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DETERMINATION OF AMERICIUM AND PLUTONIUM IN AUTOPSY TISSUE: METHODS AND PROBLEMS

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Abstract

The current methods used by the tissue analysis program at LASL for the determination of americium and plutonium in autopsy tissue are described. Problems affecting radiochemical yield are discussed. Included are problems associated with sample preparation, separation of plutonium from large amounts of bone ash, and reagent contamination. The average ^{242}Pu tracer yield for 1800 Pu determinations is 78 ± 12%. The average ^{243}Am tracer yield is 85 ± 7% for 40 determinations.

INTRODUCTION

The autopsy tissue program was established in 1959 to determine the distribution of plutonium in the tissues of occupationally exposed individuals. Later the program was extended to monitor the level of plutonium in the general population. Over the life of the program the analytical procedures have been revised several times. With the increased demand for knowledge concerning the organ distributions of Am, Th, and U, the scope of our radioanalytical capabilities has been broadened to include these radionuclides. The purpose of this report is to describe the current procedures used for the determination of Am and Pu in autopsy tissue. Some of the problems associated with these determinations are discussed.

The basic separation scheme is outlined in Fig. 1. After ashing, including treatment with $LiNO_3$-$NaNO_3$ (Mi77), an aliquot of the dissolved ash is taken to dryness and disolved in 7.8 M HNO_3. Plutonium is separated from americium and the bulk of the ash by a HNO_3- anion exchange (Ca73). Separation of plutonium from uranium and thorium is completed with a second

HNO_3-anion exchange. Americium is concentrated from the ash by an extraction into dibutyl-N, N-diethylcarbamylphosphonate (Bu70) and further purified by a methanol-nitric acid anion exchange (Kn78). The radionuclides are electroplated from an ammonium sulfate electrolyte (Ta72) and assayed by alpha spectrometry. The radiochemical yield is determined by ^{242}Pu and ^{243}Am tracers which are added at the onset.

EXPERIMENTAL

Tissue Preparation

Oven dried tissues are spiked with ^{242}Pu and/or ^{243}Am and ashed in a furnace for four days. The temperature is raised from 150 to 500°C in 50° increments at eight to twelve hour intervals, maintaining 500°C for twenty-four hours. The residue is alternately wet and dry ashed until all visible carbonized material is destroyed. A 50% weight-weight mixture of $LiNO_3$-$NaNO_3$ is added in the ratios of one gram mixture per gram soft tissue ash and one gram mixture per ten grams bone ash. The ash-salt mixture is wet and dry ashed until it will completely dissolve in 7.8 M HNO_3. Lung and lymph node samples are treated with HF before final dissolution. Samples which will not dissolve in HNO_3 after the above treatment are dissolved in HCl.

Anion Exchange Separation of Pu

An aliquot of the tissue solution is adjusted to the appropriate volume of 7.8 M HNO_3 and treated with $NaNO_2$ to stabilize Pu(IV). The solution is passed through a 1.5 X 12 cm column of AG 1 X 4, 50-100 mesh, resin (Bio-Rad Laboratories, Richmond, California)

which previously was converted to the nitrate form and conditioned with 7.8 M HNO_3. The resin is washed with ten column volumes of 7.8 M HNO_3, and Pu is eluted with seven column volumes of 0.36 M HCl-0.008 M HF. The eluate is dried and the residue is dissolved in 20 ml of 7.8 M HNO_3. After treatment with $NaNO_2$ the solution is passed through a 0.7 X 9 cm column of AG 1 X 4 resin. The resin is washed with thirty-five column volumes of 7.8 M HNO_3 and seven column volumes of 9 M HCl. Plutonium is eluted with seven column volumes of 0.36 M HCl-0.002 M HF.

Samples that are insoluble in HNO_3 are run through a chloride anion exchange. Aliquots in 9 M HCl are heated with H_2O_2 for one hour and passed through a column of AG 1 X 4 resin previously conditioned with 9 M HCl. The size of the column is selected to provide adequate exchange capacity for the iron in the sample; the usual 1.5 X 12 cm column is generally sufficient. The resin is washed with five column volumes of 9 M HCl and five column volumes of 7.8 M HNO_3. Plutonium is eluted with 0.36 M HCl-0.008 M HF. Separation is compleated with a second anion exchange as described above.

Americium Separation

The effluent from the first Pu separation is dissolved in 12 M HNO_3, and Am is extracted into dibutyl-N,N-diethylcarbamylphosphonate (DDCP, Columbia Organic Chemicals, Columbia, South Carolina) using one milliliter of undiluted DDCP per fifty milliliters of solution. After the phases separate (several hours to overnight), the DDCP is washed with ten volumes of 12 M HNO_3. The DDCP is diluted with five volumes of toluene, and Am is stripped with 2 M HNO_3 using two 20-ml volumes of acid per 6-ml volume of organic phase. Phase separation should be complete at each stage to minimize the amount of organic residue recovered in the strip solution.

Americium separation is completed with an anion exchange from a mixed solvent. The strip solution is dried, and the residue is dissolved in 6 M HNO_3. After dilution with 7.5 ml of ethanol saturated with $NaNO_2$, the solution is passed through a 0.7 X 11 cm column of AG MP-1, 100-200 mesh resin (Bio-Rad Laboratories, Richmond, California). The resin is washed in succession with two 20-ml volumes of 75/25 methanol/6 M HNO_3, two 20-ml volumes of 60/40 methanol/6 M HNO_3, and 30 ml of 60/40 methanol/2.5 M HNO_3 which elutes the Am. The column is pressurized at about

10" H_2O to maintain a flow rate of about 0.5 ml/min throughout the operation.

Electrodeposition of Am and Pu

Americium and plutonium are electrodeposited on 0.5 in stainless steel planchettes which are electropolished for seven minutes at one ampere using a solution of H_2SO_4, H_3PO_4, and H_2O with volumes of 225, 275, and 100 ml, respectively. The eluate containing Pu is heated with 0.3 ml H_2SO_4 and 5 ml HNO_3 until the H_2SO_4 fumes and for an additional ten minutes. The eluate containing Am is heated until the methanol is driven off and then similarly treated. The fumed H_2SO_4 is diluted with three ml H_2O, and the pH is adjusted to 2 using NH_3 gas and thymol blue indicator. The solution and two 1.5 ml rinses of 0.18 M H_2SO_4 are transferred to a 12-ml plastic cell, and the electrolyte is adjusted to pH 2 with NH_3 gas. The tip of a 0.1-in. platinum rod (anode) is immersed into the electrolyte to a distance of 12mm above the planchette (cathode). A constant current of 0.55 ampere is passed throught the covered cell, 2 hours for Pu and 2.5 hours for Am. One minute before removing the

cell the electrolyte is made basic by the addition of six ml 1.5 M NH_4OH. The cell is emptied and rinsed with 1% NH_4NO_3- 1% NH_4OH solution. The planchette is removed, rinsed with ethanol, air dried, and heated at 250° for 5-10 minutes.

Counting

Planchettes are assayed by alpha spectrometry. Typically, samples are counted for 50,000 seconds using 300 mm^2 silicon surface barrier detectors in 35% geometry. The background for each detector is measured for 50,000 seconds and compared to the average background of at least twenty measurements for that detector. If the result falls outside the 3-sigma control limits, the detector is cleaned and a new background is measured. If the background remains outside the control limits, the detector is replaced. The average background is updated quarterly.

The efficiency of each detector is determined weekly by a 900 second count using a secondary standard of ^{238}Pu, ^{239}Pu, and ^{242}Pu. This secondary standard is periodically calibrated by comparison with a National Bureau of Standards ^{238}Pu source. The result for each

detector is compared with the average efficiency representing at least twelve determinations. If a detector efficiency falls outside the 3-sigma control limits, the detector is replaced. Detector efficiencies are also updated quarterly.

Quality Control Samples

Analyses are performed in sets of 16; 14 tissue samples, one reagent blank, and one tissue simulant solution. The reagent blank contains no added tracers and serves to monitor reagents and equipment for contamination. The tissue simulant is spiked with about 0.1 dis/min of ^{239}Pu and/or ^{241}Am in addition to the usual tracers to determine the accuracy and precision of the analytical procedures. The amount of tissue simulant is selected to be representative of the average ash content of the set of tissues being analyzed. Periodically, unspiked tissue simulants are processed through the entire procedure, including ashing, to check for contamination. An occasional set of blank planchettes is run through the deposition procedure to insure that the electrodeposition equipment and reagents are contamination free.

RESULTS

The average radiochemical recovery for the last 4800 analyses is shown in Table 1. These analyses may be divided into two groups, those performed before and those performed subsequent to the use of the $LiNO_3$-$NaNO_3$ salt treatment. Some of the samples in the latter group may not have received the salt treatment but none of the samples in the former group received the salt treatment. While the average recovery for the group analyzed since the salt treatment was instituted is only about 10% higher then the other group, the range of recoveries has narrowed considerably. This is important because the fraction of analyses rejected for low tracer yield (less than 60%) has dropped from more than 30% to about 10%. The result for any analysis with a tracer recovery of less than 60% is rejected, and the sample is re-analyzed. The criterion for rejection was adoped because it has been shown repeatedly that the average ^{242}Pu tracer recovery from properly prepared samples or tissue simulants is 87 ± 9%. When a sample is analyzed more than once, only the analysis with the highest tracer recovery is maintained in the data base. However, the complete analytical history for each sample is recorded.

Until recently Am determinations were infrequent; hence, not much data have been accumulated. The average ^{243}Am tracer recovery for the 40 tissues that have been analyzed is 85 ± 7%, ranging from 72 to 103%. The expected average is about the same as for Pu, 87 ± 9%.

The results of the analysis of 200 quality control tissue simulants spiked with 0.1 dis/min ^{239}Pu is 0.097 ± 0.009 dis/min. The average activity corresponding to ^{239}Pu is 0.002 ± 0.001 dis/min for 200 reagent blanks.

DISCUSSION

Sample Preparation

The complete dissolution of tissue samples is undoubtedly the most difficult and tedious part of the analytical procedure. The success of any subsequent separations no matter how elegant depends strongly on proper sample preparation. Many of the autopsy tissues come from diseased or accident victims with the result that significant differences from normal, healthy tissues may be expected. The size of tissues received for analysis may be highly variable; for example the amount of liver received may range from several hundred grams to two kilograms. In general complete destruction of organic material becomes more difficult as sample size increases. While the use of $LiNO_3$-$NaNO_3$ appears to largely eliminate the problems associated with incomplete destruction of organic material, the highly variable nature of the mineral ash presents other problems. Some tissues defy dissolution in reasonable volumes of HNO_3, the preferred solvent, but will dissolve readily in HCl. Those liver and spleen samples which are troublesome usually exhibit this behavior. Lung samples which resist dissolution are usually characterized by

the presence of undissolved soil, probably refractory phosphates and silicates.

The highly variable nature of mineral ash can best be illustrated by an extreme example. The liver from a leukemia victim was dissolved in HCl because of its insolubility in HNO_3 and analyzed using an HCl-anion exchange for the initial separation. Iron breakthrough was noted during loading so the load effluent was saved for subsequent analysis. As expected the first analysis gave a low tracer recovery. Analysis of the load effluent revealed that most of the Pu was lost during the original loading. Further analysis indicated that the sample contained 1.3 grams of iron illustrating the need for a column with at least twice the exchange capacity as the one originally used.

The typical troublesome sample run through an initial HNO_3-anion exchange is characterized not by the loss of Pu in the load effluent but by its retention on the column. In these cases the Pu may be recovered by removing the resin from the column, washing the resin with 7 M HCl, replacing the resin, washing the resin with 7.8 M HNO_3, and finally eluting in the usual manner.

The HNO_3 wash invariably elutes iron. Possible explanations for this behavior include the obvious, namely that the ash was never truly dissolved and the Pu is associated with the solid. Another possibility is that during loading, perhaps due to localized concentration changes in the resin, iron phosphate is precipitated with concomitant coprecipitation of Pu. Whatever the cause this behavior suggests that it may be wise to use an HCl-anion exchange for the initial separation of Pu from all liver ar spleen samples.

Bone Samples

The amount of ash obtained from soft tissues rarely is sufficiently large to present loading problems for Pu in anion exchange separations. Such is not the case for bones. The ash derived from bones is ten to forty times as much as for soft tissues of comparable tissue weight. The volume distribution coefficient for Pu(IV) between AG 1 X 4 resin and 7.2 M HNO_3 containing calcium phosphate is shown in Fig. 2. These data predict a significant loss of Pu when 30 column volumes of 7.2 M HNO_3 containing 100mg/ml of bone ash are passed through a column of AG 1 X 4 resin.

Fig. 3 illustrates the elution behavior of Pu(IV) from AG 1 X 4 resin using eluants of 7.2 M HNO_3 containing 50 or 100 mg/ml calcium phosphate, suggesting the results obtained in actual column operation may be even worse than predicted from the distribution coefficients. On this basis we try to limit the amount of bone ash in an aliquot to 30 grams at a concentration of no more than 50 mg/ml. In terms of the 20-ml column we use for the initial separation this corresponds to 600 ml at 50 mg/ml. In an interlaboratory comparison exercise three years ago we were required to analyze 20 and 75 gram samples of bovine shank bone ash. The twenty gram samples presented no particular problem but we realized that analysis of 75 gram samples by our usual methods would result in very low recovery of tracer. As a compromise we analyzed half of the 75 gram samples. The results, shown in Table 2, were as expected. Bone samples present no particular problems with respect to ashing and dissolution other than bulk. They are far easier to dissolve than liver or spleen.

Because we desire to analyze 50% aliquots of bone samples at a constant ^{242}Pu spike level, we need to know

the ash weight - wet weight ratio for various bone types. This knowledge is necessary only if the wet tissue is to be spiked and the 50% aliquot is to contain no more than 30 grams of ash. As a matter of routine we now record the ash and wet weights. Table 3 summarizes the more recent data for defleshed bones. Earlier data showed lower and even more variable values which may be attributed to incomplete removal of soft tissue. It may be seen that using the data in Table 3 will make it difficult to limit the size of bone samples to those which will produce no more than 60 grams of ash without being overly restrictive. However knowledge of the ash weight can be used to point to the need to adjust column size or to predict a low tracer recovery.

The Am procedure is much less sensitive to total bone ash. Ninety percent or more of the ^{243}Am in solutions containing up to 100 mg/ml calcium phosphate can be extracted by DDCP. Although iron is extractable, amounts up to at least 60 mg do not interfere. Plutonium is also highly extracted by DDCP but it is retained nearly quantitatively by DDCP under the conditions used

to strip Am. Because virtually no calcium is extracted, it would appear to be a very effective way to concentrate Pu from bone samples. However, we have not found a consistently effective method to strip Pu from DDCP.

Electrodeposition and Reagent Contamination

In the process of switching to an ammonium sulfate electrolyte several years ago we found a contaminant repeatedly appearing on the planchettes. A typical alpha energy spectrum obtained at that time is shown in Fig. 4. The spurious peak at 5.30 Mev was eventually traced to a contaminant in H_3PO_4 which is a constituent of the electropolishing solution. At that time the same electrodes were used for both polishing and plating. This contaminant, later identified as ^{210}Po, was found in several different brands of H_3PO_4 including a previously unopened bottle obtained directly from stock. While the contaminant does not seriously interfere with Pu determinations, its presence would produce serious errors in the determination of Am, falling in the peak area of ^{243}Am. The contaminant plates on the platinum electrode during electropolishing and subsequently plates on the planchette during electrodeposition. Once on the electrode normal rinsing procedures

will not completely remove it. This behavior is highly reproducible if the electrode is rinsed only with water. Although we have found at least one brand of H_3PO_4 that has thus far been free of this contaminant, we now maintain a separate set of electrodes which are used exclusively for electropolishing. In addition to the reagent blank which is run with each set of samples, we periodically run a set of blank planchettes through the electrodeposition procedure to check for contamination.

The need to constantly monitor the reagents used for electrodeposition was dramatically illustrated by an incident that occurred recently. At the time, we were evaluating the effectiveness of the Am procedure. The recovery of ^{243}Am tracer was consistently running at 25% or less. Planchettes were characterized by heavy deposits which rendered them essentially useless. Current regulation during the electrodeposition was difficult to maintain. The source of the difficulty was traced to a particular batch of HNO_3 in stock at the time. The acid was found to contain about 8µg of iron per ml of acid. Thus on adding 5 ml of the acid to the eluate during the preparation step, 40 µg of iron was introduced into the sample.

Summary

The measurement of americium and plutonium in autopsy tissue is complicated by a number of problems including some that have been discussed here. The methods used provide adequate separation of Pu and Am from most other interfering radionuclides at the levels expected for this type of sample; in exceptional cases methods and techniques are modified on an individual sample basis. These methods provide a reliable means of detection of Pu and Am down to tissue ash concentrations of approximately 0.5 pCi/kg.

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Captions for Tables and Figures

Table 1. ^{242}Pu Tracer Recovery from Autopsy Tissue.

Table 2. ^{242}Pu Tracer Recovery from Bovine Shank Bone

Table 3. Ash Weight-Wet Weight Ratio for Several Bones

Fig. 1. Separation scheme for the determination of Am and Pu in autopsy tissue.

Fig. 2. Volume distribution coefficient (D_v) for Pu(IV) between AG 1 X 4 resin and 7.2 M HNO_3 containing $Ca_3(PO_4)_2$.

Fig. 3. Elution of Pu(IV) from AG 1 X 4 resin with 7.2 M HNO_3 containing (O) 50 or (X) 100 mg/ml $Ca_3(PO_4)_2$.

Fig. 4. Alpha energy spectrum from a liver simulant solution spiked with ^{242}Pu, ^{239}Pu, and ^{238}Pu.

Table 1. ^{242}Pu Tracer Recovery from Autopsy Tissue

Method	Number	Percent
Without $LiNO_3$-$NaNO_3$	3000	70 ± 20
With $LiNO_3$-$NaNO_3$	1800	78 ± 12
Total	4800	73 ± 18

Table 2. ^{242}Pu Tracer Recovery from Bovine Shank Bone

Ash Concentration ng/ml	Volume ml	N	Percent
40	500	15	87.0 ± 6.2
75	500	5	50.8 ± 12.4

Table 3. Ash Weight-Wet Weight Ratio for Several Bones

Bone Type	Number	Ash Weight/Wet Weight
Long Bone	2	0.42 ± 0.004
Rib	19	0.205 ± 0.051
Sternum	9	0 141 ± 0.036
Vertebra	23	0.121 ± 0.035

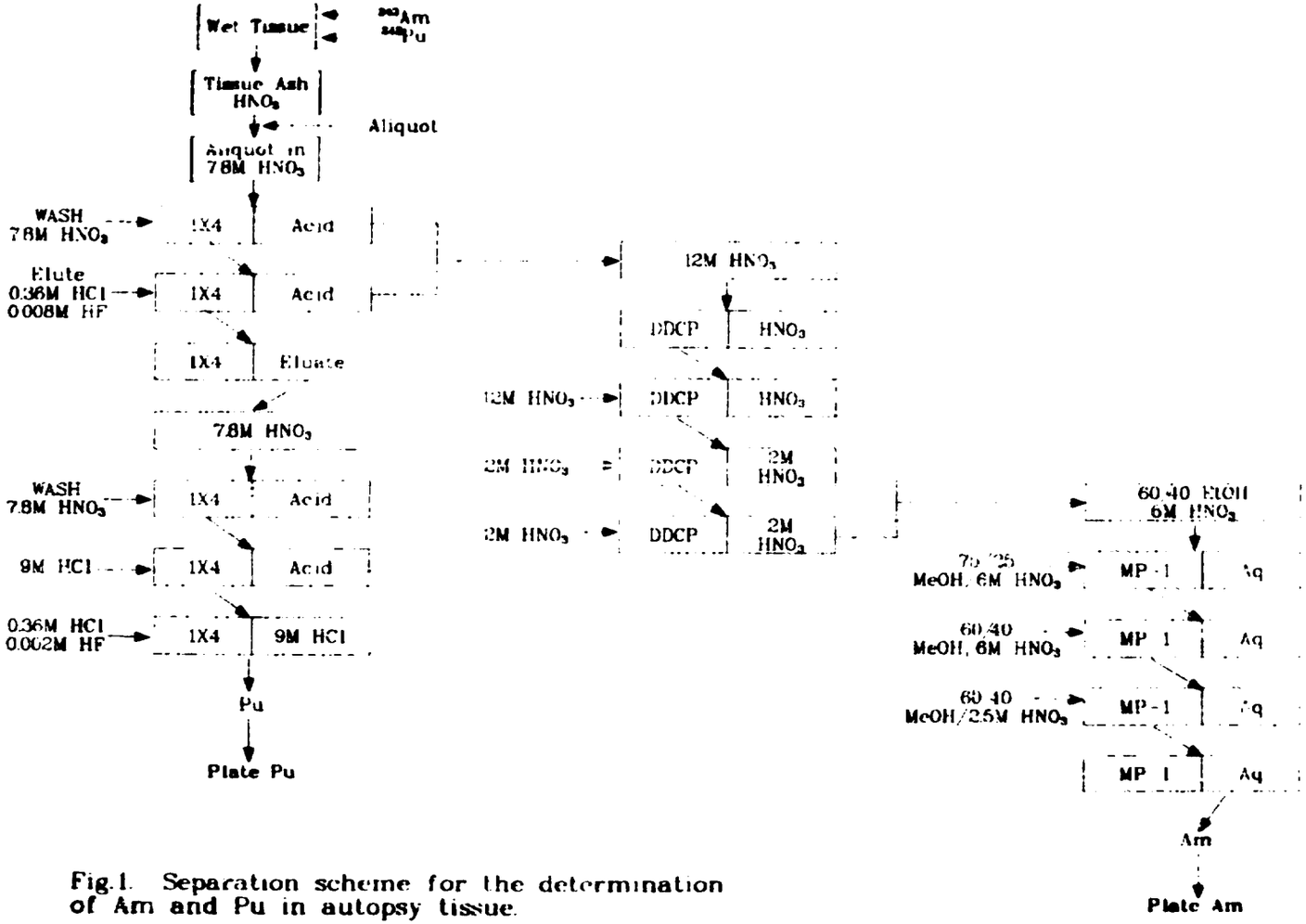

Fig.1. Separation scheme for the determination of Am and Pu in autopsy tissue.

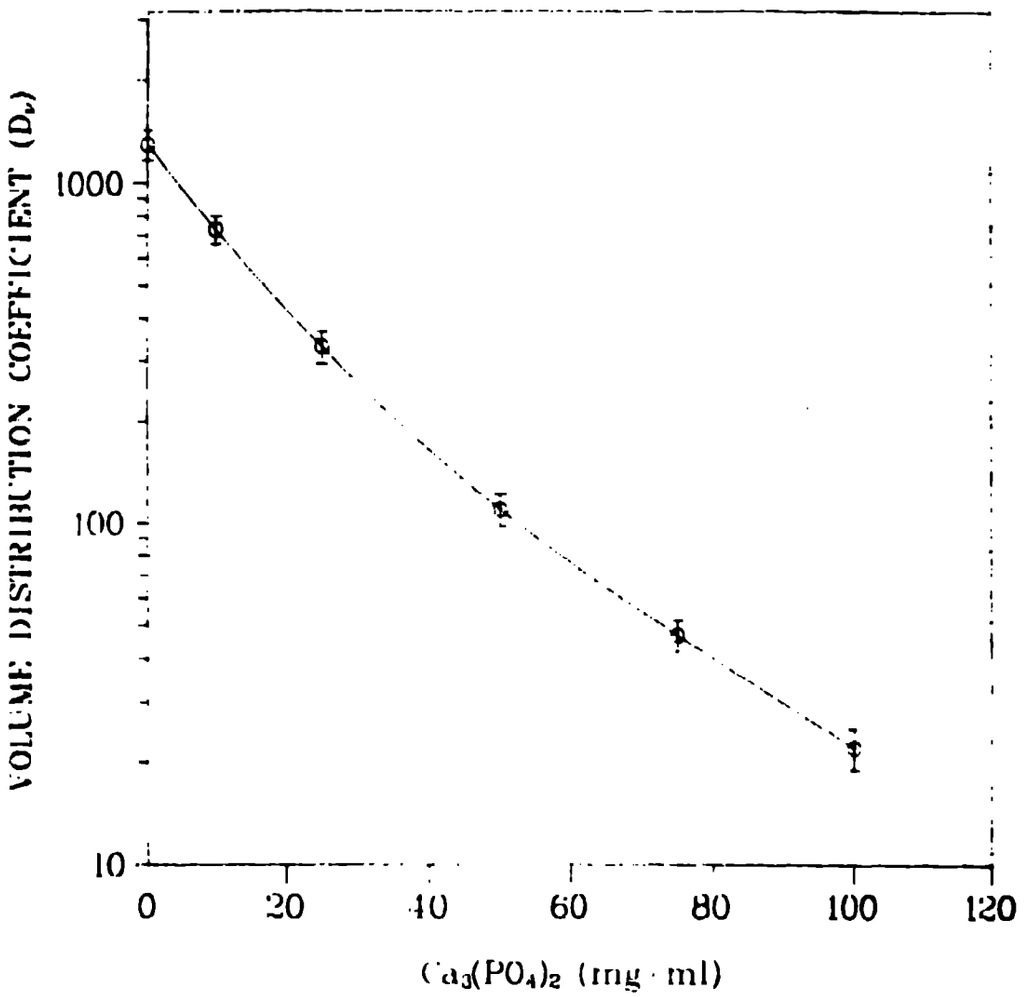

Fig. 2 Volume distribution coefficint of Pu (IV) between AG 1 X 4 and 7.2M HNO_3 containing $Ca_3(PO_4)_2$.

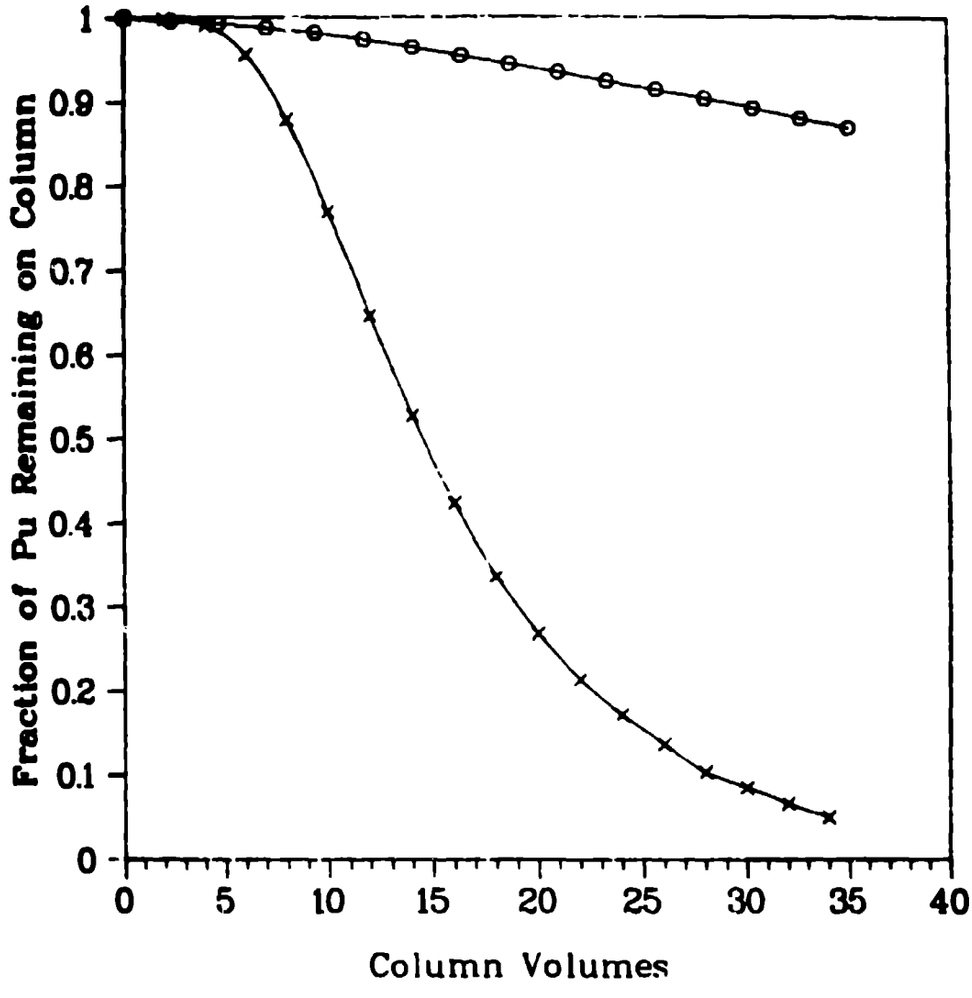

Fig. 3. Elution of Pu (IV) from AG 1 X 4 with 7.2M HNO_3 containing (o) 50 mg/ml or (x) 100 mg/ml $Ca_3(PO_4)_2$.

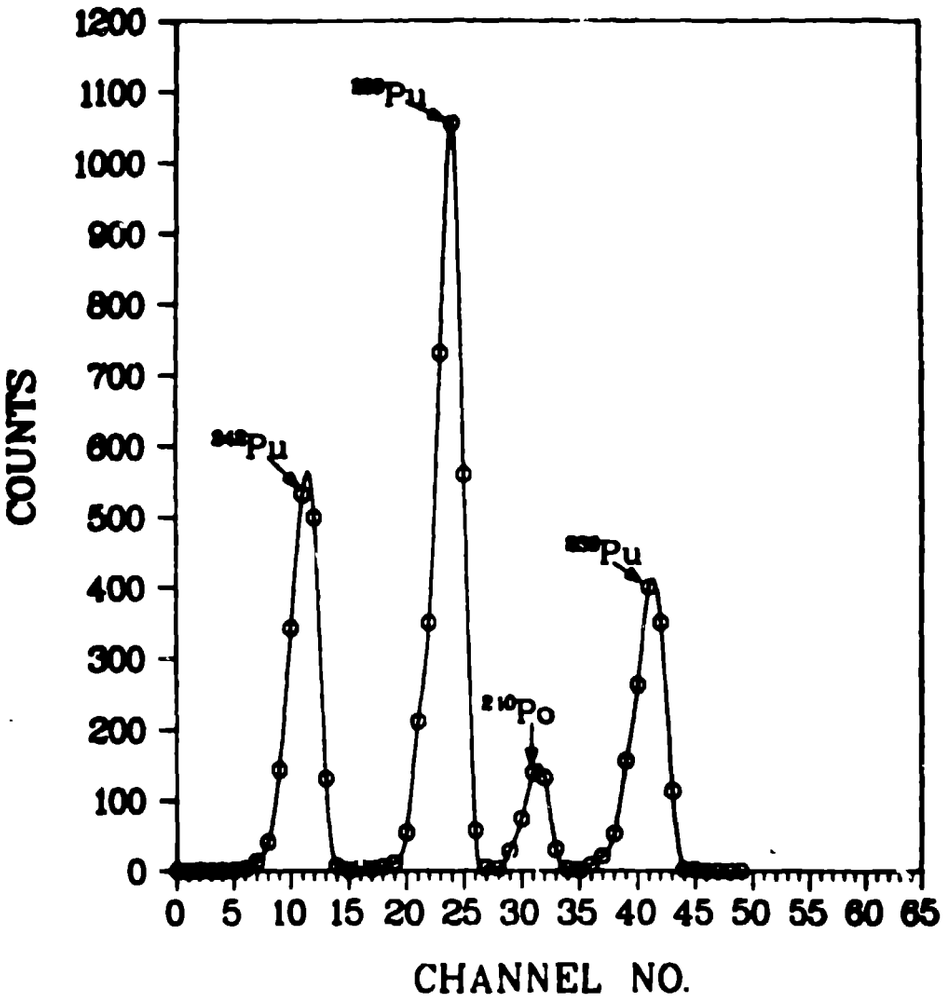

Fig. 4. Alpha energy spectrum from a liver simulant solution spiked with ^{242}Pu, ^{239}Pu, and ^{238}Pu.