0 0 0 0 4 8 0 2 5 4 7

UC-37

LBL-6451
c.1

Presented at the 26th Annual Conference
of Applications of X-Ray Analysis,
Denver, CO, August 2 - 5, 1977;
Advances in X-Ray Analysis, Vol. 21

X-RAY FLUORESCENCE ANALYSIS APPLIED TO SMALL SAMPLES

J. M. Jaklevic, W. R. French,
T. W. Clarkson, and M. R. Greenwood

RECEIVED
LAWRENCE
BERKELEY LABORATORY

APR 20 1978

LIBRARY AND
DOCUMENTS SECTION

August 1977

Prepared for the U. S. Department of Energy
under Contract W-7405-ENG-48

For Reference

Not to be taken from this room

LAWRENCE BERKELEY LABORATORY • UNIVERSITY OF CALIFORNIA • BERKELEY
37

LBL-6451
c.1

LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

0 0 0 0 4 8 0 2 3 4 8

LBL-6451

Presented at the Twenty-Sixth Annual
Conference of Applications of X-ray
Analysis, Denver, August 2-5, 1977

X-RAY FLUORESCENCE ANALYSIS APPLIED TO SMALL SAMPLES

J. M. Jaklevic and W. R. French
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

T. W. Clarkson and M. R. Greenwood
Environmental Health Sciences Center
University of Rochester
Rochester, New York 14642

August, 1977

0 0 0 0 4 8 0 2 3 4 9

X-RAY FLUORESCENCE ANALYSIS APPLIED TO SMALL SAMPLES

J.M. Jaklevic and W.R. French*
Lawrence Berkeley Laboratory, University of California
Berkeley, California 94720

T.W. Clarkson and M.R. Greenwood
Environmental Health Sciences Center, University of Rochester
Rochester, New York 14642

ABSTRACT

We describe an adaptation of photon excited x-ray fluorescence analysis which is optimized for the analysis of small samples. A fine focus x-ray tube is used in conjunction with small diameter detector collimators in order to focus on a small sample volume with as high sensitivity as possible. Sample areas of less than 1 mm diameter can be analyzed with ppm detectability. In applications involving the analysis of human hair samples, a minimum detectable limit of 10 ppm Hg can be realized in a 1 mm long segment of a single hair in a counting time of 200 seconds. Simultaneous measurements of the sample mass can be obtained from the intensity of the incoherent scattering. An automated x-ray fluorescence analysis system using the technique for the scanning of elemental profiles in such hair samples will be described.

INTRODUCTION

Present methods of photon excited x-ray fluorescence are oriented towards the analysis of large area (1 cm^2 or greater) homogeneous samples. Fluorescence analysis of smaller samples or the scanning of spatial distribution of elements within samples is normally performed using focused charged particle beams. Measurements using proton beams a few μm in diameter have been reported (1) and electron beam resolutions of 100 Å or less are routine. This high spatial resolution is achieved at some sacrifice in sensitivity, particularly for electron beams, and with the added complexity of the beam handling system and vacuum sample chambers.

We describe the design of a photon excited x-ray fluorescence system which has been optimized for the accurate and sensitive analysis of small (1 mm^2 or less) areas. Although not competitive with fine-focused particle beams in spatial resolution, the method is convenient to use and sensitive enough for a number of interesting applications. These include the scanning of inhomogeneous biological samples, measurement of residues obtained from the ashing of small samples, and the analysis of samples which are inherently in this size range. Measurements performed with a prototype instrument have indicated absolute detectable limits of 5×10^{-12} gm under realistic operating conditions.

The application of the method to the measurement of elemental distribution along the length of human hair segments has been explored using an automatic scanning device which advances the sample past the analysis region. The relationship between the incoherent scattering intensity and the mass of the hair segment has been shown to be a convenient and accurate means of converting the observed elemental concentrations to ppm by weight as preferred to the hair matrix. Scans of elemental concentration vs lengths can easily be obtained and their relationship with biological effects in the growing hair established.

DESCRIPTION OF INSTRUMENT

The spatial resolution of the x-ray fluorescence system is achieved using a small area collimator in a very closely coupled geometry. The complete spectrometer consisting of x-ray tube, collimator, and Si(Li) semiconductor spectrometer is illustrated in Fig. 1. High sensitivities are achieved by the close geometry between x-ray tube anode and sample (1.4 cm) and sample to detector (1.7 cm). The x-ray anode is a 6 mm diameter Mo rod which is contained at the end of an 8 mm diameter tube which projects beyond the end of the x-ray tube housing. A cathode assembly within this housing is operating at a negative accelerating potential causing the electron beam to be projected down the tube to the anode. The output radiation from the anode is filtered with a 25 μm Mo absorber which reduces the continum background relative to the characteristic Mo K x-rays. The tube can be operated on 100 watts dissipation using forced air cooling.

The 1 mm collimator shown in the figure is placed as close as possible to the sample while not obstructing the excitation flux from the anode. This design achieves the necessary spatial resolution with a sharp cutoff at the edges of the collimator while maintaining the full sample to detector solid angle. The collimator can easily be changed to provide finer or coarser resolution; the choice of 1 mm was a compromise between the needs of spatial reso-

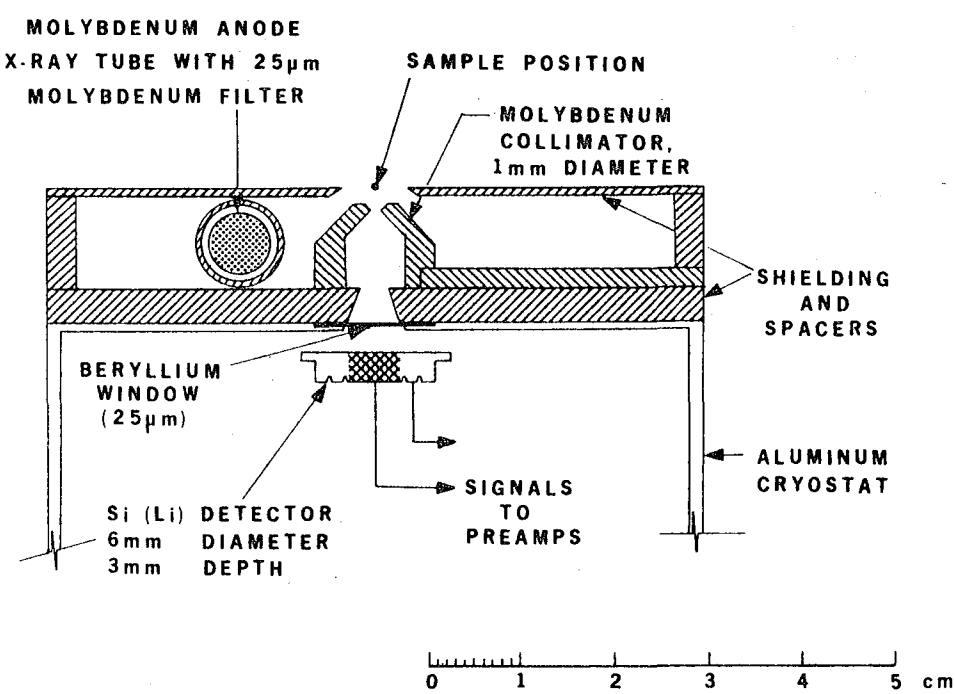

Fig. 1. Cross sectional view of the X-ray Spectrometer

lution and analytical sensitivity required for the scanning of hair samples.

A 6 mm diameter, 4 mm depletion depth, anti-coincidence guard-ring detector is used. Normal counting rates of 3000 cps were achieved with hair samples using 100 watts of x-ray tube power. For more massive samples or ones with higher atomic number constituents, counting rates of 10 Kcps or greater can easily be achieved.

The design of the collimator and shielding provides a relatively accessible sample plane which lends itself to scanning of larger samples. In the direction perpendicular to the view of Fig. 1, the maximum scan range of 10 cm is limited by the x-ray tube housing. However, scanning can be performed left to right with virtually no limit in length. For the case of the hair samples, individual segments up to 20 cm in length were scanned using a lead screw driven by a stepping motor under computer control. Spectra from 1 mm segments could be accumulated and stored followed by the automatic stepping to the next segment.

The ability to easily interchange collimators provides a possible mode of operation which can facilitate large scale scanning programs. A large collimator can be used to provide rapid survey

data until a region of interest is recognized by the presence or absence of certain elements. A smaller collimator could then be automatically substituted and a more detailed scan performed over the region of interest. The advantages of this mode of operation will become obvious in the case of certain types of human hair scans presented later in this paper.

CALIBRATION PROCEDURES

The calibration of highly collimated spectrometer system requires a different approach than typically used in energy dispersive instruments (2). Thin film standards are not directly applicable since the sample does not normally cover the entire field of view. Discrete standards are difficult to prepare because of the physical size of 1 mm^2 makes accurate standardization difficult. We employed two different calibration procedures which were then checked by intercomparison with atomic absorption results from two different laboratories.

Since the calibration methods require a precise measurement of the collimator field of view, we have performed scans of the sample region using a microscopic $Hg(NO_3)_2$ particle which was advanced across the sensitive region in 0.031 mm steps. The measurement of the spatial resolution response curve is shown in Fig. 2. From these data, the effective length of the field of view for a continuous filament sample was determined to be 1.10 mm and the effective area of an extended sample was calculated to be 1.30 x 10^{-2} cm.

The first calibration method used results of measurements performed with a carefully calibrated x-ray fluorescence unit at LBL which is normally used for the analysis of large area aerosol filters (3.7 cm diameter) (3). Human scalp hairs were artificially spiked with mercury or zinc in solutions of $Hg(NO_3)$ or $ZnCl_2$. These hairs were then scanned at 1.0 mm intervals to select 5.0 mm lengths over which the loadings were uniform. Measurement of these 5 mm lengths in the calibrated XRF system provided accurate total zinc and mercury concentrations. Subsequent scans of these segments on the collimated system provided sensitivities for zinc and mercury in counts/sec per ngm/mm of sample length. Relative sensitivities for several other elements were determined using available thin multielement standards. These data were normalized to the zinc values and converted to absolute sensitivities assuming a 1.10 mm linear field of view. The results are shown in Fig. 3.

The second calibration method used carefully validated thin film standards together with an assumed active area of 1.30 x 10^{-2} cm^2 as determined from the scan data. Table 1 compares the results

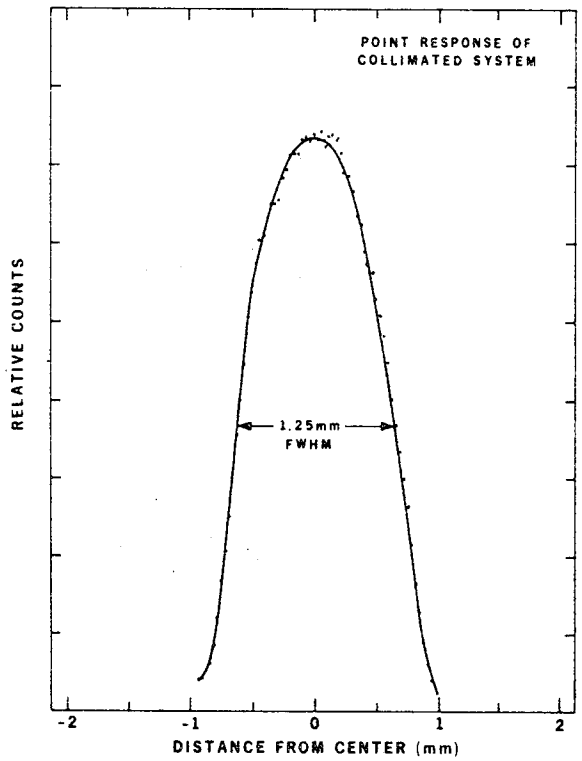

Fig. 2. Collimator field of view as determined by scanning a microscopic sized particle.

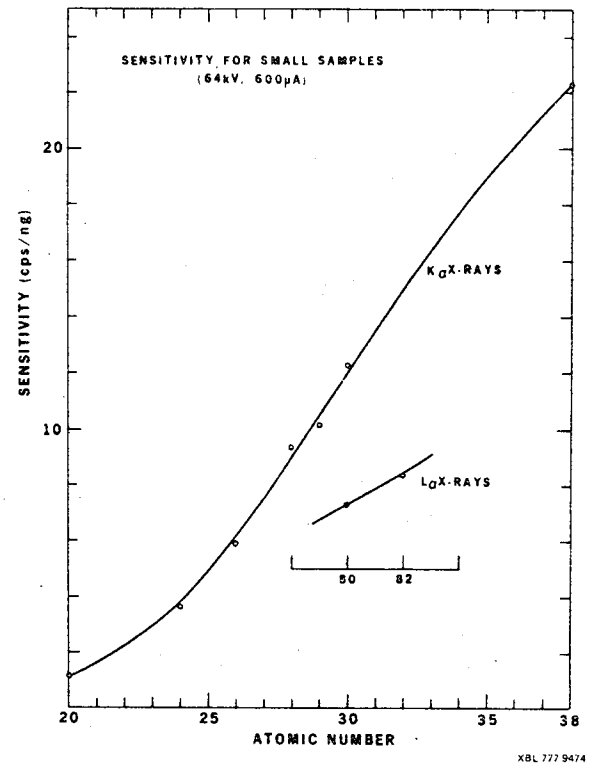

Fig. 3. Sensitivity of the spectrometer for various elements. Data taken at 64 kV and 600 μA.

0 0 0 0 2 8 0 2 5 5 1

of these two methods.

The observed discrepancies between the two methods can be explained as due either to uncertainties resulting from the determinations of the effective length and area of the field of view or due to local non-uniformity in the thin film standards. More accurate calibrations can no doubt be achieved with greater effort, but the observed agreement is adequate for present purposes.

Using these measured sensitivities, we have calculated minimum detectable limits for various elements assuming 1000 second counting times and an X-ray tube power of 100 watts. The results are given in Table 2. They assume a limit equal to 3 σ where σ is the statistical uncertainty in the background in the region of the peak of interest. The background was obtained from air scatter alone and would therefore only be achieved with samples supported by very thin substrates. In the case of single hair strands of approximately 4 μg/mm mass, the minimum detectable limit would be increased above these values by 20% for iron and 40% for mercury and lead.

Z	Element	Method #1	Method #2	Δ%
28	Ni	9.0	7.8	-13
30	Zn	12.3	10.5	-15
32	Ge	15.0	15.5	+ 3

Table 1. Comparison of sensitivities in cps/ng/mm for the two calibration methods discussed.

Z	Element Radiation	Background (30 channels)	$3\sigma_\beta$	S cps/ng	MDL pg
20	Ca Kα, Kβ	5979	232	3.15	74
24	Cr Kα	5159	215	9.6	22
25	Mn Kα	4560	203	13.2	15
26	Fe Kα	4513	202	15.3	13
28	Ni Kα	3138	169	24.5	7
29	Cu Kα	3036	165	26.4	6
30	Zn Kα	2701	156	32.1	5
35	Br Kα	12,047	329	53.1	6
38	Sr Kα	22,557	451	58.1	8
80	Hg Lα	4669	205	19.2	11
82	Pb Lα	6378	240	22.0	11
48	Cd*Kα	1177	103	1.15	90

Table 2. Sensitivities and Minimum Detectable Limit Levels in Air. 1000 sec counting times, X-ray tube power 100 watts. *Taken with 100 μm Mo foil to reduce Mo K X-ray intensity.

MASS MEASUREMENTS

Since many biomedical applications require the results to be expressed in ppm by weight, the measurement of very small masses by conventional gravimetric methods can become one of the more difficult steps in the analysis process. Because of the desire to obtain analyses directly in ppm from a single measurement, we have explored the possibility of using the net incoherent scattered intensity of Mo Kα radiation as an indicator of the mass of the sample viewed by the collimator. Since the incoherent scatter cross section is proportional to the total number of electrons, a simple relationship with mass should result.

Figure 4 shows the net incoherent scatter intensity as a function of mass per unit length for a variety of samples, including human hair and synthetic fibers. Additional data points carry the linearity of this relationship beyond 60 μgm/mm. Using this calibration curve, it is possible to perform accurate mass determinations of filaments in the 1 - 100 μgm/mm range.

In order to explore the general applicability of the method, it is necessary to consider the atomic number dependence of the scattering cross section as shown in Fig. 5 (4). For samples containing predominantly carbon, nitrogen and oxygen by mass, the results should be invariant with respect to small variations in composition.

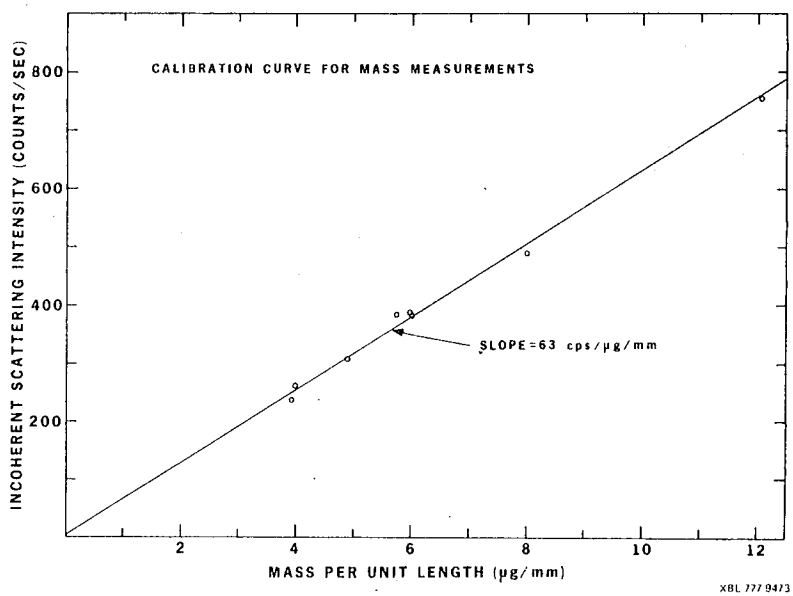

Fig. 4. Calibration curve for determination of mass per unit length of filament samples using incoherent scattering.

Excessive mass concentrations of either hydrogen or heavier elements cause changes in the slope obtained from Fig. 4. However, if the substances to be analyzed are of the same general composition, accuracies of 10% or less can easily be achieved.

In a comparison of results obtained by the scatering method with gravimetric determinations performed at the University of Rochester, the average results for several 1.0 cm hair segments were 4.10 ± 0.14 μg/mm for the x-ray method verses 4.29 ± 0.32 μg/mm for the gravimetric.

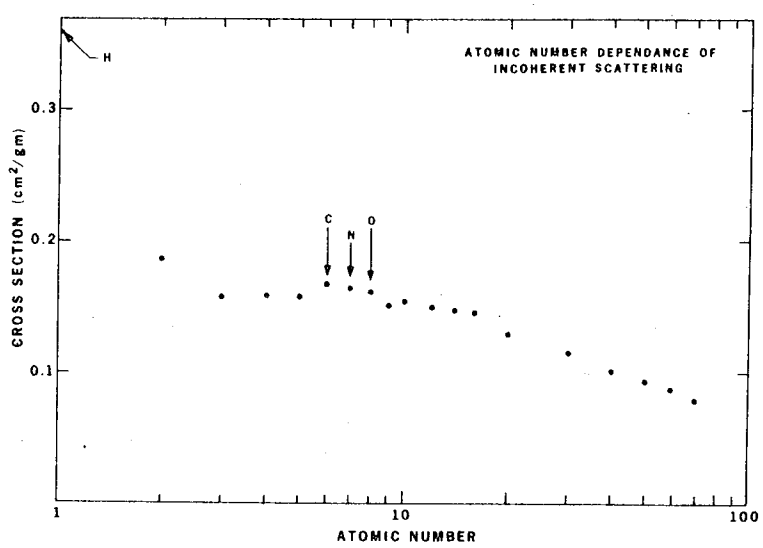

Fig. 5. Atomic number dependence of the incoherent scattering cross section.

APPLICATIONS AND RESULTS

The initial motivation for designing the scanning x-ray fluorescence system was the problem involved in measuring trace element profiles along the length of individual human hairs. A clinical study at the University of Rochester requires the ability to measure the mercury concentrations present in a large number of samples obtained from subjects exposed to mercury in various incidents.

The advantages of the X-ray method described here are numerous in this application:

1) Results can be obtained on single hair strands eliminating uncertainties due to variable growth rates among individual hairs.

2) The measurement of several elements simultaneously permits the study of possible synergistic effects among the elements.

3) The mass of the segment can be measured simultaneous with the elemental determinations.

4) Since it is non-destructive, clinical samples can be reanalyzed by other techniques.

5) It is easily automated to handle large numbers of samples.

Figure 6 shows X-ray fluorescence spectra obtained from 1 mm segment of single hair strands of five different individuals. The Mo Kα and Ar Kα peaks are due to the system itself. The Mo Kα X-ray scatter peaks are not shown.

The more obvious elements which are observed in single hair strands and which could be scanned with the 1 mm collimator are: calcium, iron, copper, zinc, mercury, lead, bromine, and strontium. The variations in concentrations, particularly those of mercury and lead are easily related to environmental factors. The diet of the Canadian fishing guide is known to contain a significant portion of fish containing mercury. The LBL laboratory technician exhibits a significant lead content, possibly due to deposits either from job related activities or freeway driving. Obviously, a detailed analysis of the significance of the difference would require a more extensive and systematic study.

These data were obtained using single hair strands and the 1 mm collimator. In order to observe additional constituents with a greater sensitivity it is necessary to increase either the number of hair strands or the area of the collimator (at some loss in spatial resolution). These options can easily be exercised in cases where more sensitive analyses are required.

The data of Fig. 7 show the results of the scanning of two hair segments for the elemental concentrations of Hg and Ca. The samples were obtained from a woman who was exposed to methylmercury contaminated grain in a February, 1972 incident in Iraq (5). The distances indicated are from the root ends of the hairs. These samples were collected in February 1973; one can assume an approximate

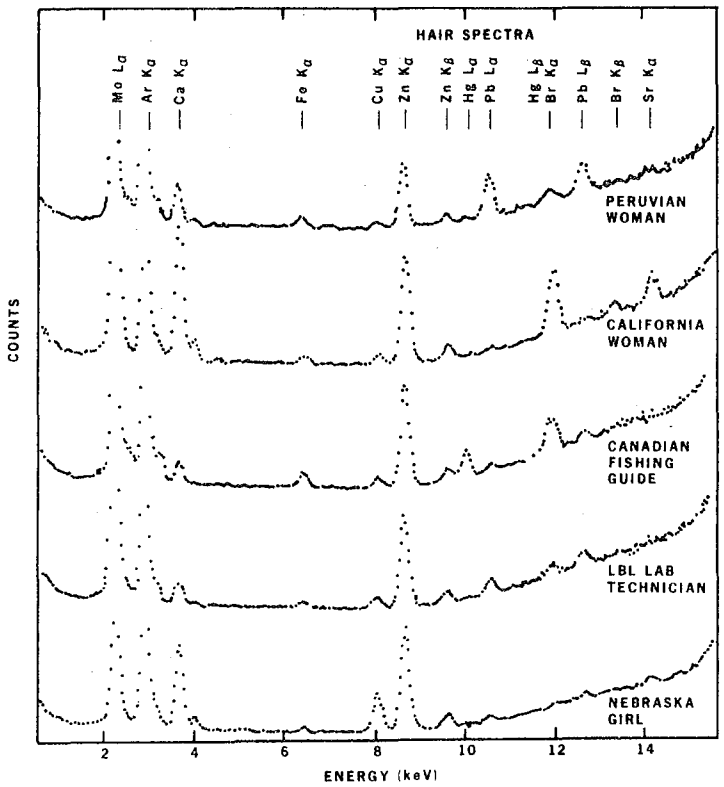

Fig. 6. X-ray spectra of single scalp hairs from several individuals.

growth rate of 1 cm/month. While the curves are displaced slightly relative to one another, they virtually coincide when overlaid. Note the rapid onset of the mercury and the approximate exponential shape of the decreasing levels. The time scale would be from right to left in the plots ; 1 mm equals approximately 3 days growth at normal rates. Also note the general decline in the calcium levels for both hairs as a function of time. Table 3 is a list of ppm levels for the principal elements observed in these samples at various positions along the length.

In order to check the accuracy of the x-ray fluorescence results, comparisons of results for mercury were made with two independent atomic absorption methods. In the first comparison, mercury spiked hair samples were analyzed by XRF and by Zeeman Atomic Absorption Analysis (ZAA) performed at LBL (6). The first three samples were chemically digested before the ZAA analysis whereas the second three were directly burned in the ZAA spectrometer. The results are given in Table 4. The improved agreement for the latter measurement indicate that some mercury may be lost in the

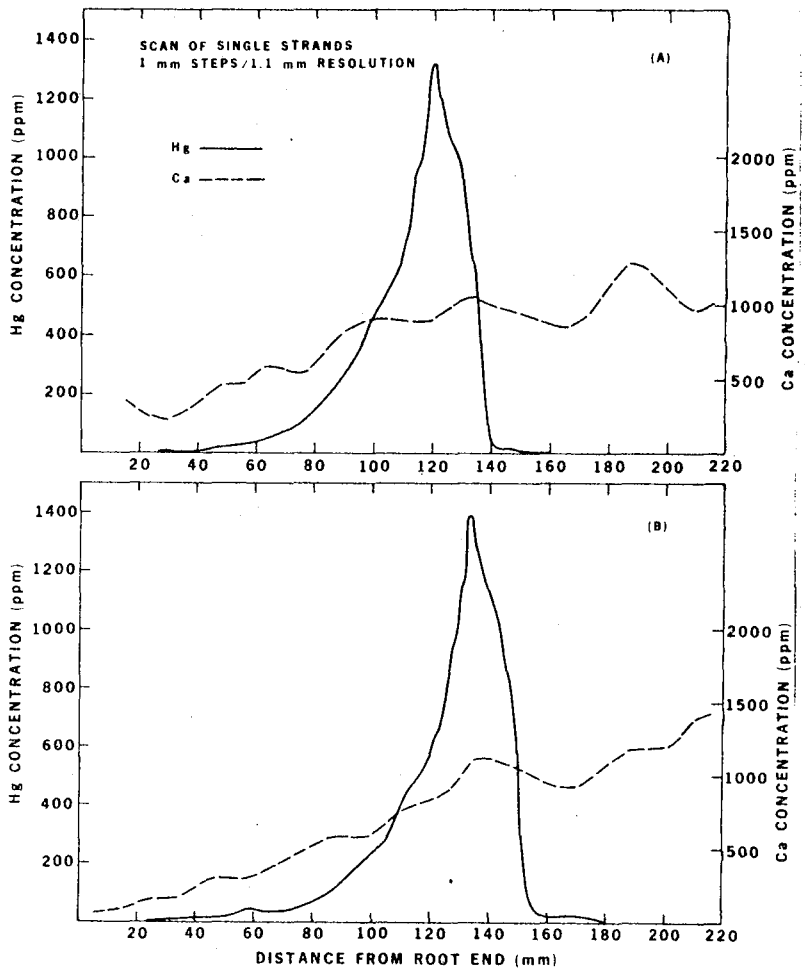

Fig. 7. Scans of two different hairs for mercury and calcium illustrating an abnormally high mercury concentration.

digestive process.

A second intercomparison involved actual mercury containing hair samples which were scanned at LBL and then sent to the University of Rochester for analysis. This comparison is shown in Table 5. Although the discrepancies are larger, it should be noted that these include errors in the total mass measurement as performed by the X-ray scatter technique at LBL and gravimetrically at Rochester. Since this was the first intercomparison between two methods at two independent laboratories, the results are quite good.

Dist. From Root End, Cm	Time Relative to Peak	Ca	Fe	Cu	Zn	Hg	Pb
20	Before	950	40	4	117	<10	5
11.8	Peak	750	46	5	190	1230	10
5.0	After	800	160	5	157	35	10
1.0	After	188	40	4	240	<10	80

Table 3. PPM levels for principle elements observed in the sample whose mercury and calcium scans are shown in Fig. 7. Relative times refer to peak mercury concentration.

Sample	LBL/XRF ng Hg	LBL/ZAA ng Hg	Δ%
1[a)]	7443	6600	+11
2	5052	3800	+25
3	151	48	+68
4[b)]	17.1	17.0	+0.6
5	22.4	21.7	+3.2
6	30.5	29.3	+4.1

a) Mercury spiked hairs in acid and an aliquot of solution used for the ZAA measurement.

b) 5.0 mm hair segments burned directly for ZAA measurements.

Table 4. Intercomparison of LBL/XRF and LBL/ZAA results.

Sample	LBL/XRF ng Hg	ROCH AA ng Hg	Δ%
7	98.6	88.8	+10
8	235.0	209.4	+11
9	345.3	277.0	+20
10	577.9	498.8	+14
11	1094.7	947.8	+13
12	1141.9	924.4	+19
13	442.0	430.7	+ 3

Table 5. Intercomparison of LBL/XRF results with the University of Rochester Atomic Absorption measurements.

CONCLUSIONS

We have demonstrated that the use of a highly collimated photon excited energy-dispersive X-ray fluorescence unit provides a sensitive and accurate method for the analysis of small samples. The detectable limits of 5 pgm is competitive with other X-ray fluorescence methods employing more elaborate excitation schemes. The simple design allows for versatility in the choice of collimator and facilitates the automatic scanning of samples with computer data acquisition and central systems. No vacuum hardware is required for sample handling, eliminating the problems associated with sample volatility and the necessity for elaborate manipulators.

The use of the incoherent scattering intensity as a measure of sample mass has been demonstrated to be a useful method for many applications. This is particularly significant for the case of small samples where direct gravimetric weighing is difficult.

Improvements in the design of the instrument would include a more elaborate on-line data acquisitor and control system to accomodate many of the potential features discussed above. As the unit becomes operational in full scale studies of large numbers of samples, the full potential of the method should be realized.

ACKNOWLEDGMENTS

The authors wish to acknowledge the participation of W. Searles and N. Madden in the design and construction of the spectrometer system.

The Lawrence Berkeley Laboratory work was supported in part by the United States Energy Research and Development Administration.

Work at the University of Rochester was supported in part by a center grant (EF-01247) and program project grant (EF-0128) from the National Institute of Environmental Health Sciences and in part under contract to the United States Energy Research and Development Administration at the University of Rochester Biomedical and Environmental Research Project and has been assigned report number UR-3490-1236.

REFERENCES

1. J.A. Cookson and F.D. Pilling, "Trace Element Distributions Across The Diameter of Human Hair," Phys. Med Biol. 20, 1015-1020 (1975).

2. "X-ray Fluorescence Analysis of Environmental Samples," T.G. Dzubay, Editor, Ann Arbor Science Publishers, Ann Arbor, Michigan (1977).

3. F.S. Goulding, J.M. Jaklevic and B.W. Loo, "Development of Air Particulate Monitoring Systems," Progress Report to EPA, LBL Report, LBL-6497 (1977).

4. F. Bakir, S.F. Damluji, L. Amin-Zaki, M. Murtadha, A. Khalidi, N.Y. Al-Rawi, S. Tikriti, H.I. Dhadir, T.W. Clarkson, J.C. Smith and R.A. Doherty, "Methylmercury Poisoning in Iraq," Science 181, 230 (1973).

5. W.H. McMaster, N.K. Del Grande, J.H. Mallett and J.H. Hubbell, "Compilation of X-ray Cross Sections," University of California, LBL Report UCRL-50174, Section II, Revision I, National Technical Information Service (1969).

6. T. Hadeishi, D.A. Church, R.D. McLaughlin, B.D. Zak, M. Nakamura and B. Chang, "Mercury Monitor for Ambient Air," Science 187, 348 (1975).

---

*Dr. W.R. French was on leave from Nebraska Wesleyan University, Lincoln, Nebraska 68504.

This report was done with support from the United States Energy Research and Development Administration. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the United States Energy Research and Development Administration.

0 0 0 0 4 5 0 2 2 9 4