Analysis of Human Hair by Spark Source Mass Spectrometry

J. P. Yurachek,[1] G. G. Clemena, and W. W. Harrison

Department of Chemistry, University of Virginia, Charlottesville, Va. 22901

TRACE ELEMENTS in human hair have not been extensively studied. The low concentrations encountered in such samples have limited many of the previous analyses to the more predominant elements only. Spectrophotometric methods (*1–3*) have been used as has atomic absorption (*4, 5*) to examine specific trace metals. Emission spectrographic techniques (*6*) have been used to survey 14 different elements in human hair. The more recent use of neutron activation analysis (*7, 8*) as a tool in forensic studies of hair has shown the presence of at least 18 trace elements in human hair.

Anomalously high concentrations of certain elements may show up in the hair—*e.g.*, lead and mercury in cases of poisoning. The hair seems to be capable of storing quantities of elements present in excess concentration, thus providing a valuable time dependent record of possible diagnostic use. In a joint program with the Medical School of the University of Virginia, various elements have been monitored by atomic absorption (*5*) for a number of patients over periods ranging up to a year. Interest in the results of this study pointed to the desirability of a broad ranging survey method which would not only simultaneously analyze qualitatively and quantitatively for those elements of suspected abnormality but would also clearly point out any unusual patterns in the other elements present, metals and nonmetals, down to the parts-per-billion level.

Spark source mass spectrometry has been shown to exhibit this degree of sensitivity and scope in a wide variety of matrices. Sasaki and Watanabe (*9*) have examined several different biological tissues using spark source mass spectroscopy. Wolstenholme (*10*) has determined the trace elements in dried blood plasma. Evans and Morrison (*11*) applied this method to a variety of biological materials, including human blood serum, human kidney tumor, sheep lung, and dried plant leaves. In this study, we have developed a survey method for trace elemental analysis of human hair. Sample preparation and a comparison of several hair samples are discussed.

EXPERIMENTAL

Apparatus. Table I shows a summary of the equipment and experimental conditions used.

[1] Present address, E. I. du Pont de Nemours and Co., Inc. Richmond, Va. 22901

(1) E. W. Rice and N. P. Goldstein, *Metabolism*, **10**, 1085 (1961).
(2) G. M. Martin, *Nature*, **202**, 903 (1964).
(3) T. F. Dutcher and S. Rothman, *J. Invest. Dermatol.*, **17**, 65 (1951).
(4) L. Kopita, R. K. Byers, and H. Schwachman, *New Engl. J. Med.*, **276**, 949 (1967).
(5) W. W. Harrison, J. P. Yurachek, and C. A. Benson, *Clin. Chim. Acta*, **23**, 83 (1969).
(6) R. W. Goldblum, S. Derby, and A. B. Lerner, *J. Invest. Dermatol.*, **20**, 13 (1953).
(7) A. K. Perkins and R. E. Jervis, *J. Forensic Sci.*, **11**, 50 (1966).
(8) L. C. Bate and F. F. Dyer, *Nucleonics*, **23**, 74 (1965).
(9) N. Sasaki and E. Watanabe, Thirteenth Annual Conference on Mass Spectrometry and Allied Topics, St. Louis, Mo., 1965.
(10) W. A. Wolstenholme, *Nature*, **203**, 1284 (1964).
(11) C. A. Evans and G. H. Morrison, ANAL. CHEM., **40**, 869 (1968).

Reagents. Silver Powder 99.999% (Gallard-Schlesinger Chemical Mfg. Corp., New York, N. Y.), and Ultra Superior Purity graphite (Ultra Carbon Corp., Bay City, Mich.) were used as matrix materials for the preparation of the electrodes. Spectrographically pure Yttrium Oxide (Johnson, Matthey and Co. Inc., London, England) was used as an internal standard. High purity acids (G. F. Smith Chemical Co., Columbus, Ohio) were used for sample wet ashing.

Sample Preparation. Procurement and pretreatment of the hair samples have been described (*5*). Two different ashing procedures were used prior to sample electrode preparation. For larger hair samples ranging in size from a few hundred milligrams to one gram, dry ashing at 450 °C in a muffle furnace was suitable.

The ash was collected, weighed, and transferred to a 25- × 12.5-mm plastic vial. The matrix material, doped with a known concentration of yttrium, was added in a ratio of 3 parts matrix material or 1 part ash. The mixture was then shaken for 30 minutes on a Wig-L-Bug (Crescent Dental Mfg. Co., Chicago, Ill.) in order to ensure homogeneity. Because only milligram quantities of the homogeneous mixture were

Table I. Equipment and Experimental Conditions

Analysis instrument	A.E.I. MS 702 spark source mass spectrometer
Other equipment	Jarrell-Ash Model 23-100 recording microphotometer, Bristol Model 570 Dynamaster recorder, Disc chart integrator Model 235A, Jarrell-Ash Model 3410 processing unit
Vacuum	Magnetic analyzer, 10^{-8} Electrostatic analyzer, 10^{-8} Source, 5×10^{-6} (when sparking)
Spectrometer parameters	Spark voltage, 30 kV Repetition rate, 300 pulses/second Spark pulse length, 100 μsec Electrostatic analyzer, 2 kV Accelerating voltage, 20 kV Primary slit, 0.002 inches Exposure range, 1×10^{-13} coulomb to 1×10^{-7} coulomb
Microphotometer parameters	Slit, 3 microns Occulter, 1.2 mm Scanning speed, 1.0 mm/sec
Recorder parameters	Response time, (full scale) 0.4 sec Range, −0.05 to +1.05 mV Chart speed, 2.5 in./min
Ion-beam chopper	Chopping frequency, 200 to 20,000 Hz Pulse length, 5 μsec
Detector	2 × 10 inch Ilford Q-2 thin glass photographic plate
Developing conditions	Developing, Eastman Kodak D-19 (1:1 ratio) at 20.0 °C for $2\frac{1}{2}$ minutes under Wratten Series 1A safelight Stop bath, 14% acetic acid solution for 30 sec Fixing, Eastman Kodak Rapid fixer with hardener for 3 minutes Washing, running water for 15 minutes, distilled-deionized water for 1 minute Drying, forced air for 15 minutes

available for analysis, the sample was tipped out on a support electrode prepared in a stainless steel molding die (Associated Electrical Industries Ltd., Manchester, England). Approximately 0.1 gram of the mixture would be required to prepare two full size electrodes. When the sample is tipped out, only about 10 mg is required for each electrode tip and approximately one-tenth of the tip is consumed in the course of an analysis. A special spring tension holder replaced the conventional screw fixed electrode holder to eliminate breakage of the somewhat brittle graphite electrodes. Each electrode was approximately 10 mm long and had a diameter of 1 mm.

For very small hair samples, the 1% or less residue resulting from ashing the sample, is so small as to be difficult to handle and requires a micro balance for proper weighing. Therefore, for 10–100 mg samples of dry hair a wet-ashing procedure was developed in which the sample in a 25-ml flask is digested in 0.5 ml of nitric acid by gently heating to a clear brown solution followed by final oxidation to a water clear solution after the addition of 0.2 ml of perchloric acid. This is heated further to a moist residue in order to remove the excess acids and then taken up into 2 ml of a 5-μg/ml standard yttrium solution. One-ml portions of the sample are added to a 10–20 mg portion of the matrix in a porcelain crucible, forming a homogeneous slurry, which is dried under an infra-red lamp after each addition. When all the digested hair sample containing the standard has been added and dried, the residue is transferred to a polyethylene vial and Wig-L-Bugged for 15 minutes, after which two electrodes are tipped out from the mixture.

Internal Standard. Yttrium was used as an internal standard because of its availability in a highly pure form, the considered improbability of its normal occurrence in the sample, and its monoisotopic nature. Standard yttrium solutions were prepared which were then used to dope the matrix to 100 ppmw yttrium by formation of a homogeneous slurry, drying, and Wig-L-Bugging to produce a stock quantity for mixing with the sample ash. Blanks were run on the matrices and standard.

Data Treatment. Density measurements were taken as per cent transmission areas with a ball and disc integrator. A computer program developed for the Burroughs B-5500 at the University of Virginia corrected equivalent peak areas of the standard and each unknown to exposure ratios which were used in a conventional expression (*12*) to calculate final concentrations. Relative sensitivity factors were computed from analyses of Jarrell-Ash SQ Powder Standards, the graphite series at 1, 10, and 100 ppm.

RESULTS AND DISCUSSION

The hair samples were not conductive to direct analysis by spark source mass spectrometry because of their nonconducting nature. Experiments with finely divided hair samples mixed with a conducting matrix also indicated that the added spectral complexity due to the organic constituents would require their elimination for meaningful qualitative and quantitative results. This led to the requirement of the previously described ashing procedures. Without ashing, a line was found at essentially every mass unit and often not sufficiently resolved from lines due to inorganic constituents to allow analysis.

Powdered silver was first examined as a matrix material because of its availability in high purity as well as its excellent conducting and electrode forming properties. However, two difficulties were encountered. Blank shots of the pure silver showed unacceptable quantities of several elements of interest, notably lead, bismuth, barium, tin, zinc, copper, iron, and aluminum. A more severe problem arose from the somewhat coarse nature of the powder which created difficulties in producing a homogeneous mixture of the ashed sample with silver, as was shown by nature of graded exposures.

A recently introduced high purity grade of graphite was then studied as a matrix material. Ultra Superior Purity graphite (Ultra Carbon Corp., Bay City, Mich.) showed very little trace element impurity and was so finely divided as to allow homogeneous mixing with the sample residue. It was necessary, on the longer exposures, to avoid the polycarbon lines at 24, 36, 48, etc., but this usually does not impose a serious limitation. A total blank of matrix and standard was also run.

Table II shows some typical data from hair samples which were also analyzed for certain elements by atomic absorption. The longest exposure used for any of the spark source mass spectrographic data was 100 nC, which represents a reasonable limitation on analysis time. Longer exposures of perhaps 1,000 nC could be useful if extreme sensitivity is required. It is difficult to compare this to previous literature values, both because of the limited number of samples run in this study by spark source mass spectrometry as well as the dearth of analytical results for many of the elements whose concentration is so low as to make analysis quite difficult by other techniques.

The reproducibility of analyzing a single sample was studied by taking a finely divided and carefully mixed hair specimen and weighing out three 10-mg samples which were then carried through separate digestion and analysis steps. The triplicate analysis is shown in Table III. Because quite small samples

Table II. Comparative Analysis of Two Different Hair Samples by Mass Spectrographic and Atomic Absorption Methods

Values given in μg/gram., dry weight

	Hair sample 1 (μg/gram)		Hair sample 2 (μg/gram)	
Element	SSMS	AA	SSMS	AA
Na	1205.0		516.4	
Mg	16.4	21.8	14.5	7.3 to 11.5[a]
Al	4.6		3.7	
Si	10.9		27.9	
P	120.2		208.9	
S	448.0		841.1	
K	72.6		225.7	
Ca	135.0	210.0	158.0	
Ti	3.9		24.0	
Cr	0.65		5.9	
Mn	1.7		1.8	
Fe	2.72	8.72	12.0	7.7 to 10.5[a]
Ni	0.45		3.4	
Co	0.24		0.34	
Cu	16.2	17.7	8.7	11.8 to 14.9[a]
Zn	246.0	236.0	181.0	122 to 172[a]
As	0.06		0.31	
Br	0.17		1.1	
Rb	0.2		0.23	
Sr	1.1		0.75	
Mo	0.13		0.28	
Ag	0.4		2.0	
Cd	0.34		0.96	
Sn	0.4		0.39	
I	0.03		0.14	
Ba	0.6		0.46	
Pb	14.5		21.8	

[a] Normal ranges for this subject from a previous atomic absorption study.

(12) W. L. Harrington, R. K. Skogerboe, and G. H. Morrison, ANAL. CHEM., **37**, 1480 (1965).

Table III. Triplicate Analysis of 10-mg Samples of a Common Hair Sample

Values in μg/grams., dry weight

Element	Sample 1	Sample 2	Sample 3
Pb	52	47	45
La	0.83	0.49	N.D.
Ba	5.0	5.6	5.0
Sn	1.5	1.5	1.6
Cd	1.5	0.48	1.8
Ag	1.9	2.6	1.4
Mo	2.2	2.6	3.1
Zr	1.9	2.3	1.0
Sr	9.4	11	12
Br	2.5	0.90	0.82
Se	0.79	0.48	N.D.
As	0.62	1.2	0.82
Ge	2.0	0.90	3.70
Ga	0.02	N.D.	0.14
Zn	153	143	148
Cu	95	114	130
Ni	28	25	20
Fe	73	110	100
Mn	7.8	8.8	5.8
Cr	31	32	33
Ca	750	750	1150
K	377	700	900
Al	N.D.	1.0	9.0

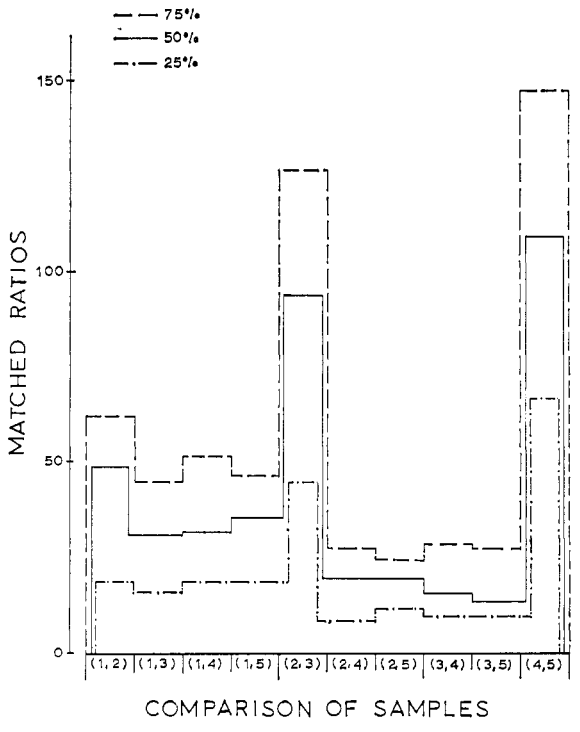

Figure 1. Effect of different limiting values on the concentration ratio comparison of 5 hair samples

Samples 2 and 3 and samples 4 and 5 are duplicates taken from two different subjects

were used, some of the variation may indicate elemental concentration differences within the hair. However, inherent imprecision in the spark source technique is quite certainly also reflected in the data.

Trace element analyses of human hair have been used in forensic studies to match or identify common origin of hair specimen (*8*, *13*). Comparisons have been made between trace element analyses by neutron activation analysis *vs.* spark source mass spectroscopy for such purpose (*14*). Perkins and Jervis (*7*) have demonstrated a ratio comparison technique using gamma ray peak heights for neutron activation studies which allowed them to identify duplicate samples from a mixed series. This method has been adapted to the integrated peak areas and resultant elemental concentrations for each mass spectrographic analysis line. Concentration ratios of a particular analysis element to other elements in the same sample are compared to the corresponding ratios in a second sample. If a relatively large number of the ratios are within a preset arbitrary comparison limit percentage of each other, the two samples are taken to be of possible common origin. For spark source mass spectrometry, the large number of elements which can be determined will permit many comparison ratios. For example, if 20 elements are determined in each of two hair samples, 190 separate and nonrepeated reference ratios may be computed. The arbitrary acceptable limit of comparison which is set is obviously very important. Perkins and Jervis studied the use of limits from 1% to a factor of two. Our data have been analyzed using 25, 50, and 75% comparison limits.

For 20 different elements, the ratio comparison was applied to the analysis data from five hair samples to determine the number of times that a particular ratio for one sample was within a specified comparison limit of the same ratio for each of the other samples. The bar graph Figure 1, shows the results for 25, 50, and 75% limit comparison figures for each sample. Samples two and three are from the same person, samples four and five are also duplicates taken from a second person. The data indicate, for example, that while approximately 100 ratios from the duplicate samples fall within 50% of each other, a much smaller number coincide for the comparison of samples taken from different persons. It is seen that although the number of ratios falling within each limit is different, of course, the qualitative conclusions would remain the same. Over a number of samples, the 50% comparison limit has been the most satisfactory. A computer program was written to evaluate directly the large number of comparison ratios which are involved when a series of samples is checked. The analysis data for each sample are read in and a printout of net comparison points is provided.

The utilization of spark source mass spectrometry to hair analysis is of interest from at least two aspects. As a clinical diagnostic aid, human hair represents a readily available, painlessly procured specimen which may reflect certain trace element conditions in the body, such as the presence of excess toxic metals. The time dependent nature of its formation produces what has been called a recording filament which may be of value in studying long term effects. In recent years, the forensic role of hair analysis has been demonstrated using neutron activation analysis. Spark source mass spectrometry, as has been shown in the present investigation, can provide analysis data for a large number of elements in human hair. This allows a rapid accumulation of a large number of ratios for the comparisons shown in Figure 1.

(13) A. K. Perkins and R. E. Jervis, *J. Forensic Sci.*, **7**, 449 (1962).
(14) T. G. Williamson and W. W. Harrison, 1968 International Conference on Modern Trends in Activation Analysis, October, 1968, Gaithersburg, Md.

RECEIVED for review July 3, 1969. Accepted August 6, 1969. Supported by OLEA Research Grant No. 154.