate at the 10 mg level, and only a slight decrease at the 50 mg level. NaCl , though insoluble, caused only slight losses in the Ca^{45} efficiency at the 10 and 50 mg levels. Only Fe^{+++} and Ba^{++} , at both the 10 and 50 mg levels, caused serious losses of Ca^{45} efficiencies, and this was primarily due to incomplete solution of these materials in the scintillation mixture. An additional source of quenching in the case of the ferric ion was the deep yellow color this ion imparted to the sample solution. None of the other ions caused any color in the scintillation solution.

All the cation interferences, except Mg^{++} and Zn^{++} , caused moderate to large losses in Sr^{89} counting efficiency at the 10 mg level. Fifty milligrams of the Al^{+++} , Ba^{++} , Fe^{+++} , Mg^{++} and Mn^{++} salts increased the quenching effect significantly over that of the

10 mg level. However, 50 mg of the K^+ , Na^+ , NH_4^+ and Zn^{++} salts caused only a slight increase in quenching over the 10 mg level samples.

Large amounts of the insoluble Fe^{+++} , Ba^{++} , K^+ , Na^+ and NH_4^+ salts in the sample to be analyzed will cause serious counting problems, and steps should be taken to remove these salts before the sample is analyzed. Though higher amounts of Al^{+++} and Mn^{++} salts increased the quenching effect, they were soluble, and thus would present less of a counting problem than would the insoluble salts. That is, homogenous samples would have better counting characteristics, and would yield more precise results than would heterogenous samples. This work indicates that this procedure, and this reagent (and others similar to it) could probably be used to solubilize Al^{+++} , Zn^{++} , Mn^{++} and possibly Mg^{++} salts for liquid scintillation counting of isotopes of these elements.

Anion Interferences. The phosphate, silicates, and sulfate salts of calcium and strontium were found to be poorly soluble under the conditions of sample preparation. Perchlorate salts were found to cause an instability of the samples, and upon short periods

of standing the occurrence of a precipitate was commonly observed. Even when this did not occur, quenching was too serious a problem in the perchlorate systems to consider these any further. The nitrate salts of calcium, although soluble, were noted to increase the quenching in Ca^{45} samples compared to the chloride salts. The chlorides were found to result in the most desirable counting system, and, for this reason, have been adopted for use in this method. Calcium acetate is as soluble as calcium chloride in the scintillator system, and causes little quenching; however, acetates would not be present after the usual mineral acid digestion of a biological sample.

Effect of Water in the Samples on Ca^{45} Detection.

The effect of water in a Ca^{45} system was studied by preparing samples of Ca^{45} and 100 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ dried at 130°C . After cooling, a given volume of water was added, and then 15 ml of the DNP-toluene-fluor reagent was added. Only after 0.2 ml of water had been added did the Ca^{45} count rate decrease considerably. But even at 0.1 ml, the lowest level H_2O studied, shaking for 3 hours still resulted in a very small droplet of water in the sample. The water phase was quite obvious in all the other samples.

Reagent and Sample Stability. Scintillation solutions, DNP-toluene (1:3, v/v) containing PPO-NPO fluor, of different ages were compared. These reagents had been stored in the dark at room temperature (24° C). The results (Table 9) showed that no loss in Ca^{45} or Sr^{89} detection efficiency, and no loss in ability to solubilize $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ or $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ occurred in solutions that were aged up to 7 months.

Samples stored in the dark at 0° C for periods up to one month showed no signs of deterioration. No check of sample stability was made beyond one month storage, or at different storage temperatures.

D. Radioassay of Sr^{89} Samples

The instrument operating conditions for the Ca^{45} - Sr^{89} double isotope counting technique were such that the maximum possible counting efficiency (for this isotope with this instrument) was not obtained. However, it is possible, when counting this isotope singly, to re-adjust the instrument settings to yield a maximum counting efficiency of more than 90 per cent. This section describes a study of the factors affecting the efficiency of counting Sr^{89} singly labelled samples.

Table 9. Stability of the DNP:Toluene (1:3) Scintillation Solution

A. Ca^{45} Detection Efficiency vs Age of Scintillation Solution			
Age of Scintillation Solution	No $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	500 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	
Months	%	%	
0	84.1	81.2	
1	84.9	82.2	
2-1/2	84.2	81.7	
7	84.1	81.6	

B. Sr^{89} Detection Efficiency vs Age of Scintillation Solution			
Age of Scintillation Solution	No $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$	50 mg $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$	
Months	%	%	
0	62.1	61.7	
1	61.8	61.4	
2-1/2	62.0	61.5	
7	62.7	61.5	

The factors studied were (a) different fluor systems, (b) the effect of varying the ratio of DNP to toluene, (c) the effect of varying the volume, (d) the quenching effect of SrCl_2 and CaCl_2 , (e) the precision and efficiency, and (f) the linearity of counting. It was not necessary to make a study of the effect of other cations or anions, or of the effect of water on the Sr^{89} counting rate, since these factors are dependent on the reagent, and not on the isotope being counted. Also, the effect of reagent age, and stability, would not be dependent on the isotope, and thus, were not included in this study. The effects of these factors on Sr^{89} counting can be evaluated from the previous discussion in connection with the $\text{Ca}^{45}\text{-Sr}^{89}$ method.

In this study, the Sr^{89} was essentially pure, containing less than 0.15 per cent Sr^{90} . Thus, it was not necessary to make any corrections for this isotope, and the total count collected was taken to be that from Sr^{89} alone. Commercial samples of Sr^{89} do contain up to 10 per cent Sr^{90} , and in such cases, it would be necessary to make a suitable correction when determining activity due to Sr^{89} . The formula

for this correction is obtained by solving the two simultaneous equations,

$$\begin{aligned} (\text{Total Count})_{\text{I}} &= (\text{Activity of Sr}^{90})(\text{Efficiency Sr}^{90})_{\text{I}} \\ &+ (\text{Activity of Sr}^{89})(\text{Efficiency of Sr}^{89})_{\text{I}} \end{aligned} \quad (1)$$

and,

$$\begin{aligned} (\text{Total Count})_{\text{II}} &= (\text{Activity of Sr}^{90})(\text{Efficiency Sr}^{90})_{\text{II}} \\ &+ (\text{Activity of Sr}^{89})(\text{Efficiency Sr}^{89})_{\text{II}} \end{aligned} \quad (2)$$

The solution is,

$$\begin{aligned} \text{Activity of Sr}^{89} &= \\ &\frac{(\text{Total Count})_{\text{I}} \frac{(\text{Efficiency Sr}^{90})_{\text{II}}}{(\text{Efficiency Sr}^{90})_{\text{I}}} - (\text{Total Count})_{\text{II}}}{(\text{Efficiency Sr}^{89})_{\text{I}} \frac{(\text{Efficiency Sr}^{90})_{\text{II}}}{(\text{Efficiency Sr}^{90})_{\text{I}}} - (\text{Efficiency Sr}^{89})_{\text{I}}} \end{aligned} \quad (3)$$

In these equations, the subscript I refers to measurements in channel I of the spectrometer, and the subscript II refers to measurements in channel II. The lower energy isotope, Sr^{90} (0.51 Mev), is counted primarily

in channel I, and the higher energy isotope, Sr⁸⁹ (1.47 Mev), is counted primarily in channel II.

The ratio $\frac{(\text{Efficiency of Sr}^{90})_{\text{II}}}{(\text{Efficiency of Sr}^{90})_{\text{I}}}$ was about one-fourth with the instrumental settings and counting conditions used, and the $(\text{Efficiency of Sr}^{89})_{\text{I}}$ was about one-fifth of the $(\text{Efficiency of Sr}^{89})_{\text{II}}$. Thus, the first term in the denominator of equation (3) was about one-twentieth of the $(\text{Efficiency of Sr}^{89})_{\text{II}}$, and can be ignored. Then, the formula that corrects the Sr⁸⁹ activity for any Sr⁹⁰ that may be present is,

$$\text{Activity of Sr}^{89} = \frac{(\text{Total Count})_{\text{I}} \frac{(\text{Efficiency of Sr}^{90})_{\text{II}}}{(\text{Efficiency of Sr}^{90})_{\text{I}}} - (\text{Total Count})_{\text{II}}}{(\text{Efficiency of Sr}^{89})_{\text{II}}}$$

This formula should be used when the Sr⁹⁰ activity is between 5 and 10 per cent that of Sr⁸⁹.

Another simplification can be made if the Sr⁹⁰ activity is less than 5 per cent of the Sr⁸⁹ activity. Since the Sr⁹⁰ efficiency in channel II actually was around 20 per cent, the first term on the right in equation (2) would be less than one per cent of the second term. Under these circumstances, the Sr⁹⁰ is

contributing a negligible amount to the total count in channel II, and thus, may be ignored when calculating the Sr^{89} activity.

Fluor Systems. The results of a comparative study of four fluor systems are given in Table 10. The most efficient system was found to be the PPO (5.0 g/l). However, these tests were made on samples containing only trace quantities of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, and previous work has shown that a secondary solute was needed when assaying samples containing higher amounts of the salt. The PPO-NPO (5.0-0.5 g/l) system could be used with only a small reduction in overall counting efficiency, and would be less quenched by higher salt concentrations.

Table 10. Comparison of Fluor Systems for Optimum Sr^{89} Counting in Di-n-propyl Phosphate:Toluene Solution (1:3, v/v)

Items of Measurement	Fluor System			
	PT	PT-NPO	PPO	PPO-NPO
HVT	5.00	5.00	5.00	5.00
Gain, %	10	10	10	10
Efficiency, %	88.0	85.3	95.0	92.6
Background, cpm	61	60	58	57

Optimum DNP-Toluene Ratio and Sample Volume. To determine the quenching effect of DNP, carrier-free samples of Sr^{89} were prepared in 1:1, 1:2, 1:3, and 1:4 DNP:toluene scintillation solutions. The 1:4 mixture (Figure 10) was found to be most efficient, and the 1:1 least efficient for Sr^{89} counting. The 1:1 mixture gave a counting efficiency that was 95 per cent that of the 1:4 mixture.

Carrier-free samples of Sr^{89} were prepared in various volumes (from 10 to 20 ml) of DNP:toluene (1:3, v/v). Figure 11 shows that there was a slight, almost linear decrease in count rate as the volume of reagent was increased. This decrease was about 0.06 per cent per ml of reagent. Volume, then, would be a negligible factor in Sr^{89} analysis by this technique.

Quenching Effect of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$.

The maximum amounts of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ soluble in the DNP:toluene reagent was established in an earlier study. The purpose of this section was to determine the quenching effect of these salts under conditions of maximum Sr^{89} counting. The samples, containing from 0 to 100 mg $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ and 0 to 1000 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$,

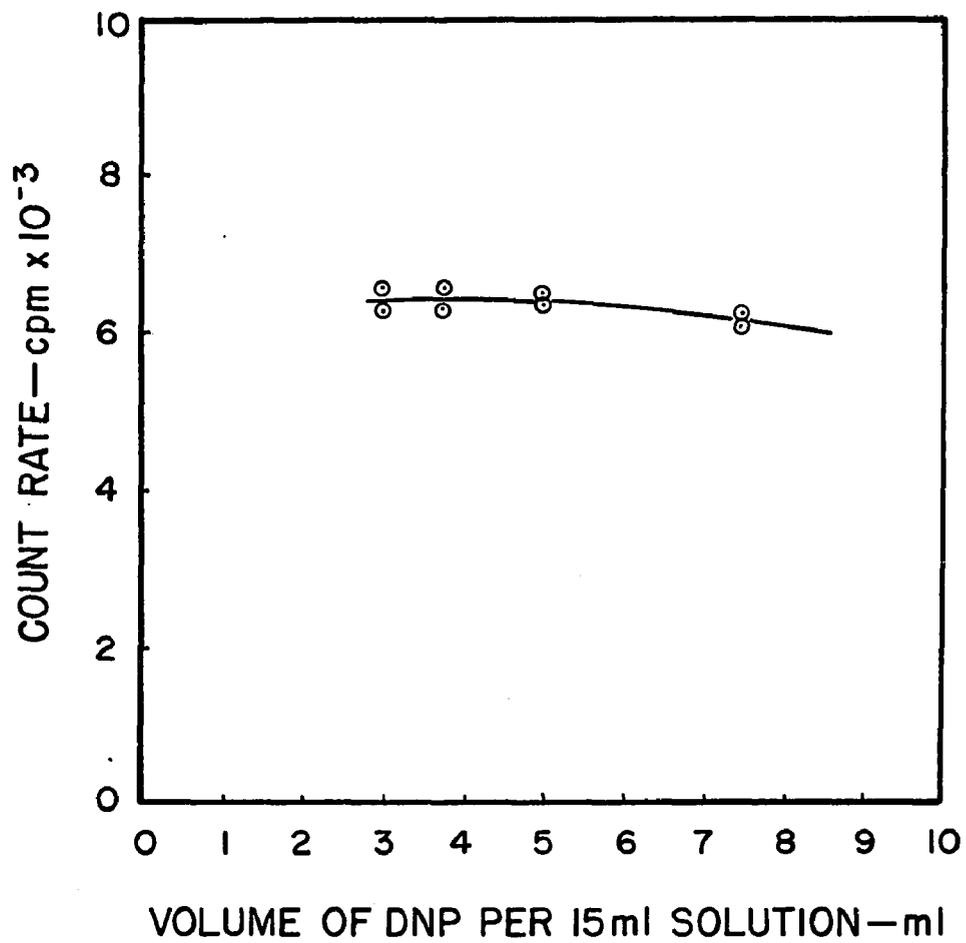


Figure 10. Effect of DNP on Sr⁸⁹ Count Rate.

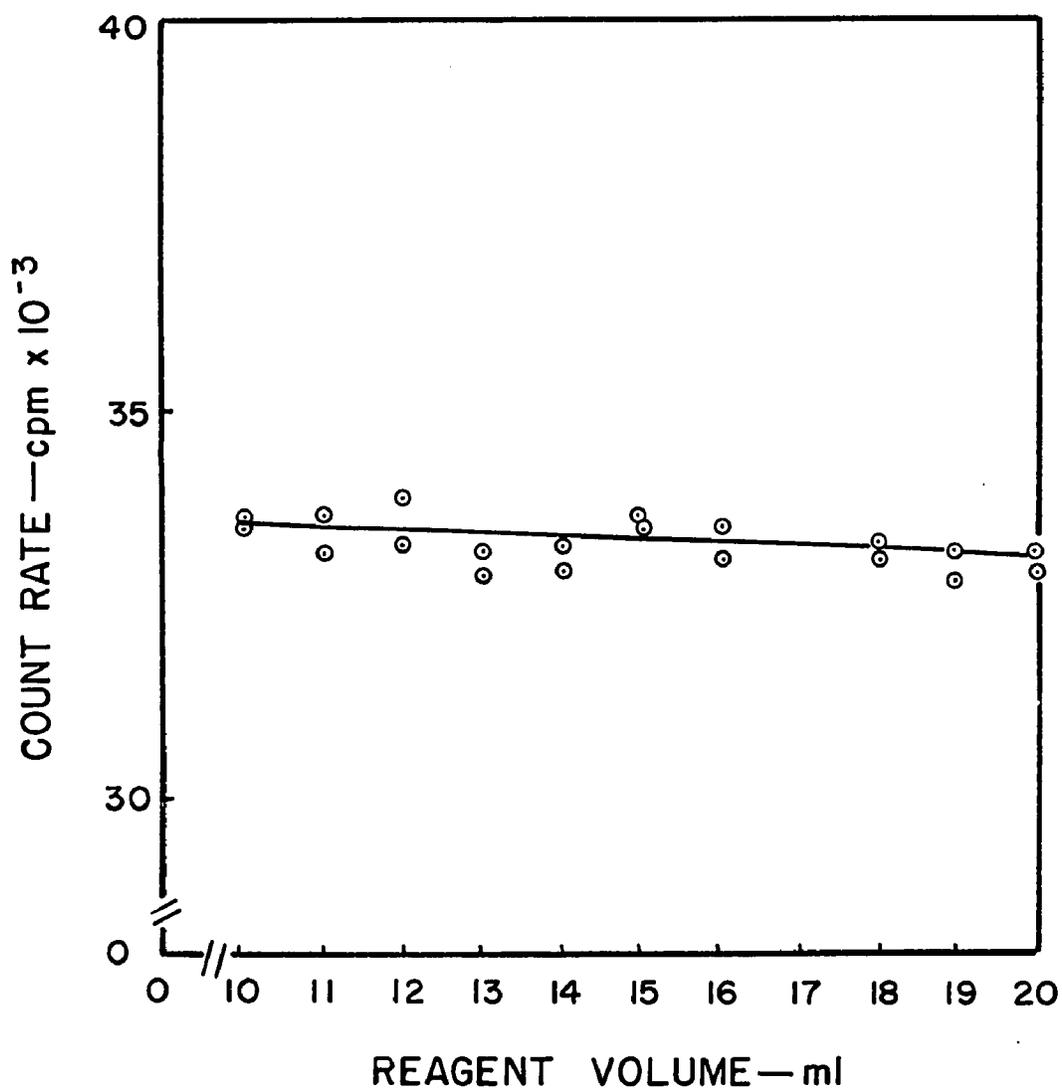


Figure 11. Effect of Reagent Volume on Sr⁸⁹ Count Rate.

were prepared in 20 ml of the DNP:toluene (1:3, v/v) reagent. In this increased volume of reagent, 70 mg of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ and 700 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were soluble. Increased concentrations of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ caused a decrease in Sr^{89} counting efficiency of about 0.02 per cent per mg. The $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ caused a decrease of about 0.015 per cent per mg. These results are illustrated in Figures 12 and 13.

Samples of strontium from natural sources (soils, plants, etc.) would be associated with large quantities of calcium, and it is not an easy task to separate these elements for analysis. In this procedure, then, calcium salts would probably cause the most difficulty.

Linearity of Counting. To test the linearity of counting two series of Sr^{89} labelled samples were prepared in the 1:3 reagent. One series contained 25 mg of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ and the other series contained only trace levels of this salt. The activity of the samples in each series ranged from 170 dpm to 68,000 dpm. The samples were counted and the observed counting rates plotted against the actual disintegration rates (Figure 14). The counting rate was quite linear

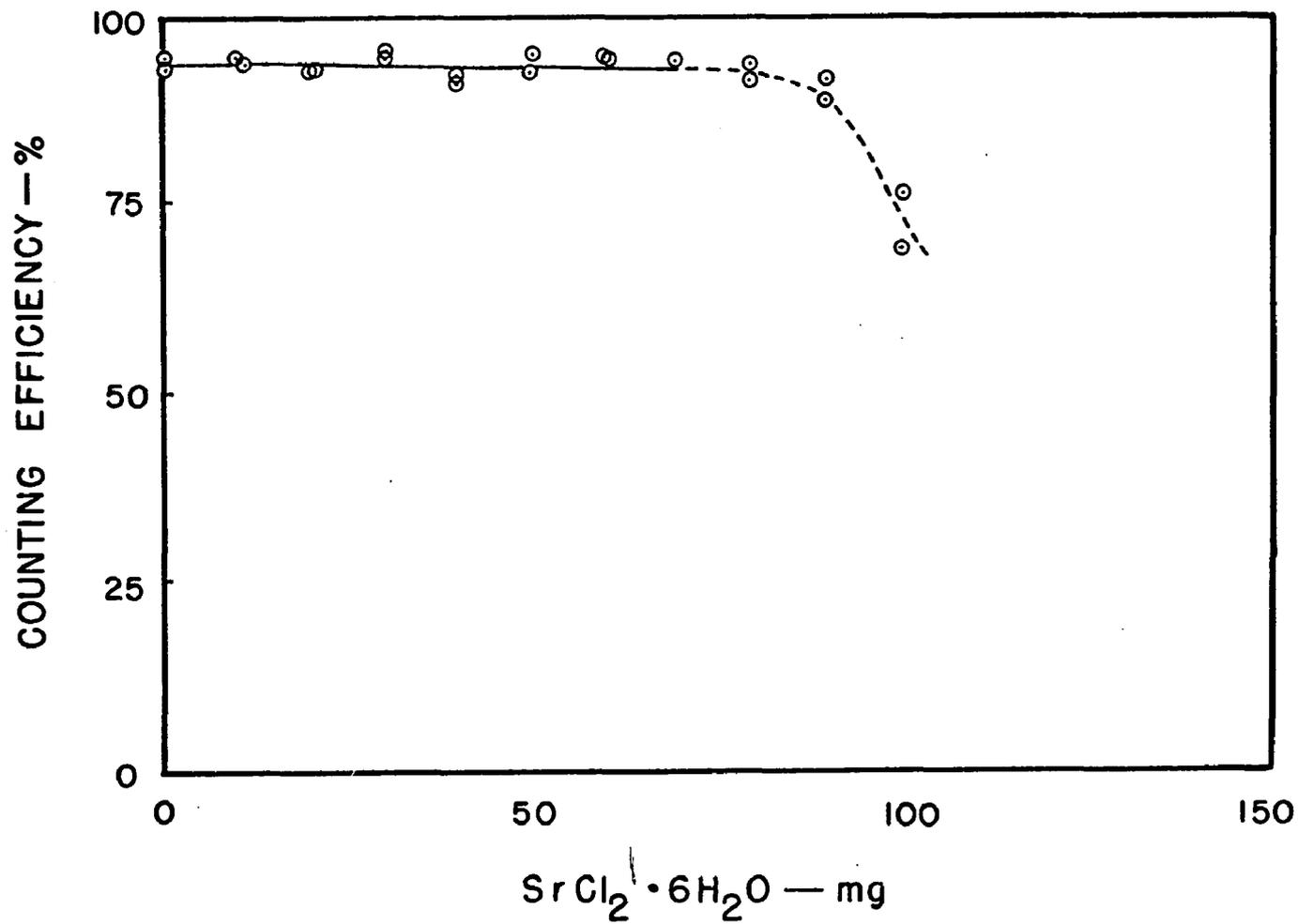


Figure 12. Quenching Effect of SrCl₂·6H₂O on Sr⁸⁹ Counting Efficiencies.

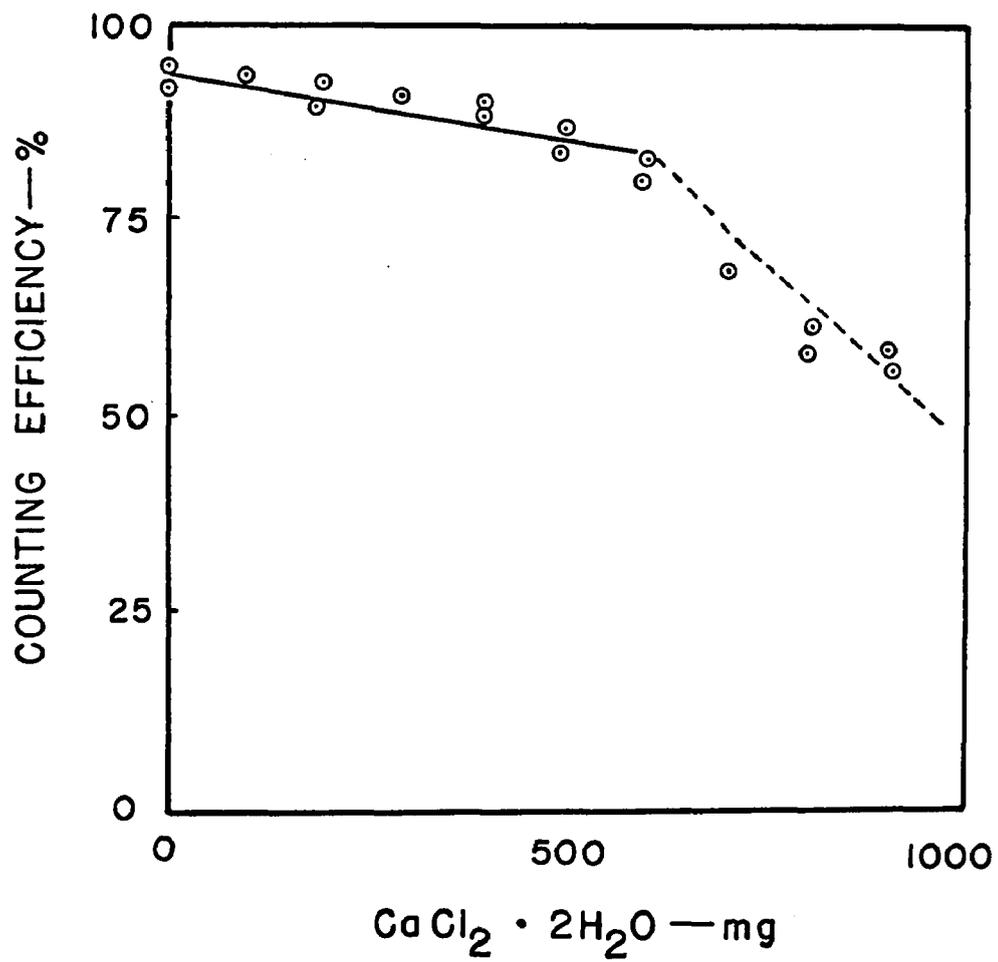


Figure 13. Quenching Effect of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ on Sr^{89} Counting Efficiencies.

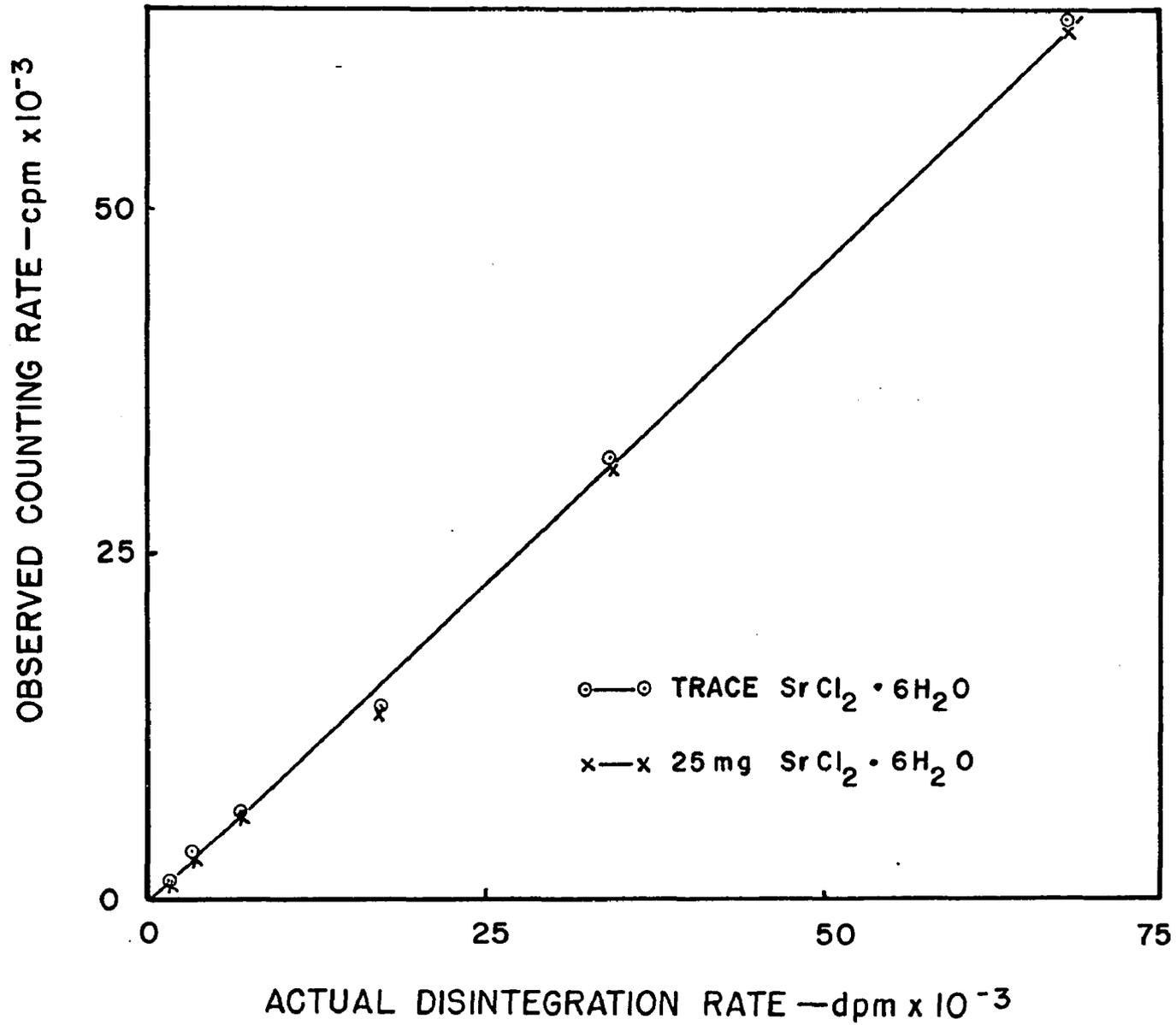


Figure 14. Linearity of Sr^{89} Counting.

over a range of specific activities of 7 to 2700 dpm per mg $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$.

Precision and Efficiency. Ten samples of a calibrated Sr^{89} standard, obtained from the Radiochemical Centre, Amersham, England, were counted in the 1:3 reagent, and ten were counted in the 1:1 reagent. The results, given in Table 11, show that a precision of better than one per cent is possible in both the 1:3 and 1:1 reagents.

Table 11. Precision of Sr^{89} Counting in Two Different DNP-Toluene Mixtures

		Ratio of DNP to Toluene	
	1 - 3*		1 - 1*
	Counts		Counts
	66272		66144
	66768		65709
	66513		65928
	66052		65673
	67417		66070
	67224		65704
	66958		64945
	66684		65636
	66412		65077
	66712		65643
Avg	66701		65648
S.D.**	0.7%		0.7%

The standard Sr^{89} had an activity of 7020 dpm on the counting date. Thus, the Sr^{89} counting efficiency was calculated to be 95.0 in the 1:3 reagent and 93.5 in the 1:1 reagent.

E. Preparation of Plant and Soil Samples for Radioassay by Liquid Scintillation

Before biological Ca^{45} and Sr^{89} samples can be radioassayed by this procedure, they must be digested and converted to chloride salts. The commonly used perchloric acid digestion method could not be used since it was found that $\text{Ca}(\text{ClO}_4)_2$ and $\text{Sr}(\text{ClO}_4)_2$ were not soluble in the DNP-toluene mixture. The dry combustion technique is also commonly used to convert organic samples to the inorganic form. This technique was tested and found to give poor recovery efficiency and precision.

The method finally adopted is a modification of the nitric acid digestion technique. Samples are heated with 5 to 10 ml of concentrated HNO_3 , depending on the sample size. Up to one gram of a biological sample can be digested by this procedure. The temperature of the digestion is controlled to just a mild boiling, and is continued until the volume is reduced

nearly to dryness. If, at this point, the digestion does not appear to be complete, more nitric acid is added, and the digestion repeated. When the digestion is near completion, a few drops of 30% H_2O_2 are added to aid the digestion and to decolorize the samples. Now, concentrated HCl is added to the samples, and they are then heated to dryness. However, this heating must be stopped before the samples are "baked". This last step converts the salt residues to the chloride form. The chloride salts are now dissolved in dilute HCl, and it is from this solution that aliquots are taken for radioassaying.

The digestion must be done carefully or losses will occur (20). There is a certain amount of "art" or technique required to perform a successful digestion. However, when the technique is mastered, the digestion can result in high recoveries and good precision. To determine the recovery and precision of the digestion procedure, a standard sample of Ca^{45} - Sr^{89} (containing 4500 dpm of Ca^{45} and 6760 dpm of Sr^{89}) was added to each of 5 one gram samples of barley straw. These samples were then digested, and assayed for Ca^{45} and Sr^{89} . The results listed in Table 12 show a precision of about 5% and a recovery of over 90% for each isotope is possible.

Table 12. Recovery Efficiency and Precision of Digestion Technique

Sample	Ca-45	Sr-89
	dpm	dpm
1	3949	5820
2	4407	6287
3	4019	5996
4	4307	6191
5	4470	6450
Avg	4270	6149
S.D.	5.6%	4.1%
Recovery	95%	91%

In some cases, the digested samples were not completely soluble in the scintillation solution, and this caused a reduction in the detection efficiency. It is likely that this sample insolubility was caused by the presence of sulfate, silicate and phosphate salts. Interferences of this sort were not serious since internal standards are used to determine efficiencies and recoveries in the samples.

SUMMARY

The primary purpose of this research was to develop a general method for the simultaneous radio-assay of Ca^{45} and Sr^{89} from biological samples. Such a method was developed, and has been described in this dissertation. First, the sample is digested in HNO_3 - H_2O_2 , and is then converted to the chloride form with HCl . The chloride salts are then dissolved in a di-n-propyl phosphate-toluene-fluor reagent, and are then counted in a liquid scintillation spectrometer.

Formulas have been derived for the calculation of the individual isotope activities from the Ca^{45} - Sr^{89} double labelled samples. These formulas can be written into a FORTRAN program to facilitate rapid calculations of large quantities of data.

The technique is simple, fairly rapid and can yield high precision. The maximum counting efficiency for Ca^{45} is about 85 per cent, and that for Sr^{89} , about 65 per cent. Furthermore, up to 500 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 50 mg of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ can be solubilized in the reagent with little loss in counting efficiency. These

facts represent improvements over other methods described in the literature. The factors affecting the procedure were studied, and the major factor of concern is perhaps the digestion step.

The procedure can, of course, be used with equal ease and efficiency for the radioassay of Ca^{45} or Sr^{89} singly labelled samples.

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