JOURNAL OF NUCLEAR MEDICINE 7:140-149, 1966

Fluorine Microdetermination By Neutron Activation Analysis; Application to Bacteriological Media

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In the course of metabolic studies on the relation of various oral bacteria to oral disease, it was considered desirable to study the influence of fluoride on the microorganisms under investigation. Such a study could necessitate the analysis of many samples for microfluorine content. The usual procedures for the determination of fluorine in biological materials depend on wet digestion, chemical conversion to hydrogen fluoride, and distillation or diffusion of HF into a suitable medium, followed by titration or colorimetric estimation (1,2,3,4), Microdetermination of fluorine in an organic matrix is difficult (4). It is not clear that the usual procedures would determine organically bound fluorine if present. Activation analysis procedures for fluorine have been described (5,6,7,8,), but not in the sensitivity range desired, nor with the necessary potential for automation.

It was desired to develop a procedure for the instrumental determination of fluorine in bacteriological media which was not only sufficiently sensitive, but also rapid, free of prior sample preparation, and amenable to automated and computerized procedures. It was felt that such a procedure, accomplished entirely by instrumentation, could be developed using neutron activation analysis. The nuclide ^{19}F occurs naturally (9), and two nuclear reactions are conveniently available in the Walter Reed Army Institute of Research nuclear reactor, a 50KW homogenous thermal reactor of nominal $10^{12}n/cm^2$ sec thermal and fast flux.

1) ^{19}F (n, 2n) ^{18}F
2) ^{19}F (n, γ) ^{20}F

Fluorine-20, a 10.7 sec activity, requires a short irradiation time, a thermal neutron flux and rapid delivery to the counting room via a pneumatic tube. The pneumatic system used for rapid delivery of samples passes through a region of the reactor having a thermal neutron flux of 10^{11} n/cm^2 sec. Fluorine-20 seemed to be the nuclide of choice, given the original goal, a sensitive, rapid, instrumental method.

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MATERIALS AND EQUIPMENT

Standard fluoride solutions were made up in distilled water with ACS reagent grade sodium fluoride to contain 5 to 1000 μg fluorine per ml. Cooked meat media (Difco) was prepared in distilled water with two per cent phytone (BBL), 0.5 per cent fructose and 0.5 per cent glucose added. Total solids constituted approximately 5.5 per cent by weight of the media. Media-fluoride mixtures having a desired 5 to 100 μg per ml fluorine content were made up by diluting an appropriate fluoride solution 1:10 with the prepared media. The final media concentration was thus 90 per cent of its initial concentration.

Polyethylene microcentrifuge tubes of approximately 0.35 ml capacity and polyethylene vials of 2 ml capacity were used to contain 0.25 ml and 1.0 ml samples respectively and were irradiated in a sample carrier (rabbit). The rabbit used initially in the pneumatic tube was constructed of polyethylene and had a bayonet-lock, rapid-opening head. However, a later nylon model with a hinged, snap-open head, was developed which proved faster in use (10).

The dual sodium iodide crystal arrangement shown in Figure 1 consisted of two five-inch diameter well crystals. Information from the two crystals could be taken simultaneously in separate 200 channel segments of the memory of a 400 channel RIDL pulse height analyzer (PHA), or the information from one crystal could be taken in 100 channel segments of the memory so that the decay of short-lived activities could be followed by use of successive 100 channel sections of the memory.

Data readout was accomplished by three methods: x-y plots, digital tape and punch tape for computer analysis. An RIDL magnetic tape recorder was

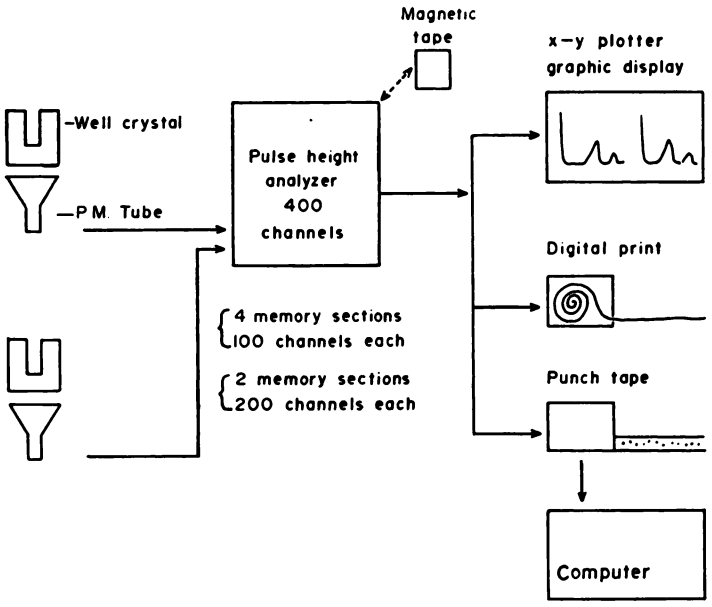

Fig. 1. Schematic of instrumentation two crystal input.

also used as an additional automatic memory for the pulse height analyzer.
I. *Dual Crystal Method:* The procedure consisted essentially of a comparison of a standard and *unknown* which were irradiated for a short period (12-24 sec), transferred to separate crystals and counted simultaneously for a convenient period. The data was stored in the PHA memory as described above and readout later by means of a Moseley x-y plotter.

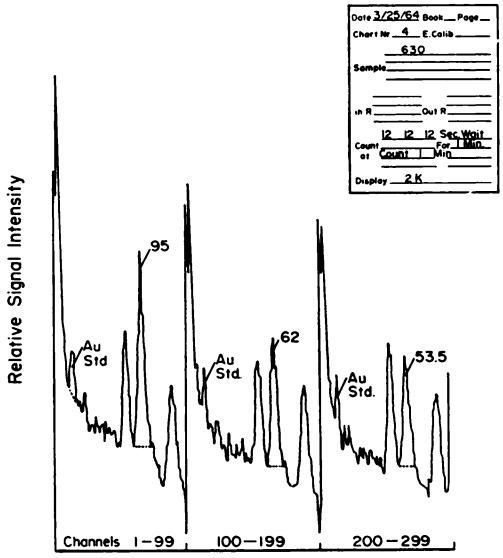

Fig. 2. Spectrum observed in successive 12 sec counts. The numbers 95, 62 and 53.5 are the 1.6 MeV γ intensity.

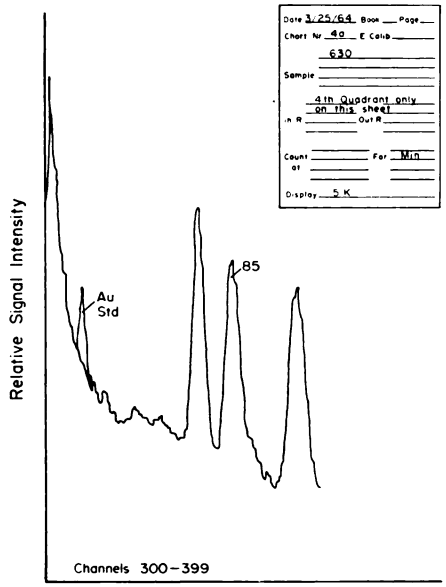

Fig. 3. Fourth quadrant spectrum observed in 1 min count after one min decay (Fig. 2).

II. *Single Crystal, Internal Standard Method*

A. *Long Procedure.*

In this procedure, known amounts of a suitable nuclide, serving as an internal standard, were added to media samples containing 5 to 100 μg fluorine. Single samples were irradiated for short periods (6-24 sec) and counted for similar short periods. The data was stored in the PHA memory as described above. It was essential that the internal standard chosen meet three criteria; (a) a half-life of sufficient duration such that its decay was negligible over the time intervals used; (b) a suitable gamma energy, and (c) a cross-section adequate to produce measurable activity over the short irradiation period used. Both gold and manganese met these criteria. Since the 0.41 MeV gamma energy of ^{198}Au fell within the area of general Compton scatter, the 0.85 MeV gamma energy of ^{56}Mn was considered preferable. However, a 5 μg Au internal standard can be used successfully (Figs. 2,3). When Mn was used as an internal standard 4 to 8μg was found to be convenient.

By counting an irradiated sample in successive 100 channel sections of the PHA memory it was possible to obtain a decay curve on the shortlived ^{20}F and thus distinguish it from a longer lived interference having approximately the same gamma energy (1.6 MeV). The clock time from the beginning of irradiation to the beginning of the first count was recorded in order to establish the interval from zero time after irradiation to the beginning of the first count. As shown in Figure 2 sample #630 was counted for 12 seconds in 100 channels of the PHA memory; immediately counted for 12 more seconds in the next 100

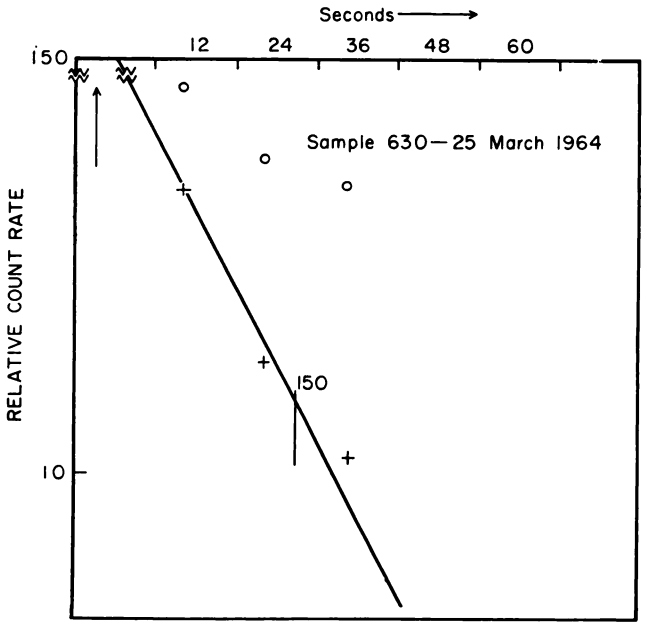

Fig. 4. Analysis of data exhibited in Figs. 2 and 3.

channels, and finally counted for an additional 12 seconds in the third 100 channel section. After a suitable decay period (one to two min), the fourth 100 channel section as shown in Figure 3 was used to count for a longer period (usually one min) to establish the intensity of the interference signal occurring at 1.6 MeV. The half-life of this contaminant was determined to be on the order of 30 minutes. The relative values of the internal standard, fluorine and contaminate peaks were determined from the x, y plots in terms of peak heights measured in millimeters from the base of each peak. It is seen in Figure 2 that the 1.6 MeV peak is decaying from 95 to 63 to 53.5 while the peak of the gold internal standard remains effectively constant. The spectrum of the fully decayed sample, *i.e.* no further ^{20}F remaining (Fig. 3) is seen to still have a 1.6 MeV peak of significant intensity which must be used to correct the 1.6 MeV peaks obtained in the first there quadrants. Thus, in analyzing the data from Figures 2 and 3, as shown in Figure 4, the extrapolation of the background and contaminant give essentially a zero slope for purposes of correcting the observed count rates used over a 36 sec period. The data obtained and exhibited in Figures 2 and 3, and analyzed in Figure 4, closely fit a half-life of 10.7 seconds. A similar set of data taken by a magnetic tape recorder, as a means of doing automatically on sample #766 what was done manually on sample #630 is shown analyzed in Figure 5. It is possible with the magnetic tape recorder to obtain more points on the decay curve, since data may be taken in a given section of the PHA memory for a given period, scanned and stored on tape within approximately 2.5 sec, cleared, and new data stored in the same channels. The capacity of the

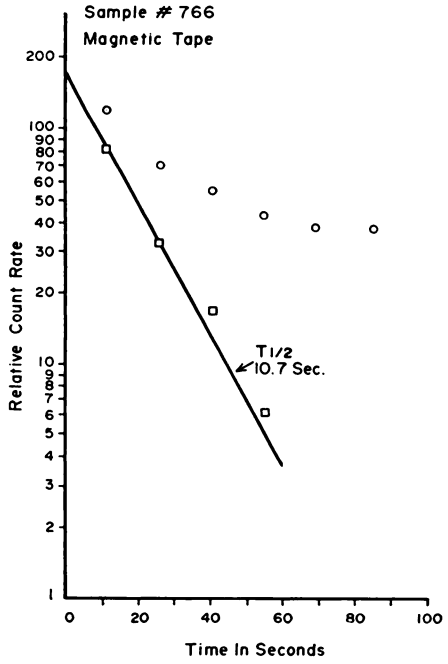

Fig. 5. Analysis of data obtained by magnetic tape readout.

magnetic tape device for repetitive scannings far exceeds the requirement of the technique used for this nuclide since in 30 to 40 seconds very little ^{20}F was left. Because of the rapid decay of ^{20}F, the 2.5 sec data scanning period of the tape recorder and a limitation of no less than 6 sec time units for data accumulation in a single PHA measurement, only four decay points were obtained. Thus, the principal advantage of the magnetic tape recorder in this work was in its automation potential rather than in obtaining more data.

Since the time interval between the beginning of the first count and the end of irradiation was recorded, it was possible to extrapolate the calculated fluorine half-life back to zero-time after irradiation. The fluorine value could then be calculated as a ratio of its extrapolated zero-time value to the internal standard value which was also a zero-time number since its decay was negligible within the short counting period used. In actual practice the internal standard value was taken as an average of the four peaks obtained by the counting procedure used (Figs. 2,3). By plotting the ratio of ^{20}F signal/internal standard signal vs μg fluorine added to the media, a standard curve can be prepared as shown in Figure 6.

B. *Short Procedure.*

Once the feasibility of the method was established as described above, it became apparent that the procedure could be shortened and calculations simplified by taking only two counts; one for 18 seconds immediately after removal from the reactor and the other for one to two minutes after a one minute hold.

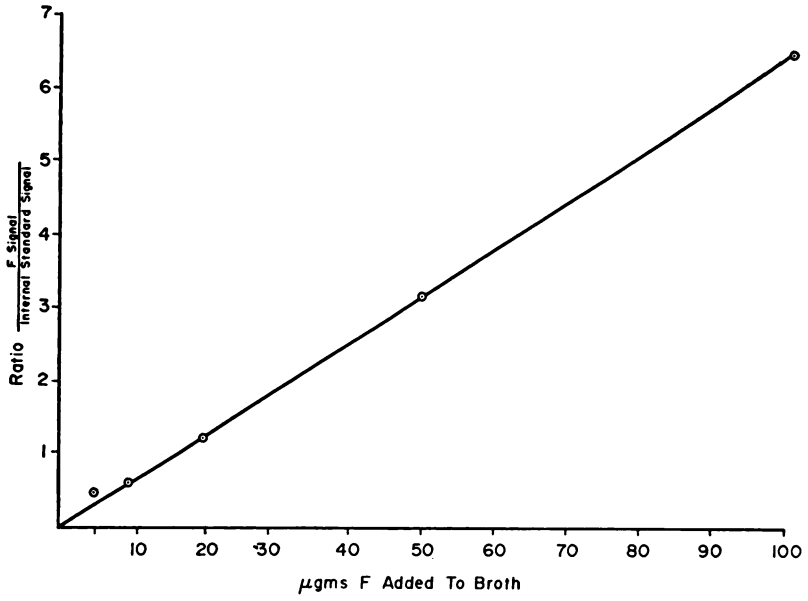

Fig. 6. Results using small samples (0.2 ml) 24 seconds in reactor. Long procedure.

Such a procedure would improve the statistical reliability of the first count and the single fluorine point overlayed with an ideal 10.7 second half-life could then be extrapolated to zero time after irradiation. Using this procedure it would also be possible to prepare tables relating single fluorine decay points to the appropriate zero-time extrapolation, thus simplifying calculations.

RESULTS

I. *Two-Crystal Systems:* Good agreement was obtained between the *unknown* and the actual fluorine content (Table I). These comparisons were made on solutions of reagent grade sodium fluoride in distilled water. There was no apparent trend in the results departing from proportionality when comparing various fluorine ratios.

As seen in Table I, reasonable precision was obtained by manual computation of the graphical data readout. The variability of the results (±4.9%) was considered due, in part, to experimental variability in sample preparation, timing, reactor flux patterns and the inherent imprecision of the graphical method of computation.

By use of a simple computer program, the punch tape data readout was interpreted by electronic data processing. The data shown under *computer* in Table I are the results of a program which establishes the low points on either side of a designated peak, then sums the peak between the low points. Such a procedure is better than one which finds the *peak* and then sums the signals over a given number of channels on either side of the peak (7,8).

It is apparent in Table I that the comparison of standard *vs.* unknown fluoride solutions using the dual crystal system is possible with a precision of approximately five to ten per cent depending on whether manual or computer data handling is used. However, the dual crystal method could not be used for bacteriological media because of the presence of an interference whose peak occurs almost precisely at the 1.6 MeV fluorine peak.

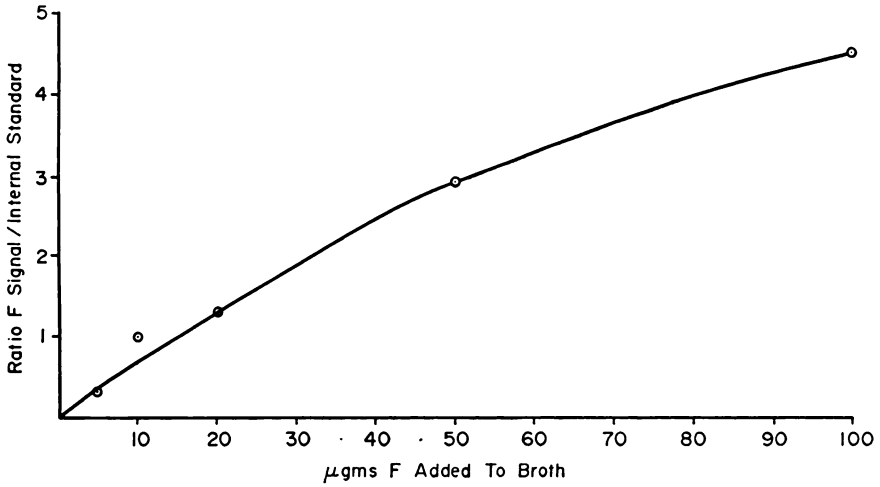

Fig. 7. Results using large samples (1 ml) six seconds in reactor. One point procedure.

II. *Single Crystal, Internal Standard, Fluorine Decay Analysis:*

Varying in-reactor times and sample sizes were used to ascertain an efficient combination of these factors with respect to the accuracy and sensitivity of the method.

In Figure 6 a typical curve is shown by results obtained by the long procedure using 0.25 ml fluorine-media samples with an in-reactor time of 24 seconds. By using the fluorine ratio signal to the internal standard signal as a normalizing reference, an estimation of the fluorine content in various samples is feasible. Good precision was obtained down to the 5 μg level. The working thermal neutron flux was approximately 10^{11}n/cm^{2}sec; whereas, the reactor is rated at about 10^{12}n/cm^{2}sec. It is probable that if the pneumatic tube were more conveniently located in the maximum flux, sensitivity would be increased since a more intense fluorine photopeak would be obtainable.

A series of runs was made by the short procedure using 1 ml samples and a six second in-reactor time. The results shown in Figure 7 indicate that six seconds is not a desirable in-reactor time but the analytical procedure would nevertheless be usable. A subsequent set of runs made by the short data handling procedure using 1 ml samples, but a longer in-reactor time (12 sec) gave superior results (Figure 8). The results shown in Figs. 6,7,8 all seem to go through the origin indicating no significant contribution to the apparent F result by other possible reactions (^{23}Na (n,γ) ^{20}F). This is in accord with the known sodium content of the media and with known flux of the reactor used and cross section data for this reaction.

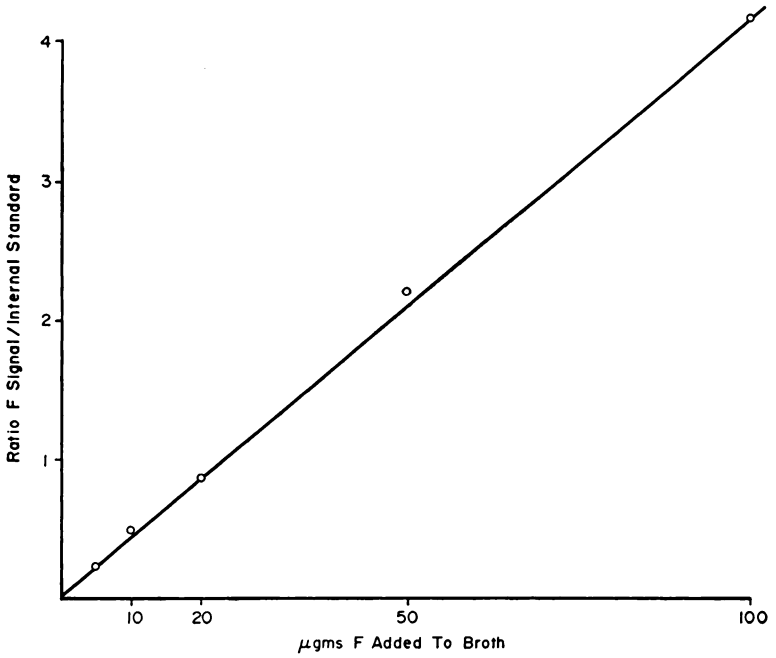

Fig. 8. Results using large samples (1 ml) 12 seconds in reactor. One point procedure.

The procedure as used is adequate for the metabolic study. It is believed that further increase in the sample size, increasing the in-reactor time and the flux, all would lead to an even more accurate estimation of the fluorine at levels down to 1 μg/g, as long as the total activity of the sample system does not cause unduly high dead time in the PHA system, and as long as new interferences do not appear in the energy region of interest.

SUMMARY AND CONCLUSIONS

A rapid repetitive instrumental method for the microdetermination of fluorine in a complex organic matrix has been developed. The method is independent of the type of fluorine bonding and is applicable to bacteriological media without prior sample preparation. Under the conditions used, the method is applicable to fluorine concentrations about 5μg/ml. The method is amenable to automation and electronic data processing.

TABLE I

TWO-CRYSTAL WEIGHING OF 10.7 SECOND ACTIVITY

Sample	*Size* (μgms)	*Graphic*	Δ%	*Computer*	Δ%
1	50/50	0.96	4	.963	3.7
2	50/100	0.49	2	.513	2.6
3	125/50	2.49	0.4	1.95	19.8
4	150/50	2.89	4	2.5	20
5	100/100	1.02	2	No Ans.	—
6	100/125	.88	1	.93	17
7	150/100	1.54 (1.63)[1]	2.65	1.47	2
8	125/125	0.92 (1.02)[1]	8	97	3
9	150/125	1.25	4	1.11	8.1
10	150/150	1.02	2	No Ans.	—
11	30/30	1.05	5	1.20	20
12	30/30	1.08	8	.83	20.4
13	30/90	.389 (.375)[1]	15	.36	9
14	90/30	3.04	1	3.1	3
15	90/90	0.90	10	.95	5
16	90/90	1.00	0	1.06	6
			$\bar{\Delta}$ = 4.9%		$\bar{\Delta}$ = 10%

[1]Replicate data readout on another scale.

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