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A COUNTER FOR THE DIRECT MEASUREMENT OF ^{55}Fe
IN BLOOD AND OTHER LIQUID SAMPLES ^(a)

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Summary

Iron-55 can be accurately determined directly in blood or other liquid samples by placing the sample in a long length of fine-bore thin-walled polyethylene tubing which is located between two large-area, low background proportional counters. This method reduces the required analysis time from days to minutes with only a slight reduction in sensitivity.

Introduction

Iron-55 is used extensively in clinical measurements of iron absorption in medicine^{1,2} and has also been one of the more abundant fallout isotopes. It is measurable in many living organisms of the biosphere.^{3,4} Iron-55 emits only a 5.9 keV X-ray and low energy auger electrons and this radiation is almost entirely absorbed within even a small sample of material. The procedures commonly used for ^{55}Fe analysis requires the separation of the ^{55}Fe from the sample mass to reduce self absorption of the X-rays, and requires at least two days for analysis. This includes the steps of ashing the sample (either wet or dry ashing), chemical separation of the ^{55}Fe and stable Fe, electrodeposition of the iron on a planchet, and counting the planchet on a proportional counter or thin crystal NaI(Tl) detector.^{1,3,5} Another method uses liquid scintillation counting, after the ashing and chemical separation steps.^{6,7} This paper describes a method in which the ^{55}Fe is counted directly in the blood sample from humans, animals or fish, or in a solution of the ashed tissue sample. The method was developed for use in iron absorption studies in clinical medicine but it has sufficient sensitivity for use in environmental radioactivity studies.

5.9 keV X-Ray Absorption Studies

Although a 5.9 keV X-ray is easily absorbed in biological tissue or water, the transmission can be made relatively high if the sample is in the configuration of a thin layer having a large surface area. The half thickness for absorption of a 5.9 keV X-ray by water is about 0.3 mm and such a sample thickness would allow a relatively high counting efficiency.

The sample container walls must be not only thin but also rigid so that the geometry and absorption by the sample remains constant. Figure 1 shows the absorption coefficients of various elements in the energy region of several keV. Although a beryllium container would provide a low absorption for 5.9 keV X-rays, the problems of construction, decontamination, and cost eliminate it as the first choice. Carbon is the next best element for a sample container material and the absorption of the ^{55}Fe X-rays by polyethylene (half thickness = 0.76 mm) is only 40% of that for water. After testing several materials and geometries it was found that thin-wall, fine-bore, polyethylene tubing provided a container which gave a reproducible

geometry, low absorption of X-rays and could be easily filled with a liquid sample and decontaminated between the counting of different samples.

Counter Design

A counter designed to count ^{55}Fe directly in blood or liquid samples is shown in figures 2 and 3. The unique feature of this counter is the sample holder. One hundred fifty feet of polyethylene tubing are coiled onto a lucite frame. The tubing has an inside diameter of .023 inches and a wall thickness of .0075 inches. The tubing is divided into lengths each 50 feet long and having a volume of about 4 ml. The three sections of tubing can be filled simultaneously or separately to give a choice of sample size 4, 8, or 12 ml. The tubing is charged with the liquid sample under a pressure of 20 lbs/in². The tubing easily withstands this pressure and charging times are one minute for a dilute water solution and about three minutes for a more viscous blood sample.

For counting, the sample holder is placed between two large area, thin window proportional counters. The upper and lower sections of the proportional counter each consist of a main counting chamber made up of four 2 x 2 x 16 1/2 inch counters each separated by a grid of ground wires. A ring of 1 x 1 x 16 1/2 inch anticoincidence guard counters surround the main chambers except for the entrance side which faces the opposite counter. The anticoincidence guard chambers are separated from the main chambers by a grid of .001 inch wire spaced 1/8" apart. The thin entrance windows are made from 0.00015 inch thick aluminized mylar. The counters are constructed from 1/4 inch thick lucite painted on the inside with colloidal graphite to provide a conducting surface. The ends of the counter are not painted and are non-conducting. A mixture of 90% argon, 10% methane gas flows through the counter at atmospheric pressure during operation. The counter has excellent resolution and a low background when operated inside a 2 inch thick lead shield and in anticoincidence with the guard chambers. The background is 3 counts per minute in the energy region of 4.6 to 7.2 keV. Twenty-five percent of the X-rays emitted in an ^{55}Fe solution contained in the tubing interact with the counting gas. The 2 inch thick main chambers absorb about 93 percent of the X-rays which come through the entrance windows.

Procedure

This method has been shown to be a fast, accurate, and reliable method for measuring ^{55}Fe in blood samples from any human or animal. The blood sample must be free from small blood clots which may clog the fine-bore polyethylene tubing. The blood is forced into the tubing which has been rinsed with water but not dried. If the blood is forced through a long length

of dry tubing, some of the water in the blood is used to wet the walls of the tubing and a very viscous or almost dry plug of blood cells forms at the head of the blood stream and this may clog the tubing. When the tubing is filled with blood an additional one milliliter volume is allowed to pass through so that any blood at the head of the blood stream which has become diluted by the water will be removed. After counting, the blood is removed from the tubing and the tubing is rinsed in turn with 10 ml of dilute saline solution (9 gram NaCl per liter), and finally with 5 ml of distilled water. The tubing is now ready for the next sample and residual contamination from previous blood samples has not been encountered with this procedure. The ^{55}Fe incorporated in the hemoglobin molecule does not seem to adhere to the tubing, however, after counting inorganic acid solutions of ^{55}Fe , more than 10 ml of acid ferric nitrate solution (2 molar each of HNO_3 and $\text{Fe}(\text{NO}_3)_3$) is sometimes needed to remove all the previous ^{55}Fe . In practice several sample holders are used so

that they can be cleaned and refilled with a sample while the one is being counted between the two proportional counters.

Results and Discussion

The sensitivity of this method is compared to that of other methods in Table 1 where the results of analyzing identical ^{55}Fe solutions by four methods are presented. The method is not quite as sensitive as that of counting an electrodeposited planchet on a low background proportional counter, but the ease of sample preparation for counting far outweighs this lower sensitivity for many types of samples. The precision of counting samples is excellent and limited only by the statistical counting error. An X-ray spectrum from a blood sample measured in the counter is shown in Figure 3.

Sedimentation of the red blood cells during a period of two hours has not produced any detectable

TABLE 1

COMPARISON OF A SAMPLE OF ^{55}Fe

SOLUTION COUNTED BY THREE DIFFERENT METHODS

	<u>Liquid Scintillation</u>	<u>Planchet Counting (Geiger Counter)</u>	<u>Planchet Counting (Proportional Counter)</u>	<u>^{55}Fe Liquid Sample Counter</u>
c/m	805	66	660	380
Bkg (c/m)	20	20	1	3
Detection Limit* for 20-Min Count (pCi)	7.5	100	2.4	6.2

* 95% Confidence Detection Limit Calculated By $\frac{2\sqrt{B}}{\frac{C}{\text{pCi}}}$

TABLE 2

COMPARISON OF COUNTS MADE ON SIX DIFFERENT ALIQUOTS OF A BLOOD SAMPLE

	<u>Counts/10 min</u>	<u>^{55}Fe Content (nCi/ml)</u>
1. Whole Blood	18,192	1.82
2. Whole Blood	18,217	1.82
3. Whole Blood	18,152	1.82
4. Diluted 1:1 With Isotonic Saline Solution	9,389	1.88
5. Diluted 1:1 With Isotonic Saline Solution	9,108	1.82
6. Diluted 1:1 With Isotonic Saline Solution	8,952	1.79

TABLE 3

ANALYSIS OF BIOLOGICAL SAMPLES FROM BIKINI ATOLL
BY ELECTRODEPOSITION AND BY DIRECT COUNTING OF A SOLUTION OF THE ASH

<u>Common Name</u>	<u>Tissue</u>	<u>⁵⁵Fe Content (nCi per Gram Dry Weight)</u>	
		<u>Chemical Separation and Electrodeposition Method</u>	<u>Direct Counting of Solution</u>
Yellowtail Tuna	Liver	0.21	0.21
Ulua (Jack)	Dark Muscle	1.29	1.14
Ulua (Jack)	Dark Muscle	3.63	3.56
Ulua (Jack)	Light Muscle	0.35	0.37
Ocean Sediment	155 ft Depth	0.94	0.93

effect on the counting efficiency and this indicates that the counting efficiency is quite independent of the position of the activity within the tubing. The count rate of the blood is independent of the concentration of red blood cells. Figure 4 shows the change in count rate with change in concentration as ⁵⁵Fe tagged blood is diluted with isotonic saline solution. The two percent differences between some of the points and the straight line could easily be due to a combination of counting error and dilution error rather than change in absorption of the X-rays due to dilution. It is often desirable to dilute blood samples by an equal part of saline solution. This allows the sample to flow through the tubing faster which speeds up the filling and emptying of the sample holder and decreases the possibility of the sample clogging in the tubing. Table 2 shows the results of counts made of six aliquots of the same blood sample of which three were diluted by an equal part of isotonic saline solution. These results were in good agreement with liquid scintillation counts which were made on the same sample.

The determination of ⁵⁵Fe in non-liquid biological samples can be accomplished by ashing the sample, dissolving the ash in dilute nitric acid and counting this solution in the counter. Acids containing heavier elements such as chlorine, sulfur, phosphorus, etc. must not be used since they strongly absorb the 5.9 keV X-rays. Figure 5 shows that the effect on counting rate of increasing concentrations of HNO₃ are negligible up to about 4 molar whereas HCl concentrations significantly decrease the counting rate even at low concentrations. Table 3 gives a comparison of the measurement of ⁵⁵Fe in some Bikini Atoll samples by the older methods of ashing, chemical separation, electrodeposition and counting and this method where the sample was ashed, dissolved and counted.

With the use of a small pump and the addition of an anticoincident agent the blood of live animals could probably be continuously circulated through the counter and the ⁵⁵Fe measured continuously. Such studies are planned in the near future. An automatic sample changer could also be incorporated into the counter if it were desirable.

Iron-59 Interference

Many samples from iron absorption studies contain both ⁵⁵Fe and ⁵⁹Fe. The sensitivity of the counter for counting ⁵⁹Fe is very low. Most of the beta rays from ⁵⁹Fe are absorbed in the solution or the walls of the tubing and the gamma rays are too energetic to be absorbed by the Argon-Methane gas. Only 0.22 percent of the ⁵⁹Fe disintegrations produce a count in the region of the 5.9 keV peak and only samples having high levels of ⁵⁹Fe need this small correction. The ⁵⁵Fe content of the sample can be determined with a NaI(Tl) detector.

Instrumentation

The anode wires of all eight main counting chambers are connected in parallel and the single signal cable is connected to a proportional counter preamplifier which is connected to a single channel analyzer. The window of the single channel analyzer is set to cover an energy region of 4.6 to 7.2 keV. Any pulses occurring in this energy region are fed through an anticoincidence analyzer and then to a scaler. The anode wires from the 28 anticoincidence guard chambers are connected in parallel and the signal cable connected to a preamplifier which is connected to an integral discriminator. The discriminator level is set just above the noise level and all pulses above this level are fed to the coincidence analyzer. Any pulses from the main counting chambers which occur at the same time as a pulse from the anticoincidence counters are rejected by the anticoincidence module. Since the count rates are relatively low in the application of this counter, a slow coincidence unit with gate pulses adjustable from 1 to 5 microseconds is used. The background of the counter is 3 counts per minute with the anticoincidence on and 50 counts per minute with it off.

Both the main chambers and anticoincidence chamber are operated at 1700 volts from a simple high voltage supply. This appears to be about the optimum voltage for the best photoppeak resolution in the main chambers.

Summary

This counter has been in use for several types of blood and liquid samples for about one year. The background and sensitivity have remained constant. In addition to the time saving and simplicity feature of this method it should be inherently more accurate since the measurement does not depend on a constant chemical yield or recovery as do the methods involving chemical separation and electrodeposition. In addition to the extensive medical uses of this method, the counter is sensitive enough to measure ^{55}Fe from nuclear fallout in the blood of Alaskan Caribou, Eskimos, and other population groups.

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