A major purpose of the Technical Information Center is to provide the broadest dissemination possible of information contained in DOE's Research and Development Reports to business, industry, the academic community, and federal, state and local governments.

Although a small portion of this report is not reproducible, it is being made available to expedite the availability of information on the research discussed herein.

LA-UR--83-3391

DE84 003838

CONF-831195--3

Los Alamos National Laboratory is operated by the University of California for the United States Department of Energy under contract W-7405-ENG-36.

TITLE: THE DETECTION OF ELEMENTS IN BIOMEDICAL FLUIDS BY LASER-INDUCED BREAKDOWN SPECTROSCOPY

AUTHOR(S): Thomas R(obert) Loree, CHM-6

SUBMITTED TO: For the Proceedings of the International Congress on Applications of Lasers and Electro-Optics

Los Angeles, CA

November 15-17, 1983

Los Alamos National Laboratory
Los Alamos, New Mexico 87545

FORM NO 836 R4
ST NO 2629 6/81

THE DETECTION OF ELEMENTS IN BIOMEDICAL FLUIDS BY LASER-INDUCED BREAKDOWN SPECTROSCOPY

THOMAS R. LOREE

LOS ALAMOS NATIONAL LABORATORY

ABSTRACT

Laser-induced breakdown spectroscopy has been used for the qualitative elemental analysis of human blood, sweat, and serum. The detection of most trace elements, especially metals, was demonstrated.

INTRODUCTION

Laser-induced breakdown spectroscopy (LIBS) is a technique for the elemental analysis of samples in any physical state, in situ and in real time. We have applied this technique to preliminary demonstrations of feasibility for the analysis of the human body fluids blood, sweat, and serum. LIBS is a variation of atomic emission spectroscopy (AES), in which the sample is truly atomized and excited in a plasma formed by laser-induced breakdown. During the lifetime of the plasma, all the excited species (neutral atoms and ions) radiate their unique sets of spectral lines. Detecting these spectral fingerprints to deduce elemental compositions is the heart of all AES techniques. The

wavelength of the laser used to form the breakdown is not important, but the requirement of high power dictates a pulse length of some tens of nanoseconds; the lifetime of the plasma itself is tens of microseconds. The atomic emission lines are independent of the laser wavelength, but the degree of ionization does vary with energy. At laser energies of several hundreds of millijoules, only neutral and singly-ionized lines are seen.

In the course of laser development, laser-induced breakdown was observed as soon as high laser powers were available, to the detriment of early optical components. The phenemenon, and the life of the plasma established by the breakdown, are quite complex[1]. The spectral history of the plasma, however, is quite well-delineated, especially in a gaseous sample. The first several hundred nanoseconds are dominated by white light, followed by a period of strong ionic emission, relaxing after a microsecond to a long period of neutral atomic emission and recombinant molecular lines.

Quite early, it was recognized that the emission from the plasma could be used for atomic detection[2], and that improved signal-to-noise could be obtained by using time resolution[3]. By some curious turn of events, however, the only form in which lasers were commercially used for AES analysis was the laser microprobe[4], in which the breakdown only vaporized the sample. The vapor was then drawn into a conventional electric spark for excitation. LIBS itself seems to have been forgotten for some period of time.

We rediscovered LIBS in 1980 to solve the problem of probing and analyzing coal gasifier product streams. Since LIBS requires

only optical access to a hostile environment, it was ideal for that purpose[5]. When the technique was improved by adding time resolution[6,7], we found that it could be applied to a wide variety of problems; the detection of beryllium[8], the analysis of other airborne particulates[9], bulk liquids[10], metal alloys[11], and the present study, biomedical liquids.

In bulk liquids, spectral emissions from single shots were severely repressed, and it required a double-pulsed technique to elicit useful spectra. Since double-pulsing adds both complexity and expense to the apparatus, and since we wanted to avoid working with large samples, a fresh approach to liquid testing was required. We found that LIBS interrogation of a sample soaked into filter paper worked very well, giving in effect a large liquid surface without requiring a macroscopic sample. All data presented was taken in this manner, although nebulized and microdrop samples have also been successfully analyzed.

Whole blood, sweat, and blood serum were used in this study because of their ready availability. Other body liquids, such as spinal fluid, may be more appropiate for actual applications of LIBS since small sample size and rapid analysis is normally of little advantage for the studied fluids. However, for a feasibility demonstration, any of these complex biomedical fluids should illustrate the potential of LIBS detection.

THE EXPERIMENT

A schematic of the apparatus that evolved ir these

experiments is shown in Figure 1. The laser beam (200 mJ of 1.06 um in a 15-ns pulse) entered from the left and was focused onto the sample with a cylindrical lens (15-cm focal length). This produced a rectangular focal spot 5mm high by 1 mm wide, which would barely produce breakdown on the surface. Twenty shots were used on each spot before rotating the sample to a new position, and the damage to an irradiated spot was slight. This combination of a gentle breakdown and few shots minimized the spectral contribution of the Whatman 541 filter paper. Serum and sweat samples were applied to the paper at full strength, but whole blood had to be diluted with distilled water to make it flow.

The collected light was dispersed with a 1/2-meter spectrometer and detected with a Tracor Northern time-gated intensified diode array. Time-gating is normally used to good effect in LIBS studies to eliminate early-time white light[7]. In the data to be shown, the time window used was the interval 2-12 μs after the 15-ns laser pulse. The spectra were collected, manipulated, and displayed by a Tracor Northern multichannel analyzer, then plotted out on an x-y recorder for hard copy.

As appropiate for a qualitative preliminary study, sample collection and preparation were rather casual, and open to the possibilities of trace level contamination. For instance, sweat was collected during profuse perspiring by dripping onto filters, and thus had the opportunity to pick up contributions from skin and clothing. After drying the sample, the filter paper was mounted by its perimeter on a cylinder (which in turn was mounted on the rotating stage of Figure 1), so that the interrogated area was free-standing. This procedure insured that there could be no

question of backing or mounting materials contributing to the spectra. Comparisons of paper alone (wetted with distilled water and dried) with sample-soaked paper led to the conclusion that under run conditions, the paper contribution to the combined spectrum was negligible.

BLOOD AND SERUM

It is obvious that blood and serum are very complex molecular substances, and this is also true from an elemental standpoint. A list of the normal elements to be found in whole blood is given in Table I[12], and includes a fair fraction of the entire periodic table. Since we ran these samples in open air, C, O, N, and Ar from air were inherent in all spectra. We will use spectroscopic suffixes I and II (as in O I and O II) to denote the neutral atom and singly-ionized state, respectively.

At the most basic level, what differences can we expect to see between blood and serum? In Table II we list the differences in electrolyte levels between serum and blood cells. Clearly, we should see more Na and Ca in serum, while the cells in whole blood should enhance the K spectra. This last comparison can be seen in Figures 2 and 3, where we show the major K lines with a nearby O line for a standard. Relative to the O line, the amplitude of the K pair in Figure 2 (blood) is greater than that in Figure 3 (serum). In a like manner, the principal lines of Na (589.0 and 589.6) were stronger in serum than in blood. Since there were no invariant comparison lines in those spectra, they are not shown. The comparison for Ca, however, can be seen in

Figures 4 and 5. The UV portion of the spectrum is much richer in lines than the IR, so these spectra are more interesting. The serum spectrum in Figure 4 contains contributions from a number of elements; note the strong Ca lines and the weak (but definite) Fe line at 302.1 nm. In Figure 5 (blood) these two are reversed, the Ca pair subsiding and the Fe line becoming the strongest member of an emergent family of Fe lines. Other elements seen in these spectra are Al, Mg, Cd, Cu, Sn, and Si. All of these are corroborated by spectral lines detected in other regions of the spectrum, although the strength of the "Al" lines in Figure 5 makes that identification suspect. A summary of the elements detected will be given in Table III. In the trace elements, blood and serum seem to be alike, save for Co and Ni, which were not detected in serum.

Strong molecular lines (CH, CN, C_2, and NH) were seen in all samples. These are felt to be formed in recombination as the plasma cools, rather than being remnants of parent molecules. In either event they carry no analytical information, and only serve to confuse the analysis.

SWEAT

Perspiring is known to be an effective pathway for the body to rid itself of metals; so much so that one treatment for Hg poisoning is to place the subject in a sauna. Hohnadel et al.[13] measured values for Ni, Cu, Zn, and Pb, and noted that others had detected traces of Al, Cr, Co, Cu, Fe, Mn, Mo, Sn, and Hg. Of course, this list do not include the major constituents, which

would include C, H, O, N, Na, Ca, K, and Mg. Sweat would appear to be as complex in elemental composition as blood or serum.

Again, we will only give a few examples of the spectra obtained, and save a complete listing of the elements detected for Table III. Figure 6 shows a large Cu peak; Cu was seen in abundance in all samples. Also notable in this example is Fe, which contributed many lines to the sweat spectrum.

The spectrum of Figure 7 shows large Ca peaks, with an attendant pair of Al lines. Strong C and Mg lines dominate the spectrum of Figure 8, but there are some minor lines of interest. This includes Si, Mn, Co, Pb, and B. Boron is an unnatural element, (but was verified by other spectra), and probably resulted from contamination by detergent in the clothing of the subject. The results listed in Table III show that sweat is similar in elemental composition to blood; Fe is on the same order, Zn is larger, and sweat does not contain detectable amounts of S or Cl.

CONCLUSIONS

A summary of the results for all fluids is given in Table III, where (*) signifies large peaks, (√) means definitely identified, (?) shows uncertainty in identification, and (-) indicates that the element was not detected. A comparison with Table I shows that we worked our way well into the trace constituents in this preliminary survey study. One must be aware that elements vary by orders of magnitude in their spectral output, and thus one may see the same line amplitudes from 10 ng

of Fe and 10 μg of S or F. The result is an erratic pattern of detection on a list ordered by concentrations. Unscrambling these complex spectra is complicated because LIBS produces lines that are intrinsically 0.2-0.3 nm wide. This fact, coupled with unknown LIBS amplitudes and the complexity of the spectra, makes unambiguous identification of all lines quite difficult. Hence, for instance, the uncertainty in assigning the large peaks in Figure 5 to Al I.

We must emphasize the qualitative and preliminary nature of this feasibility demonstration, and the immature state of LIBS. We do not present LIBS as having improved detectabilities over a standards laboratory technique like AES in inductively-coupled plasmas, although LIBS detection limits are within two orders of magnitude in most cases. The strengths of the technique are that it is field-deployable, requires little or no sample preparation, works with very small samples, and can gather information in real time. In trying to quantify LIBS spectra for other studies, we have obtained useful working curves for a variety of situations and on several different criteria, but we have only begun to attack the analytical problems of accuracy and reproducibility at a level better than several percent. In summary, LIBS is a demonstrated detection technique, but is not yet a mature method of accurate analysis.

As applied to biomedical fluids, LIBS could be useful in a number of ways. We suggest the rapid analysis of small samples, field detection of metals (as in heavy metal contamination or poisoning), or perhaps as a quick sorting technique to select

samples for more accurate analysis. This particular study has been restricted to biomedical fluids, but LIBS can also be used for tissue analysis. In combination with microscopy, this could result in a new type of direct laser microprobe.

TABLE I

ELEMENTS IN WHOLE BLOOD

ELEMENT	WEIGHT/ml
C, H, O, N	(Also in air)
"Chlorides"	2.95 mg
Ca	970 µg
P	350 µg
Na	94 µg
K	46 µg
Mg	40 µg
S	40 µg
Zn	8.8 µg
Si	8.3 µg
Br	8.1 µg
Rb	3.0 µg
Cu	980 ng
Fe	485 ng
F	300 ng
Pb	290 ng
Mn	200 ng
Sn	130 ng
Au	120 ng
I	100 ng
Al	90 ng
Cd	<10 ng
Co	<10 ng
Ni	<10 ng
Ag	<10 ng
Hg	<10 ng

TABLE II

ELECTROLYTE LEVELS

	BLOOD CELLS	PLASMA OR SERUM
Na	25	140
K	110	4.5
Ca	0.002	11.0

(mM/liter)

TABLE III

DETECTED ELEMENTS

	BLOOD	SERUM	SWEAT
C, H, O, N	*	*	*
Al	*	*	*
B	—	—	✓
Bi	—	?	?
Br	?	?	—
Ca	*	*	*
Cd	*	*	*
Cl	✓	✓	—
Co	✓	?	✓
Cu	*	*	*
F	✓	✓	✓
Fe	*	✓	*
K	*	✓	✓
Li	—	—	?
Mg	*	*	*
Mn	*	*	✓
Na	*	*	*
Ni	✓	?	✓
P	✓	✓	✓
Pb	✓	✓	✓
S	✓	✓	—
Si	✓	✓	✓
Sn	✓	✓	✓
Zn	✓	✓	*

References

1. "Laser-Induced Breakdown of Gases," C. Grey Morgan; Reports on Progress in Physics 38, 621 (1975).

2. "Spectrochemical Analysis Using a Pulsed Laser Source," E. F. Runge, R. W. Minck, and F. R. Bryan; Spectrochim. Acta 20, 733 (1963).

3. "Time-Resolved Spectroscopy of Laser-Generated Microplasmas," E. Archbold, D. W. Harper, and T. P. Hughes; British J. Applied Physics 15, 1321 (1964).

4. Laser Micro-Spectrochemical Analysis, H. Moenke and L. Moenke-Blankenburg (translated by R. Auerbach); (Crane, Russak & Co., New York, 1973).

5. "Laser-Induced Breakdown Spectroscopy: Detecting Sodium and Potassium in Col Gasifiers," T. R. Loree and L. J. Radziemski; Proceedings of the 1981 Symposium on Instrumentation and Control for Fossil Energy Processes (ANL-81-62, CONF-810607, Argonne National Laboratory, 1982) p. 768.

6. Laser-Induced Breakdown Spectroscopy: Time-Integrated Applications," T. R. Loree and L. J. Radziemski; Plasma Chem. and Plasma Processing 1, 271 (1981).

7. "Laser-Induced Breakdown Spectroscopy: Time-Resolved Spectrochemical Applications," L. J. Radziemski and T. R. Loree, Plasma Chem. and Plasma Processing 1, 281 (1981).

8. "Detection of Beryllium by Laser-Induced Breakdown Spectroscopy," Leon J. Radziemski, David A. Cremers, and Thomas R. Loree; Spectrochim. Acta 38B, 349 (1983).

9. "Time-Resolved Laser-Induced Breakdown Spectrometry of Aerosols," Leon J. Radziemski, Thomas R. Loree, David A. Cremers, and Nelson M. Hoffman; Analytical Chem. 55, 1246 (1983).

10. "Spectrochemical Analysis of Liquids Using the Laser Spark," David A. Cremers, Leon J. Radziemski, and Thomas R. Loree; Applied Spectroscopy, in press (1983).

11. "The Identification of Impurities in Metals by Laser-Induced Breakdown Spectroscopy," T. R. Loree and L. J. Radziemski; Proceedings of the Technical Program, Electro-Optics/Laser 81 Conference & Exposition (Cahners Exposition Group, Chicago, 1981), p. 28

12. Blood and Other Body Fluids, P.L. Altman and D.S. Dittmer (Biological Handbooks series, Federation of American Societies for Experimental Biology, Washington D.C., 1962).

13. "Atomic Absorption Spectroscopy of Nickel, Copper, Zinc, and Lead in Sweat Collected from Healthy Subjects During Sauna Bathing", D.C. Hohnadel, F.W. Sunderman, Jr., M.W. Nechay, and M.D. McNeely; Clin. Chem. 19, 1288 (1973).

CAPTIONS

Figure 1: A schematic of the LIBS apparatus used to analyze blood, serum, and sweat. The sample was soaked into Whatman 541 ashless filter paper, dried, and mounted on a rotating stage. The laser beam (100-200 millijoules, 15 ns, 1.06 um) was focused only on one axis with a cylindrical lens, resulting in a rectangular breakdown spot on the sample parallel to the input slit of the spectrometer. With a 1/2-m spectrometer, a 1200 groove/mm grating, and a 2.54-cm-wide diode array, a 30-nm wide spectrum was detected for each setting. The diode array was time-gated to eliminate the early white light background, and the data were stored in a Tracor Northern multichannel analyzer.

Figure 2: The spectrum of blood from 740 to 780 nm, showing the most prominent K lines. Note that they are larger than the nearby O line.

Figure 3: The spectrum of serum from 760 to 800 nm. As expected from the lower concentration of K in serum, the K peaks are smaller than in blood.

Figure 4: The spectrum of serum from 270 to 330 nm. Mg dominates, but note the large Ca peaks in the vertically expanded superior plot. Serum is rich in Ca and Na, so the 285.2/.3 peak is a composite of Mg and Na lines. Note also the presence of Sn, Cu, a small Fe line, and Al.

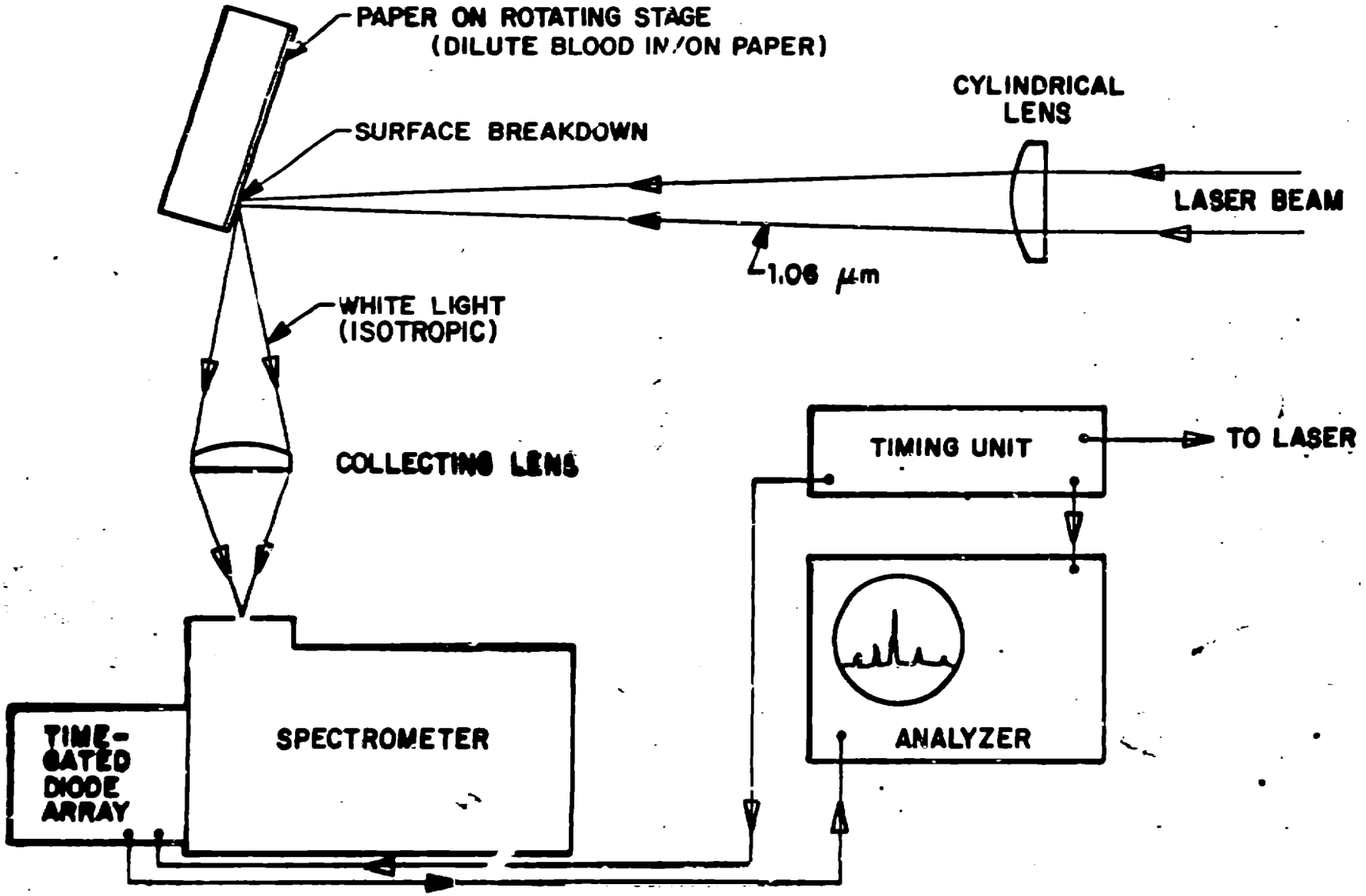
Figure 5: The spectrum of blood in the same region as Figure 4. The reduction of Ca and increase in Fe are obvious. The great increase in the lines assigned to Al is suspicious, casting doubt on this line assignment.

Figure 6: The sweat spectrum from 490-530 nm. The point of this example is to show the large Cu peak. Fe is has several nice lines; Ca and Mg were abundant, as will be shown following.

Figure 7: Sweat, 380-420 nm region. The large line pair from Ca contains a believable pair from Al. All molecules are believed to be formed in recombination.

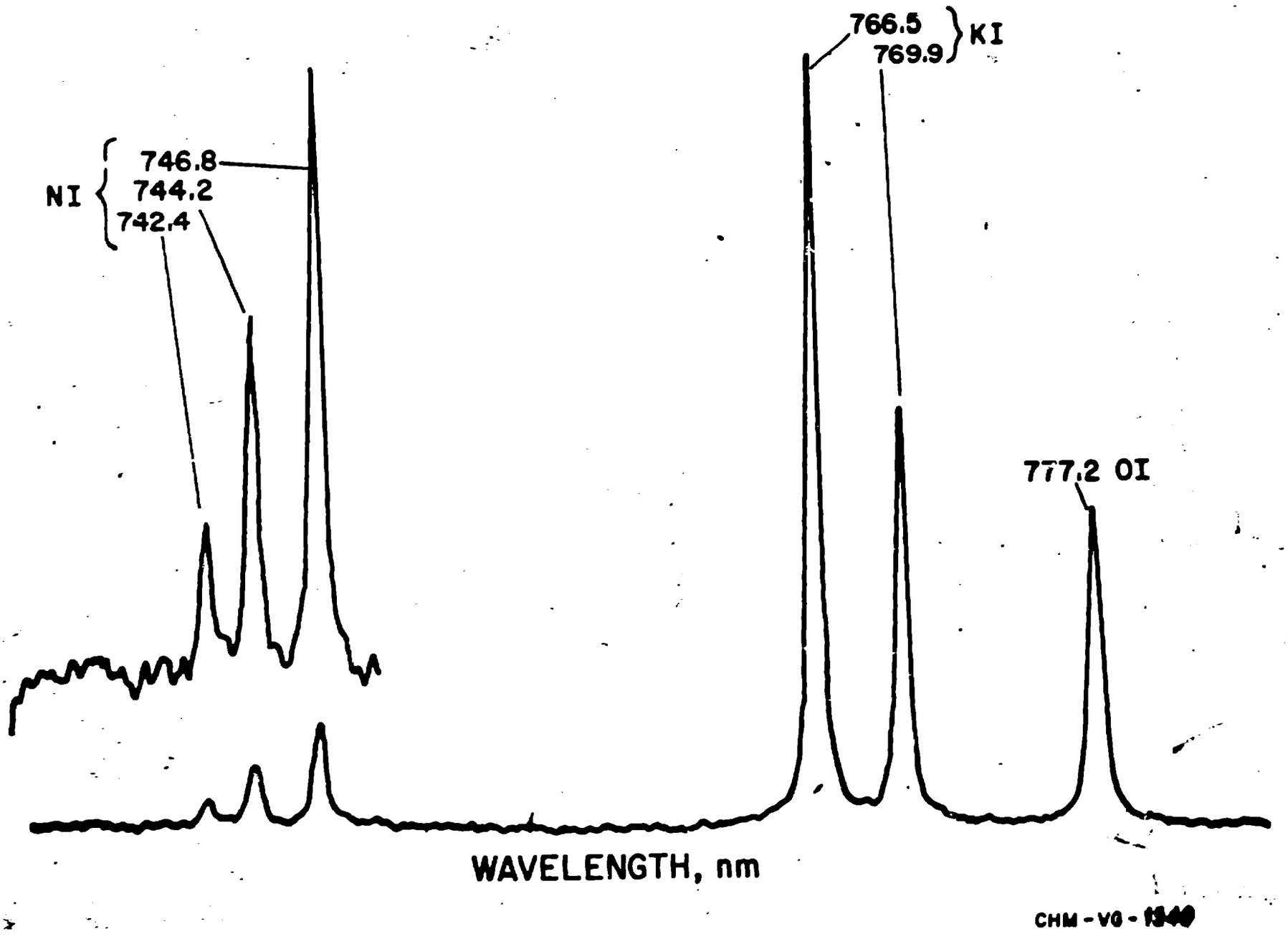
Figure 8: A section of the UV spectrum of sweat, from 245 to 285 nm. Many elements are seen in this rich spectral region, including a group from Si, lines from Mn, Co, Pb, and large Mg and C lines. The detection of B was confirmed in other traces, and must come from some contamination of the sample, such as a detergent.

LIBS SCHEMATIC



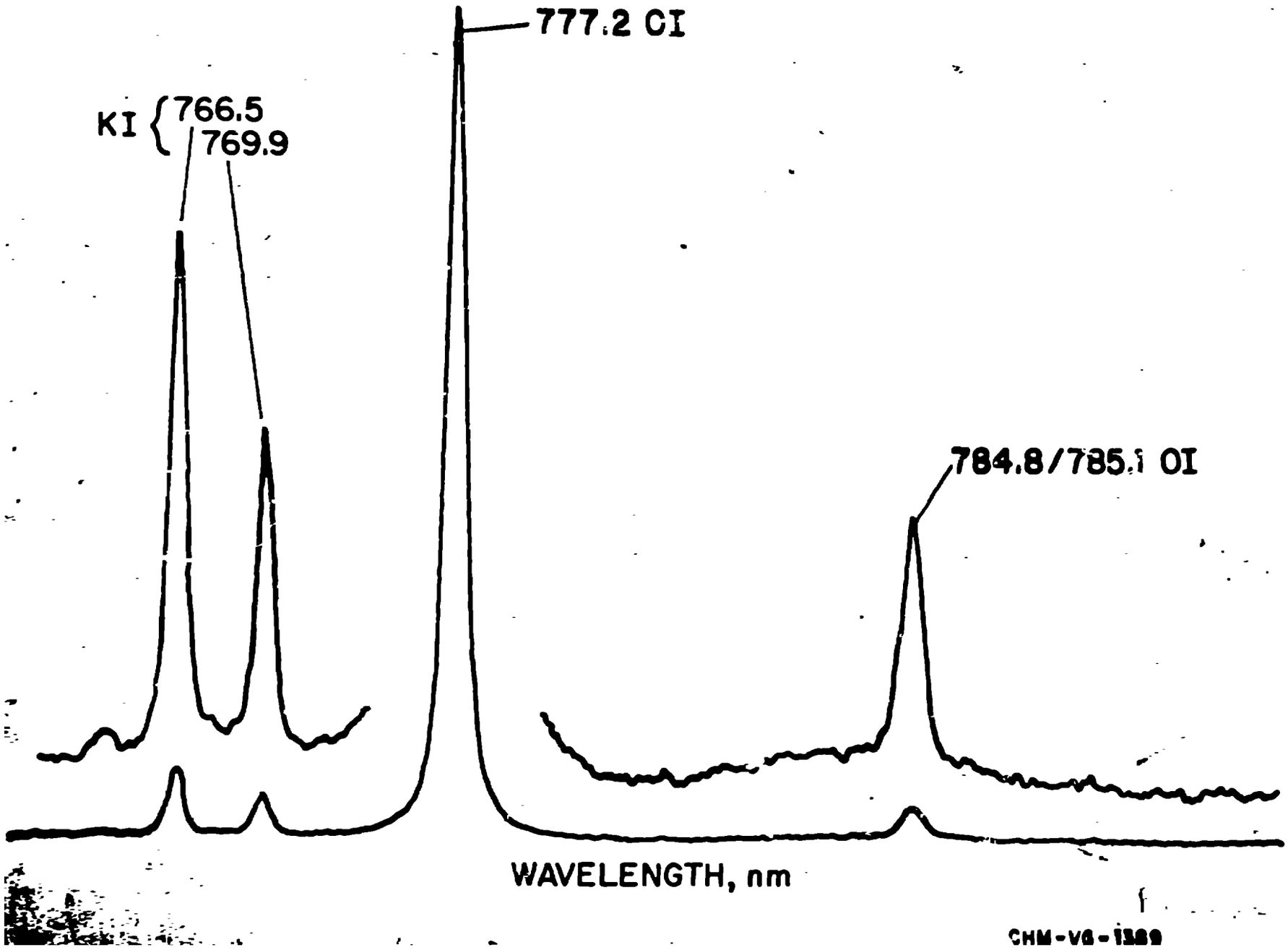
CHM-VG-1338

LIBS OF BLOOD: 740-780 nm



CHM - VG - 1340

LIBS OF SERUM: 760 – 800 nm



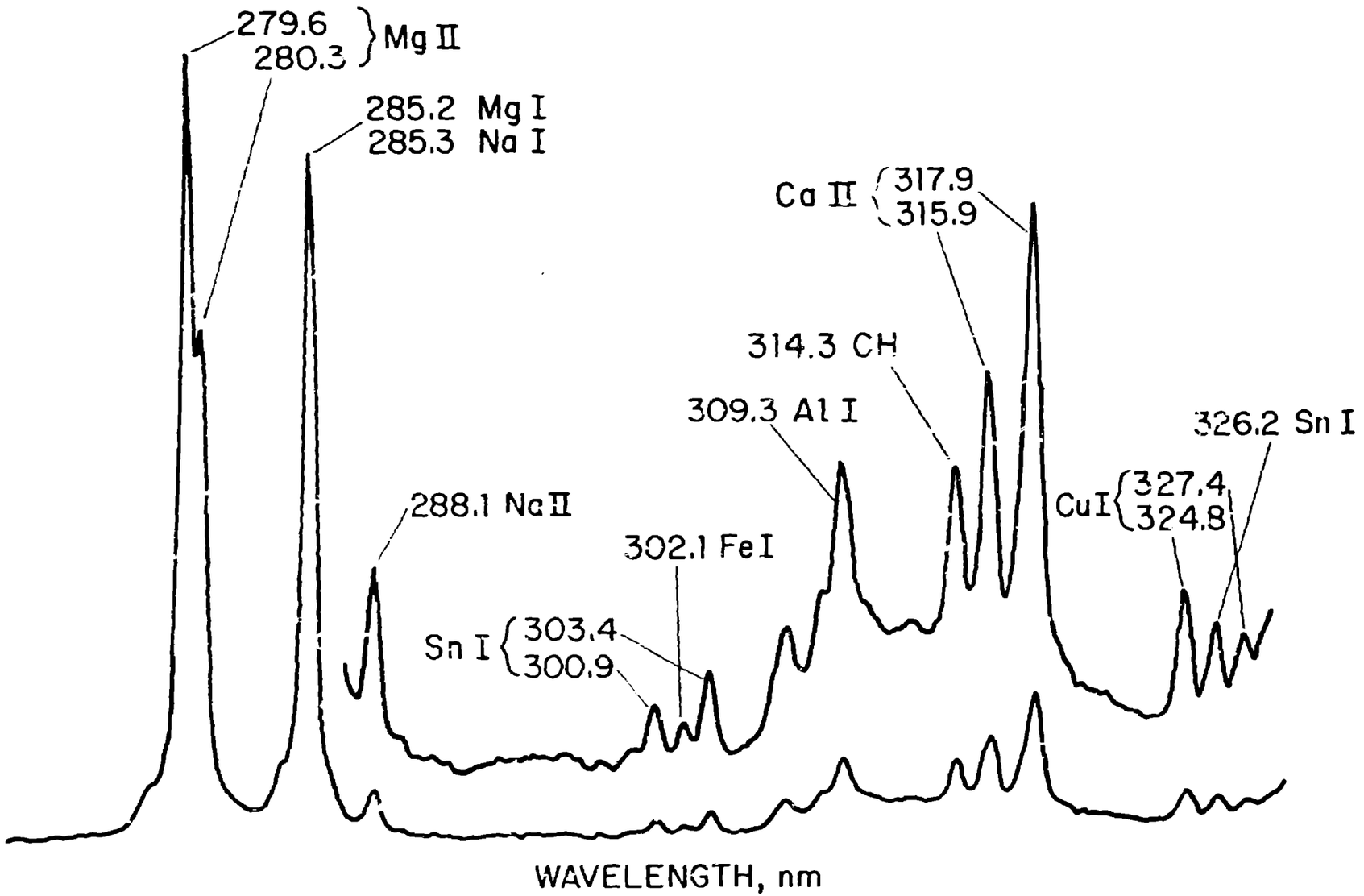

279.6
280.3
Mg II
285.2 Mg I
285.3 Na I
Ca II
317.9
315.9
314.3 CH
309.3 Al I
326.2 Sn I
288.1 Na II
302.1 Fe I
Cu I
327.4
324.8
Sn I
303.4
300.9
WAVELENGTH, nm

FIGURE 4

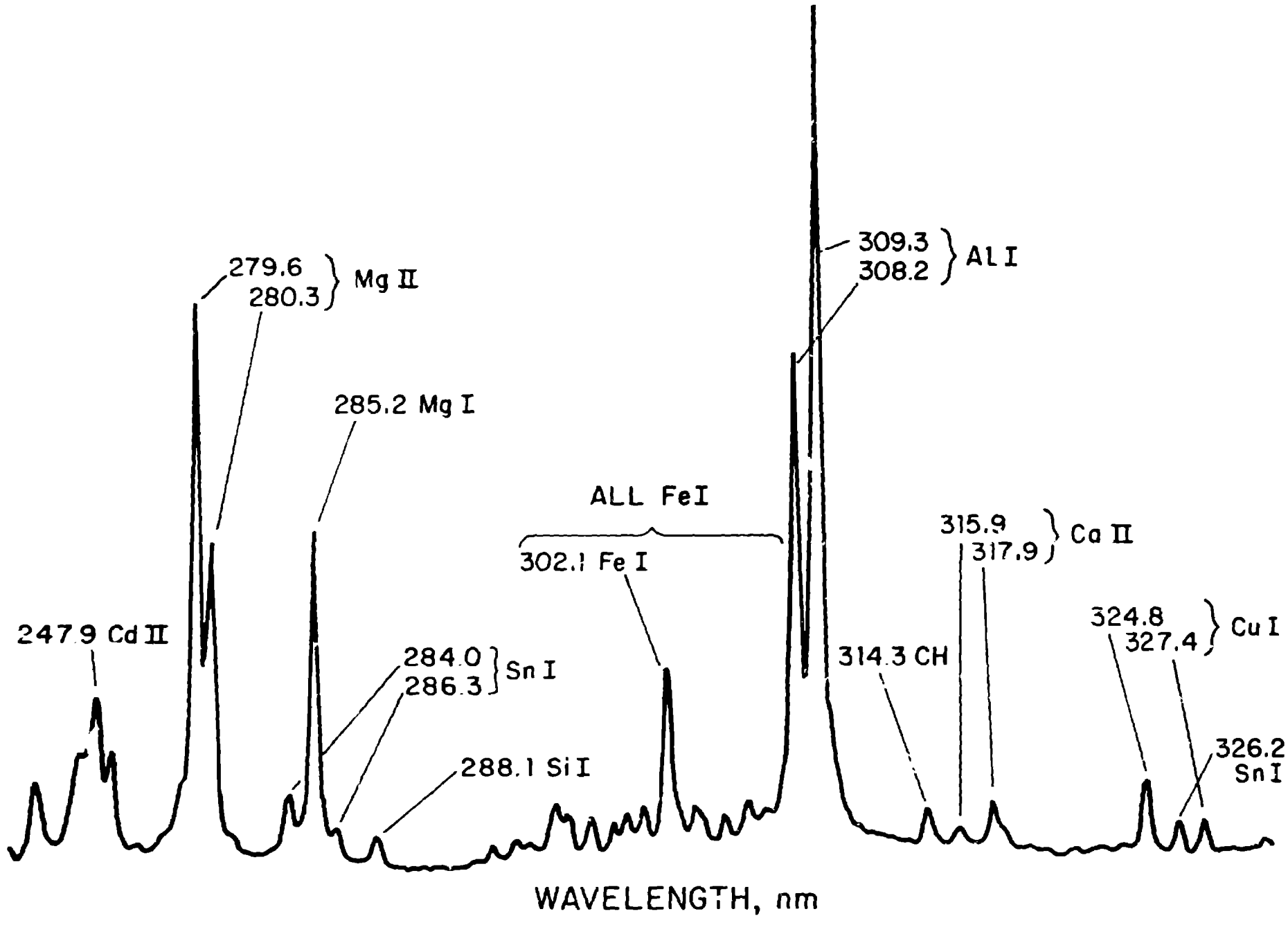

279.6
280.3
Mg II
285.2 Mg I
309.3
308.2
Al I
ALL FeI
302.1 Fe I
315.9
317.9
Ca II
247.9 Cd II
284.0
286.3
Sn I
314.3 CH
324.8
327.4
Cu I
288.1 Si I
326.2
Sn I
WAVELENGTH, nm

FIGURE 5

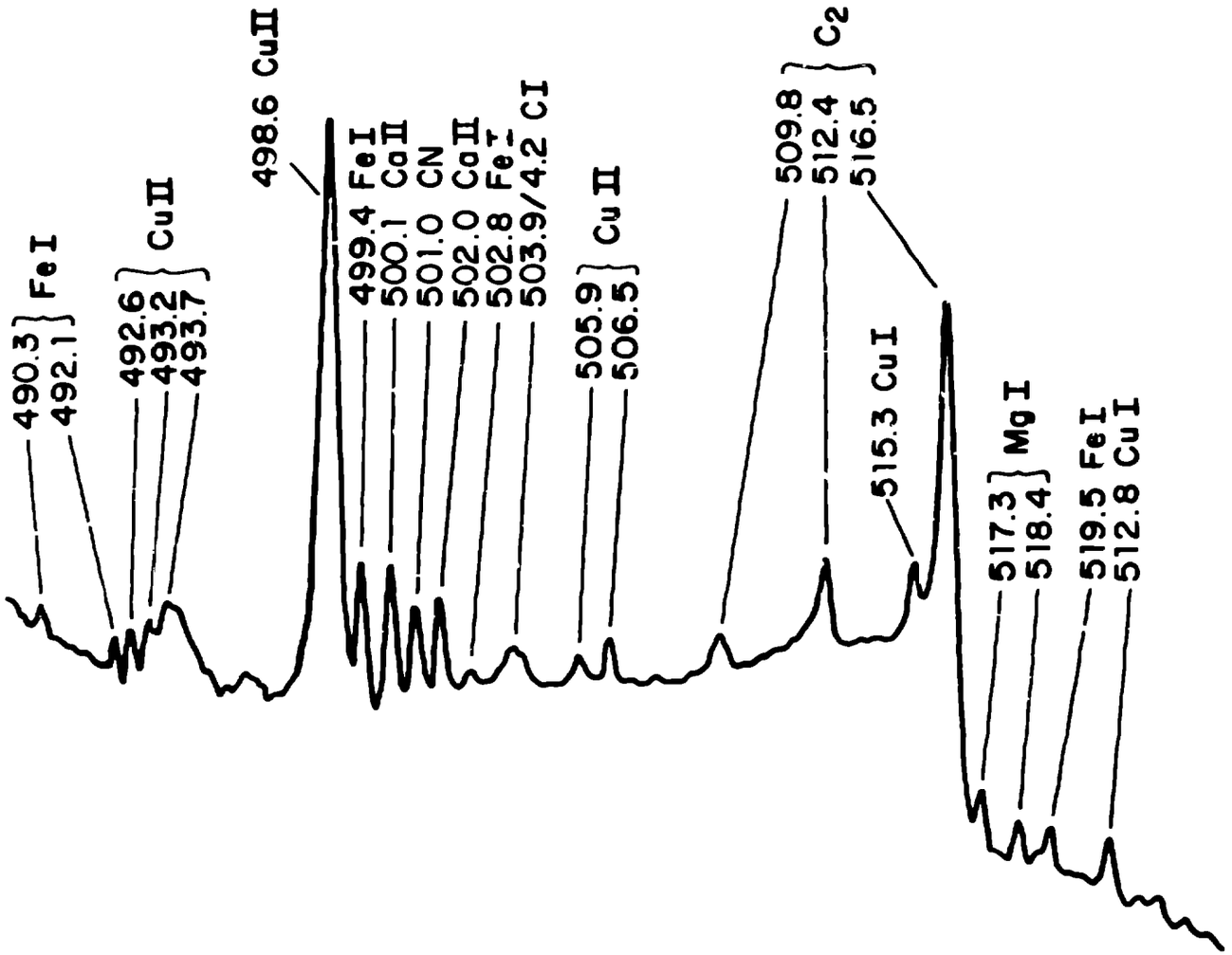
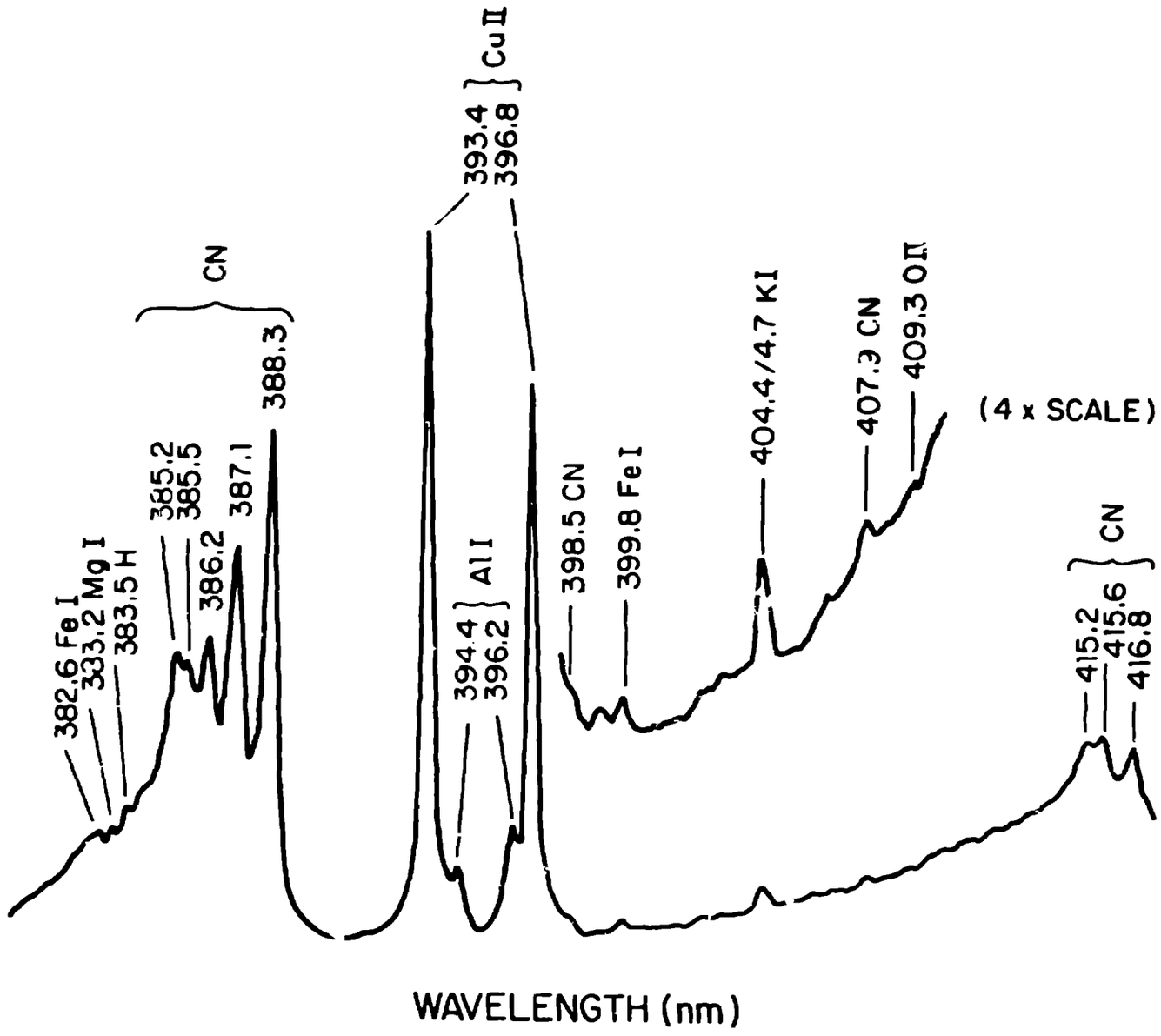
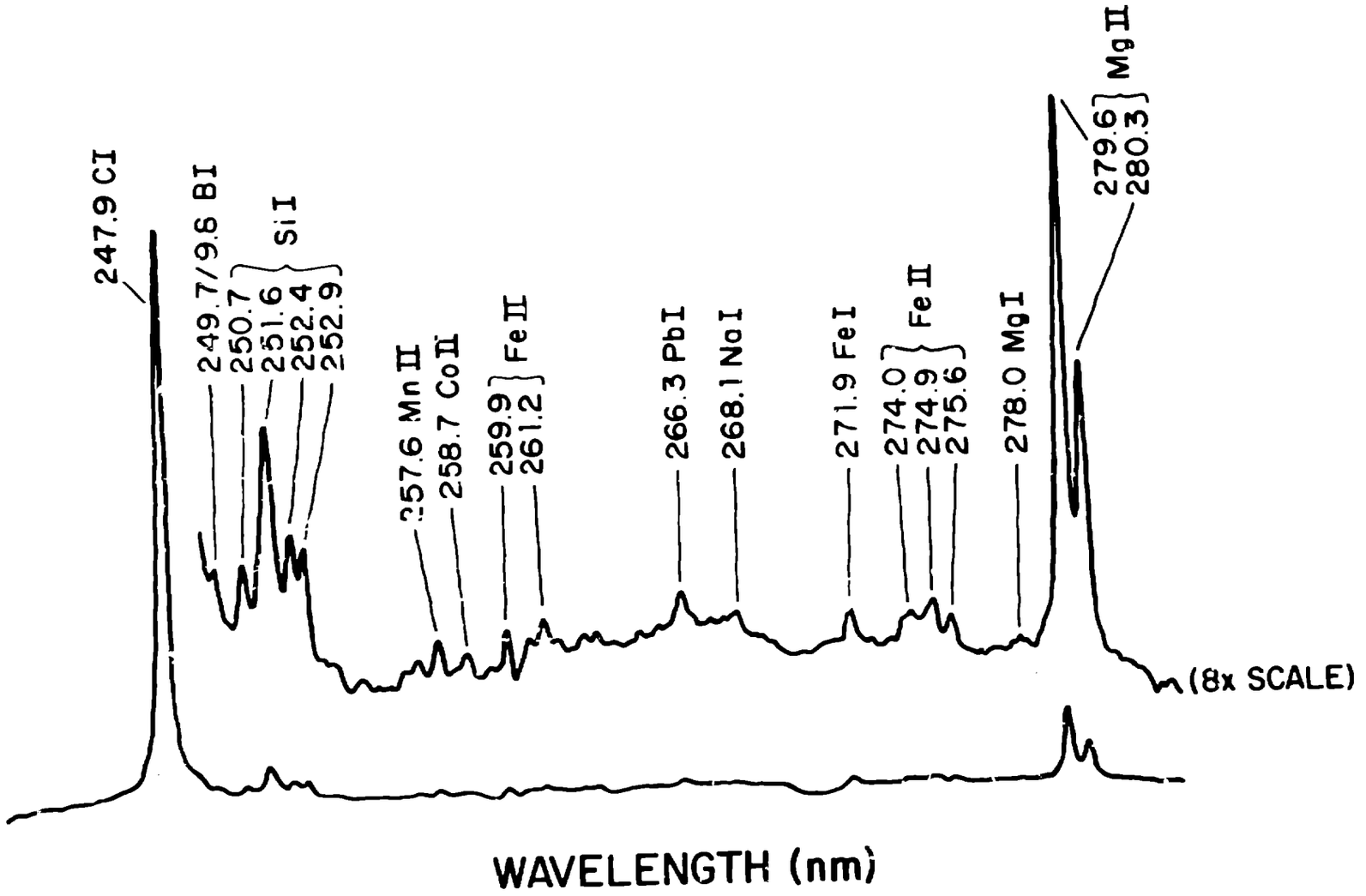

490.3
492.1
Fe I
492.6
493.2
493.7
Cu II
498.6 Cu II
499.4 Fe I
500.1 Ca II
501.0 CN
502.0 Ca II
502.8 Fe I
503.9/4.2 C I
505.9
506.5
Cu II
509.8
512.4
516.5
C2
515.3 Cu I
517.3
518.4
Mg I
519.5 Fe I
512.8 Cu I
WAVELENGTH (nm)

FIGURE 6


382.6 Fe I
383.2 Mg I
383.5 H
385.2
385.5
386.2
387.1
388.3
CN
393.4
396.8
CuII
394.4
396.2
AlI
398.5 CN
399.8 Fe I
404.4/4.7 KI
407.3 CN
409.3 OII
(4 x SCALE)
415.2
415.6
416.8
CN
WAVELENGTH (nm)


247.9 CI
249.7/9.6 BI
250.7
251.6
252.4
252.9
Si I
257.6 Mn II
258.7 Co II
259.9
261.2
Fe II
266.3 Pb I
268.1 Na I
271.9 Fe I
274.0
274.9
275.6
Fe II
278.0 Mg I
279.6
280.3
Mg II
(8x SCALE)
WAVELENGTH (nm)