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Analytical applications of inductively coupled plasma-optical emission spectroscopy

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I. INTRODUCTION

According to many dictionaries now in common use, the word "trace" when used as a noun can have a multitude of meanings. In at least one dictionary, one of the definitions of "trace" is given as "a very small quantity of a chemical constituent or component, especially when not determined because of its minuteness." This definition may have been applicable at one point in history, but it does not apply today. Trace constituents must now be determined quantitatively. Both the scientific and the lay public are now becoming aware of the critical role certain trace elements may play in a number of human activities. Materials scientists are becoming increasingly cognizant of how the physical, mechanical, optical, and electrical properties of metals, alloys, semiconductors, and luminescent materials may be strikingly influenced by trace impurities. And, at the moment, the lay public is becoming sensitized to the fact that there may be other heavy metals, other than Hg, which they are ingesting with their food, water, or air and which may be affecting their physical and mental health in subtle ways. In this context it is appropriate to note that at least 60 trace metals have been found in the blood and tissues of man and that diseases are often accompanied by changes in their concentration and distribution in the tissues and fluids of man. Some of these trace constituents are essential and are kept in balance in healthy persons by homeostatic mechanisms. Others may be essential but unequivocal evidence has not been accumulated. Still others appear to be acquired because they are not detectable when we are born but are present in measurable amounts when we die. There are

some trace elements that are essential but at higher concentrations are toxic. Alarmingly, the concentration interval between the essential and toxic levels may be very small. Finally, there are some trace constituents, such as Hg or Cd, which appear to serve no useful function but are considered toxic at surprisingly low concentrations. These relationships between trace elements and human health are discussed in more detail in Chapter IV.

Although the role of trace elements in human nutrition and health has received widespread attention, many of the studies in the past have concerned themselves with only one element at a time, primarily because viable analytical techniques were not available for performing ultratrace multielement determinations on biological samples of limited volume or weight. Because there are often subtle metal-metal interactions in biological systems, single-element studies may lead to seriously misleading interpretations.

With reference to environmental pollution, there is that nagging feeling that there may be an extended list of trace metal pollutants, which are now affecting our well being, but are not being monitored effectively because adequate technology is not available for monitoring many metals at the part per billion level. Thus, there is an increasing awareness that trace metals, occurring at unseen levels, may be playing an insidious role in our lives, while at the same time, we recognize that, in spite of the progress that has been made, the analyst must be extraordinarily successful to determine 10^{12} impurity atoms in a matrix of 10^{22} atoms on a multielement basis. The facts of life are that trace element technology is still a rather immature science. First, it has its

special hazards for the unwary. At the ultra-trace level the simple taking of the sample, its storage, and its manipulation prior to the final analytical step involves high risk of intolerable contamination of the sample or loss of the impurities of interest. The "how, why, when, and where" of these contaminations or losses are usually not obvious nor have they been adequately characterized, and all too often their consequences tend to be ignored.

Trace element analytical technology is immature from another standpoint. In many scientific or technological fields, such as solid-state materials research, the assessment of water or air pollution, geochemical prospecting, oceanography, and the evaluation of the biomedical effects of trace metals, it is often highly desirable, if not necessary, to determine many trace metals in many samples, which may be available in only very limited quantities, but there are presently no viable or practical ways of performing these analyses.

Over the past eight years several authors (1-22) have investigated the analytical utility of inductively coupled plasmas as an excitation source for optical emission spectrometry. These plasmas possess unique physical and spectroscopic properties which make them very useful for the optical emission determination of trace elements. The studies which have been reported indicate that these inductively-coupled plasma sources should be capable of solving many of the analytical problems discussed above. However, most of these investigations have dealt with the development of instrumentation, the properties of the source, and the determination of the limits of detection which can be achieved for elements in

dilute aqueous solution. There is a scarcity of information in the literature on the applications of this source to actual analytical problems. As a result, this investigation was undertaken to determine the applicability of an inductively coupled plasma optical emission spectroscopic system to practical analytical problems using metallurgical samples (steels) and biological samples (whole blood and serum) as typical examples.

II. INDUCTIVELY COUPLED PLASMAS AS EXCITATION SOURCES FOR ANALYTICAL SPECTROSCOPY

The inductively coupled plasma is an eddy (or ring-like) plasma, in which the volume filled by the ionized gas is comparable with a short-circuited secondary turn of a transformer. In the plasma generating system an induction coil surrounds a quartz tube ~ 2.5 cm in diameter through which flows argon gas. A schematic drawing of a typical plasma is shown in Figure 1. No electrodes are necessary in this system. The induction coil is connected to a high frequency generator which operates at frequencies in the 30 MHz range at generator input levels of 2 to 5 kW. To form a plasma it is necessary to plant a "seed" of electrons in the induction coil space; the simplest way to do this is to "tickle" the tube with a Tesla coil. The electrons and ions so formed may now be accelerated by the high frequency oscillating magnetic fields induced in the axial direction by the radio frequency current flowing in alternating fashion through the coils (Figure 1). The accelerated electrons and ions cause additional ionization, and as soon as the plasma is adequately ionized, there is induced by the axial magnetic fields an eddy current which flows in azimuthal circular closed paths around the periphery of the plasma. This current meets resistance to its flow and Joule heating results. The steps just discussed lead to the almost instantaneous formation of a plasma of extended dimensions. Although there does not appear to be complete agreement that local thermodynamic equilibrium prevails, the gas temperatures attained in those portions of the plasma

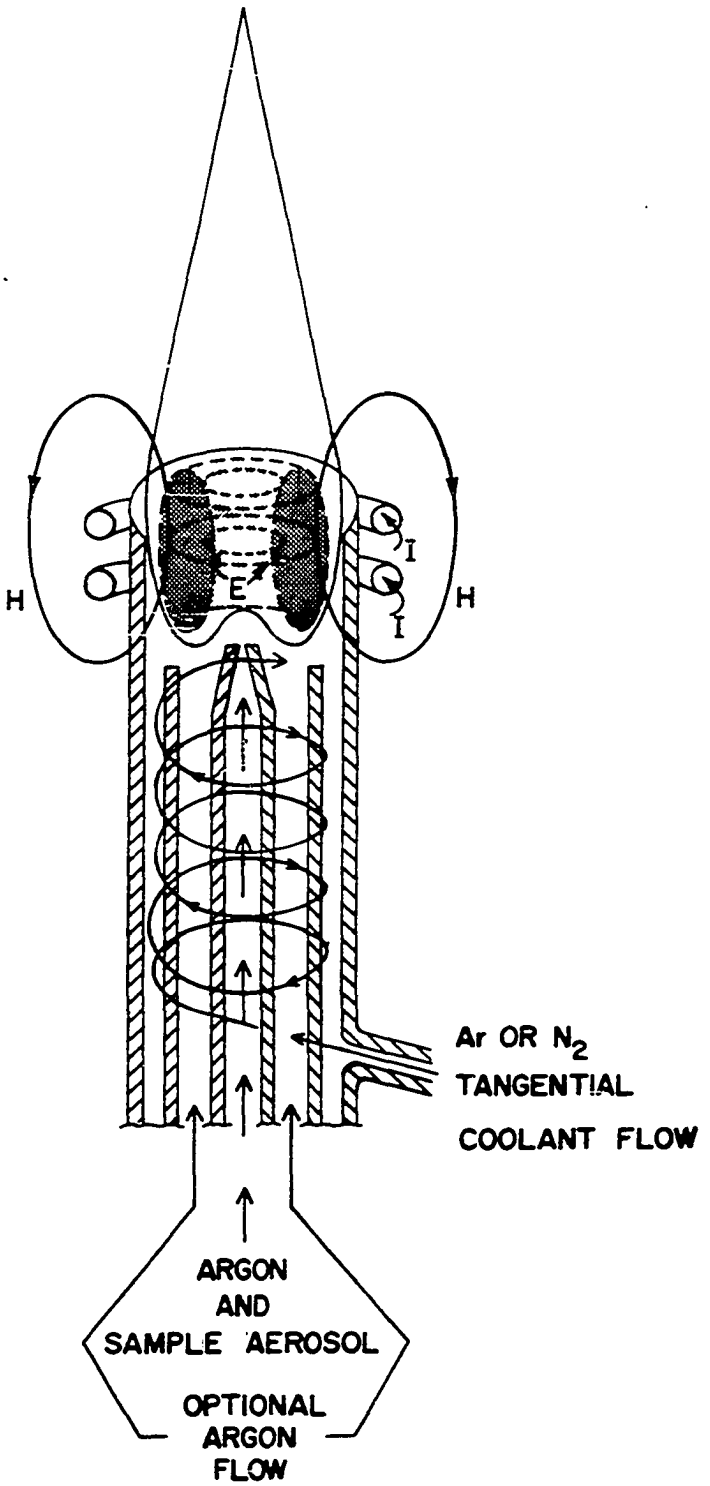

Figure 1. Schematic diagram of an inductively coupled plasma source

where the eddy currents are concentrated are probably in the 9,000 to 10,000 K range (23,24). At these temperatures, it becomes necessary to thermally isolate the plasma from the walls of the quartz tube, lest the walls collapse in a few seconds. The tangential or vortex method of stabilization, which was devised by Reed (25-27), is currently being used to both stabilize and thermally isolate the plasma. A stream of Ar gas (~ 10 l/min) is introduced tangentially so that it flows spirally up the tube. This flow cools the walls, keeps the plasma away from the walls, and in addition, creates a low-pressure zone in the axial channel of the tube. Some of this coolant gas circulates into the axial channel and becomes the plasma sustaining gas.

In addition to the tangential stabilizing flow, a second inlet with a flow of ~ 1 to 2 l/min. of Ar is needed for the introduction of samples into the plasma. If the plasma is generated at frequencies of ~ 30 MHz, the eddy current flows in closed paths closer to the outer surface of the plasma, which represents the hottest part of the plasma. An incipient cooler "doughnut hole" in the center of the plasma can then be further developed by optimizing the flow velocity of the carrier gas which injects or blows the suspended aerosol into the plasma. The degree to which a toroidal shape is developed by the plasma can be controlled by the frequency of the generator and by the orifice design and gas flow velocity of the sample injection system. The sample is injected into the center of the toroidal plasma, thus avoiding sample rejection, which is a serious problem with some plasmas (28,29).

Preliminary studies on the distribution of free atoms in the toroidal

plasma indicate that the sample particles travel through the center of the toroidal plasma. During their transit the sample particles are heated primarily by radiation and thermal diffusion to a temperature of ~ 9,000 K. The sample particles are restricted to a narrow channel through the center of the plasma by the expanding heated gas of the plasma and by thermal gradients. Thus the sample concentration, including both ground state and excited state populations, is highest along the central axial channel. This is a desirable situation from two standpoints. The free atoms produced by the high temperature of the plasma are concentrated in a small cylindrical volume up through the center of the plasma and tail flame, and if this volume fills the optical aperture of the spectrometer, the most efficient use is made of the free atoms. In addition, the atoms experience an isothermal environment, so that there are almost no atoms at lower temperatures surrounding the light-emitting excited state atoms in the central channel. Consequently, there is a very low degree of self-reversal, even for the sensitive resonance spectral lines, and linear analytical curves covering a four-order-of-magnitude concentration range can be readily obtained.

The sample aerosol droplets travel through the central channel of the plasma at a relatively moderate speed so that their residence time in the plasma is longer than for other excitation systems. With the longer residence time at temperatures in the vicinity of 9,000 K, it is believed that total decomposition of the particles into free atoms occurs. Thus few interelement effects have been observed and corrections can easily be made for those found.

Since populations of excited states increase exponentially with temperature, the induction-coupled plasma has the capability to excite useful spectra at trace concentrations of all metals and metalloids. Because the free atoms then exist in an inert (Ar) atmosphere, they have a longer lifetime, and monoxide compound formation is reduced.

The free atoms are transported in a rather narrow channel through the hotter plasma zones into the cooler tailflame, so that they experience a continuous range of temperatures from ~ 9,000 K to room temperature. The temperature region from which the free-atom light emission is taken can thus be selected by the analyst. Ordinarily, radiation from the hottest zones is avoided because of high continuum background and complex spectra. In somewhat cooler regions, the continuum is virtually absent, and the spectra are relatively simple, so that short focal length, high optical speed spectrometers provide adequate resolution of the spectra from almost any sample.

Most of the credit for the technological development of these plasmas should be given to Reed (25-27). Explorations on the use of these plasmas as excitation sources for atomic emission and as absorption cells for atomic absorption were pioneered by Greenfield and associates in the United Kingdom (6-8) and in the United States by Fassel and his associates (1-5). Later, Dunken and Pforr (9-11), Britske and associates (12), Hoare and Mostyn (13), Bordonali and Biancifiori (14,15), Veillon and Margoshes (16), Mermet and Robin (17), Goldfarb and Goikham (18), Kleinmann and Svoboda (19), Morrison and Talmi (20), Truitt and Robinson (21), and Boumans and DeBoer (22) also explored their analytical

potentialities.

The most important advantages of inductively-coupled plasma excitation, as documented by the authors cited above, may be summarized as follows: (a) effective injection of the sample into the hot portion of the plasma; (b) relatively long residence time of the sample in the plasma; (c) higher gas temperature than combustion flames; (d) continuous temperature gradient from ~ 9,000 K to room temperature, allowing greater latitude in selecting optimal temperature; (e) free atoms may be generated in hottest zones of the plasma and then observed in lower temperature zones, where background emission is lower; (f) chemical environment may be manipulated, within limits; and (g) no electrode contamination.

In view of these advantages these plasma sources should exhibit superior performance when compared with other sensitive spectroscopic techniques. One important criterion of the performance of an analytical technique is its ability to detect trace and ultratrace quantities of material. This capability is often expressed as a detection limit which is usually defined as the quantity of analyte required to provide a signal/noise ratio of 2 or 3.

The scientific value of experimentally determined detection limits is often questioned, because the values obtained depend sensitively on the experimental conditions under which they are measured. If the measurements are made under carefully described experimental conditions, with facilities that can be readily duplicated, and if the statistical basis for the detection limit determinations is properly identified, the

values obtained are of real value. The values then reflect the "state of the art." Since the analyst usually has sufficiently precise control over the excitation process to allow quantitative determinations at a concentration factor of ~ 5 above the measured detection limits, the values provide the analyst with a numerical basis for comparing analytical capabilities.

Such numerical data on experimentally determined detection limits have been provided by the lengthy list of investigators whose papers have been cited above. These data and those collected by Kniseley et al. (32) are summarized in Tables 1 - 3. To enhance the relevance of the comparative evaluations that may be drawn from the data, the most recent compilations of detection limits observed by flame atomic absorption, emission, and fluorescence techniques are also included. In drawing the comparision with flame atomic absorption or fluorescence, it is important to recognize the multielement capability of observing the emission spectra excited in the plasma, whereas for flame absorption and fluorescence, the elements usually must be determined one at a time.

A comparison of detection limits for elements whose lowest excitation potentials are > 4 eV is shown in Table 1. The lowest excited states of these elements cannot be abundantly populated, even at the gas or electron temperatures prevailing in the plasmas. Consequently, for these elements, flame atomic absorption or fluorescence possess inherent advantages, and the data in this table reflect this advantage for several of the elements. Although there are some exceptionally high powers of detection for flame atomic fluorescence (e.g., Ag, Cd, Zn), these numbers were observed in

Table 1. Comparison of detection limits (μg/ml) for elements with excitation potentials greater than 4 eV

Element	Induction Coupled Plasmas		Flame Atomic Emission (30)	Flame Atomic Absorption (31)	Flame Atomic Fluorescence (31)
Ag	0.02	(32)	0.008	0.0004	0.0001
As	0.1	(5)	10	0.1	0.1
Au	0.04	(32)	0.5	0.02	0.05
B	0.03	(5)	0.05	6	--[a]
Bi	0.05	(32)	20	0.05	0.05
Cd	0.003	(22)	0.8	0.01	0.000001
Co	0.003	(5)	0.03	0.005	0.01
Hg	0.001	(22)	10	0.5	0.02
Mg	0.00003	(22)	0.005	0.0003	0.001
P	0.07	(22)	--[a]	--[a]	--[a]
Pb	0.002	(22)	0.1	0.01	0.01
Sb	0.2	(5)	0.6	0.1	0.05
Se	0.3	(32)	100	0.1	1
Sn	0.03	(22)	0.1	0.03	0.05
Te	0.2	(32)	2	0.1	0.05
Zn	0.009	(5)	10	0.002	0.00004

[a]No data available.

Table 2. Comparison of detection limits (μg/ml) for elements with excitation potentials less than 4 eV

Element	Induction Coupled Plasma		Flame Atomic Emission (100)	Flame Atomic Absorption (101)	Flame Atomic Fluorescence (101)
Al	0.0002	(22)	0.005	0.1	50
Ba	0.00002	(22)	0.001	0.05	--[a]
Ca	0.00002	(22)	0.0001	0.002	0.02
Cr	0.0003	(22)	0.004	0.005	0.05
Cu	0.0001	(22)	0.01	0.005	0.001
Fe	0.0003	(22)	0.03	0.005	0.008
Ga	0.0006	(22)	0.06	0.07	0.3
Ge	0.004	(22)	0.4	1	20
In	0.03	(32)	0.002	0.05	0.1
Li	0.0003	(22)	0.00002	0.005	--[a]
Mn	0.00006	(22)	0.008	0.002	0.006
Mo	0.0002	(22)	0.1	0.03	--[a]
Na	0.0003	(22)	0.0005	0.002	--[a]
Ni	0.0004	(22)	0.02	0.005	0.003
Pd	0.002	(22)	0.05	0.03	--[a]
Pt	0.08	(32)	2	0.1	--[a]
Sr	0.00002	(5)	0.0001	0.01	0.03
Tl	0.2	(32)	0.02	0.03	0.008
V	0.0002	(22)	0.01	0.02	--[a]

[a]No data available.

Table 3. Comparison of detection limits (μg/ml) for elements that form stable monoxides [D_c(MO) > 7 eV]

Element	Induction Coupled Plasma		Flame Atomic Emission (100)	Flame Atomic Absorption (101)	Flame Atomic Fluorescence (101)
Be	0.0004	(22)	0.1	0.002	0.01
Ce	0.002	(22)	10	ND	--[a]
Hf	0.01	(5)	20	8	--[a]
La	0.0004	(22)	0.01	2	--[a]
Nb	0.01	(5)	1	3	--[a]
Si	0.3	(32)	3	0.1	--[a]
Ta	0.07	(5)	4	5	--[a]
Th	0.003	(5)	10	--[a]	--[a]
Ti	0.0002	(22)	0.2	0.1	--[a]
U	0.03	(5)	5	30	--[a]
W	0.001	(22)	0.5	3	--[a]
Y	0.00006	(22)	0.03	0.3	--[a]
Zr	0.0004	(22)	3	5	--[a]

[a]No data available.

relatively cool flames, such as those formed from hydrogen-argon-entrained air. For these flames the degree of atomization would surely be greatly depressed and solute vaporization interferences would undoubtedly be greatly increased when these elements are determined in the presence of an actual sample matrix.

Table 2 draws a similar comparison for a typical list of elements whose lowest excited states are below 4 eV. For these elements, exceptionally good powers of detection are observed for the inductively coupled plasma, and, with a few exceptions, are comparable to or superior to those reported for flame atomic absorption or fluorescence. The third comparison, shown in Table 3 covers a typical list of elements that form stable monoxide molecules [D_o(MO) > 7 ev]. Here the superiority of the inductively coupled plasma is striking; in many instances, the inductively-coupled plasma values are from 2 to 4 orders of magnitudes superior to those observed by the flame techniques.

The data in Tables 1 to 3 strongly support two general conclusions, namely: (a) that the metallic elements can be detected in the inductively-coupled plasma at the fractional µg/ml down to the fractional ng/ml level; (b) with only a few exceptions, the detection limits observed in the plasma are comparable to or greatly superior to the best values so far reported in the literature for flame atomic absorption, emission or fluorescence spectroscopic techniques.

III. EXPERIMENTAL FACILITIES

The experimental equipment and conditions used are summarized in Table 4. A highly schematic drawing of the sample introduction system is shown in Figure 2. The pneumatic nebulizer fits onto a Teflon spray chamber that is 15 cm long and has a diameter tapering from 35 mm to 20 mm. The desolvating system consists of an 18 mm (i.d.) glass tube, ~ 33 cm long, surrounded by a coiled Calrod heater (~ 20 cm long) which is maintained at ~ 400°C. This is followed by a Leibig condenser, ~ 28 cm long, with a side outlet to the plasma. This condenser removes the water which is evaporated from the aerosol droplets by the Calrod heater.

The plasma torch assembly is a modified version of the one described by Greenfield et al. (34) and a detailed drawing is shown in Figure 3. The torch is fabricated of fused quartz tubing and consists of concentric outer and inner tubes (called the coolant and plasma tube respectively) and a removable aerosol injection tube. Argon (~ 10 l/min) is introduced tangentially into the coolant tube and, in a few special applications, a low flow of Ar (1-2 l/min) is also introduced tangentially into the plasma tube.[1] The flow velocity of the argon coolant gas is caused to increase towards the top of the plasma tube by the constriction resulting in an increase in both the cooling efficiency and the degree of vortex

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[1] Ordinarily there is no gas flow in the plasma tube and the inlet of this tube is sealed off to prevent air from entering the plasma. In special applications, such as the analysis of lubricating oils, this low flow of Ar is used to prevent carbon deposits from forming on the top of the plasma tube.

Table 4. Experimental facilities and operating conditions

Nebulizer	Pneumatic type described by Kniseley et al. (33).
Plasma Power Supply	Lepel High Frequency Laboratories Model T-2.5-1-MC2-J-B generator with attached tuning and coupling unit, 2.5 kW, frequency set to ~ 30 MHz. The load coil was two turns of 5 mm o.d. copper tubing, i.d. of coil was 27 mm.
Gas Flows	Argon used throughout with 10 l/min through the coolant tube and 1.1 l/min through the aerosol tube. No "plasma gas" flow was used. Gas flow system described by Kniseley et al. (33).
Spectrometer	Hilger-Engis Model 1000, 1-meter Czerny-Turner mounting scanning spectrometer with 1200 rulings/mm grating blazed for 5000 Å. Reciprocal linear dispersion of 8 Å/mm in first order.
Slits and Order	Both entrance and exit slits were 25 μ wide, 4 mm long, and straight edged. First order spectra were employed throughout.
Detector Electronics	The photocurrent from an EMI 6255B phototube was amplified with a Keithley Model 410 picoammeter and recorded with a Leeds and Northrup Speedomax recorder. Time constant was ~ 3 sec.
Average sample nebulization rate	3.3 ml/min

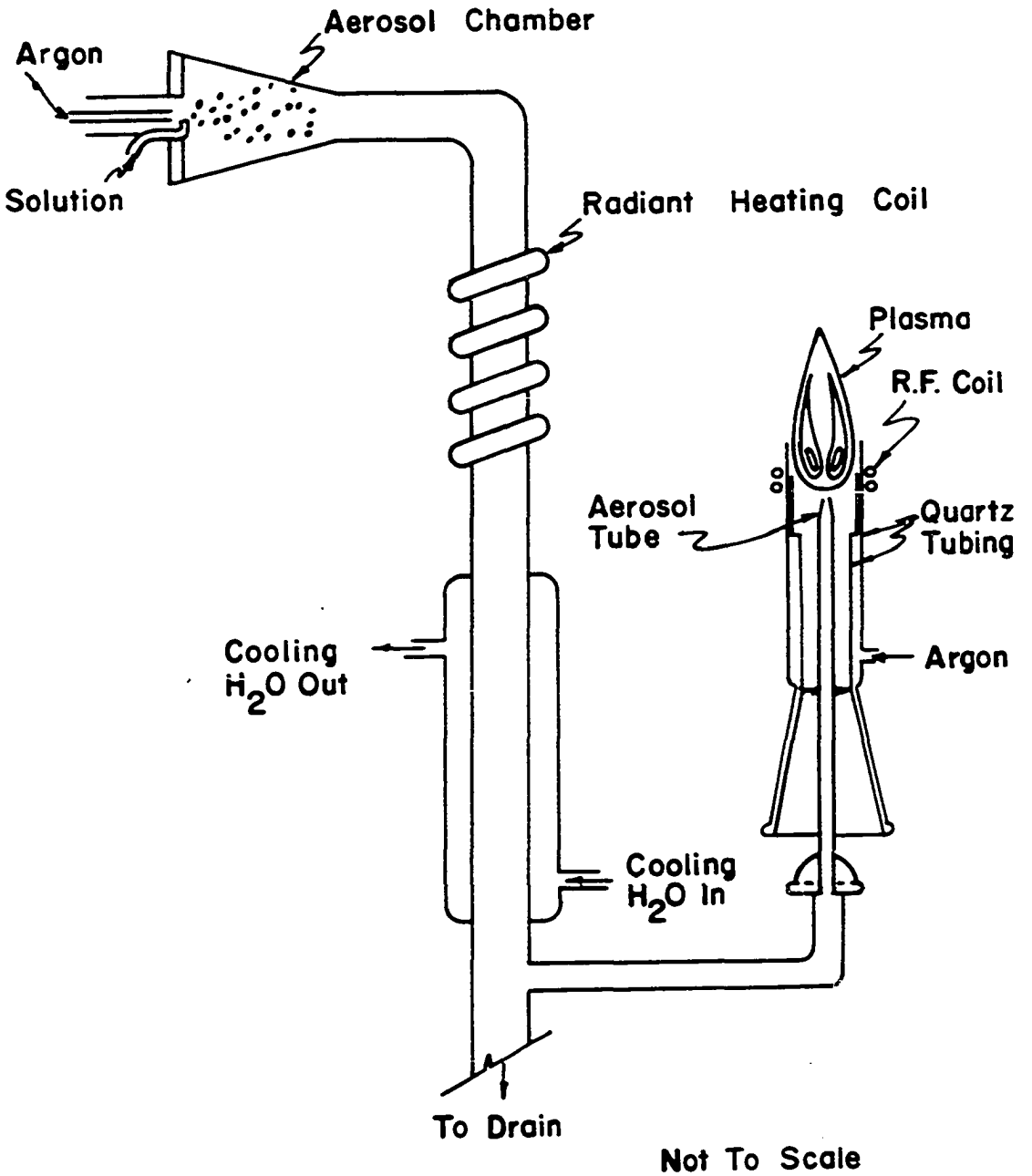

Figure 2. Schematic diagram of the sample introduction system

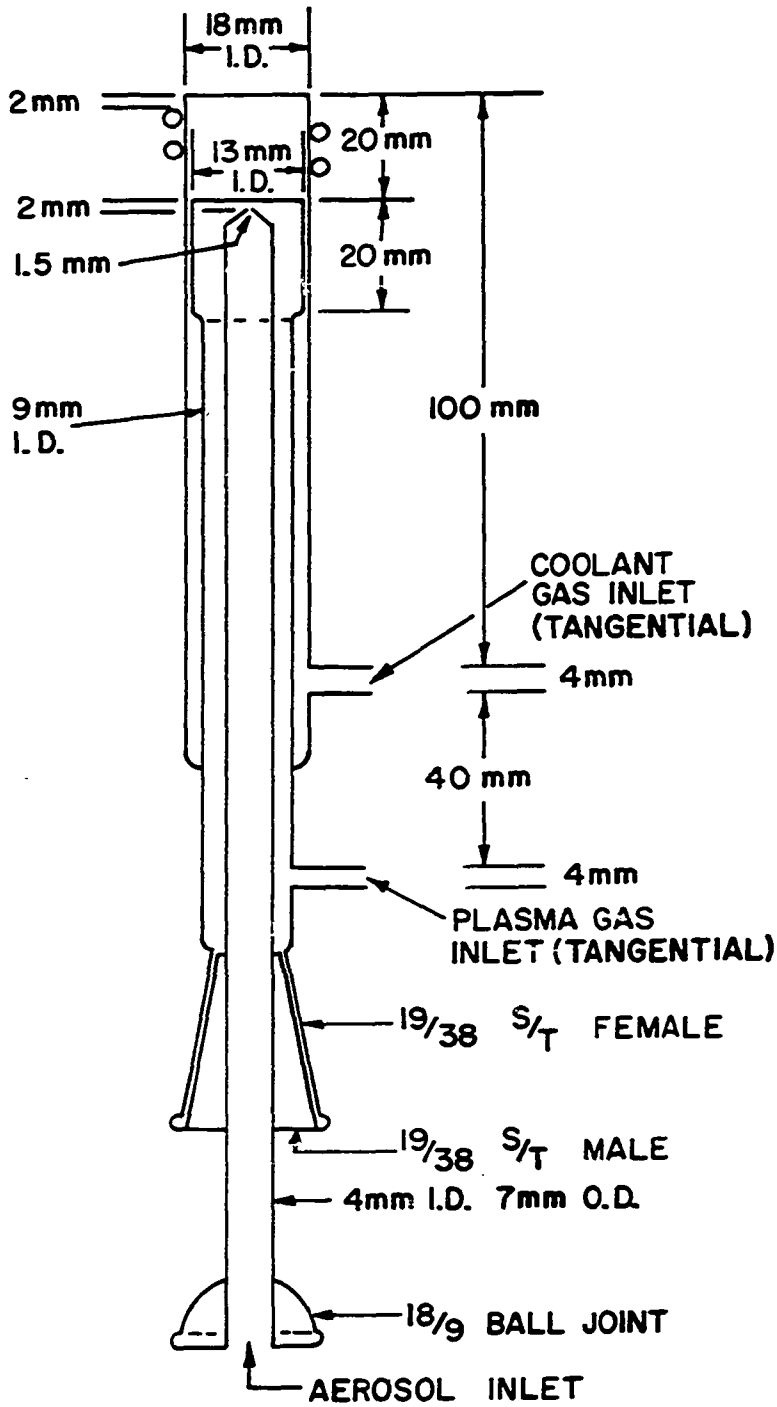

Figure 3. Plasma tube configuration

stabilization. The optimum constriction width has been empirically determined to be 1 mm for the present system. A larger spacing (2 mm) necessitated a higher argon consumption rate due to inefficient cooling of the outer tube wall, whereas a smaller spacing (0.5 mm) resulted in plasma instability.

The diameter of the orifice of the aerosol injection tube was also optimized empirically. For a constant argon flowrate through the orifice, the injection velocity of the aerosol into the plasma is inversely proportional to the square of the orifice diameter. If the injection velocity is too low, the aerosol will not penetrate the "doughnut hole" of the plasma (5,35). If the injection velocity is too high, the residence time of the particles will be unfavorably short. An orifice diameter of 1.5 mm and an argon flowrate of about 1.1 ℓ/min were found to be acceptable compromises of these parameters. Assuming that the velocity of the particles remains constant and equal to the injection velocity (which is approximately 1300 cm/sec for the above parameters), the particle residence time is approximately 2.5 msec for an observation height of 18 mm above the load coil.

Because the aerosol is injected into the "doughnut hole" of the plasma and since most of the radio frequency power is absorbed in the "doughnut" (i.e., within the skin depth), the aerosol is principally heated by conduction and radiation from the surrounding argon. This indirect heating process is favorable because major changes in sample composition would not be expected to change the absorbed power significantly.

IV. APPLICATION OF AN INDUCTIVELY COUPLED PLASMA-EMISSION SPECTROMETRIC SYSTEM TO THE DIRECT DETERMINATION OF TRACE ELEMENTS IN WHOLE BLOOD AND SERUM

A. Relationships Between Trace Elements and Human Health

In recent years, the role of the trace elements in human health has received widespread attention. Advances in biology and medicine have stimulated interest in the trace metals in body tissues and fluids, and sensitive analytical techniques and new methods for isolating metalloenzymes have made it possible to study quantities of metals that were formerly undetectable. Concern over environmental pollution has drawn much attention to the role of the trace metals in human diseases. At least sixty elements in low concentrations (less than one part per thousand) have been found in the blood and tissues of man (36-44). It will become apparent during this discussion that there is a need for a rapid, sensitive, reliable method for performing simultaneous multielement determinations of trace metals in body tissues and fluids, especially in blood. The simultaneous multielement aspect is important because of (a) the large number of elements in the body, (b) the high degree to which these elements affect the biological activities of each other, (c) and the small samples available for analysis.

The trace metals function primarily as catalysts in enzyme systems. For example, zinc is a constituent of enzymes chiefly associated with protein synthesis: carbonic anhydrase (45), pancreatic carboxypeptidase, alkaline phosphatase, alcohol, malic, lactic, and glutamic dehydrogenases, and tryptophan desmolase (46,47). In addition, zinc is a cofactor in a

variety of enzyme systems including arginase, enolase, several peptidases, oxalacetic decarboxylase, and carnosinase (46). Examples of copper enzymes are cytochrome oxidase and monoamine oxidase in blood plasma (48).

Trace metals have been implicated in disease ever since their importance in metabolism was demonstrated. Almost all diseases are accompanied by changes in the concentration of one or more trace metals in some body tissue or fluid, especially the blood (49). In particular, such changes are often associated with various forms of cancer. High serum copper levels have been correlated with high activity of Hodgkin's disease, and the measurement of serum copper levels has been proposed as a means of evaluating response to therapy (50-52). In acute leukemia, high serum copper correlated with a high percentage of blast cells in marrow is the most significant index of activity of this disease, the measurement of serum copper levels has been suggested as a supplement to bone marrow examinations for the management of leukemia (53,54). Mortazavi et al. (52) found that the serum copper level was generally elevated in patients with lymphomas (Hodgkin's disease, reticulum cell sarcoma, and lymphosarcoma). Serum copper was also found to be greatly elevated in multiple myeloma, and lymphoepithetial tumors (Schminke's tumor).

Subnormal zinc plasma levels have been reported in patients with malignant tumors (55-57). Decreased zinc levels in the whole blood of carcinoma patients have been reported by Nuryagdyev (58). Marked changes in leukocyte zinc occur in patients with chronic leukemia (48). The concentration of zinc in the peripheral leukocytes is greatly reduced below normal and cannot be raised by injections of zinc gluconase. A rise to

normal levels occurs in clinical remission and during X-ray therapy, accompanying the falling leukocyte count (48). The zinc content of the leukocytes also decreases in patients with a variety of neoplastic diseases, and this difference has been suggested as a diagnostic test for cancer (59).

Morgan (60) reported low serum zinc levels in patients with bronchogenic carcinoma. Zinc levels were high in the kidney and not significantly different from normal in the liver. In contrast, cadmium, which is closely associated with zinc in nature, was high in serum, kidney and liver.

Teitz et al. (61) reported that a wide variety of trace elements, including lead, cadmium, iron, molybdenum, chromium, silicon, and silver are at elevated concentrations in the tissues of persons with cancer. They noted that the metal content of the tumor itself was often low and that the increased metal storage involved several organs; this suggests that the increases might have occurred before, rather than after, the cancer developed.

Nuryagdyev (58) reported that the concentrations of aluminum, manganese, cobalt, copper and lead in the blood of carcinoma patients were 114-200% higher than in healthy controls. These elements were excreted at a subnormal rate.

A complication in interpreting changes in trace metal concentrations is the fact that abnormal concentrations are almost never specific indicators of only one disease. For example, hypercupremia (high blood copper) occurs in most chronic and acute infections in man, in various anemias,

collagen disorders, hemochromatosis, and myocardial infarction, as well as in leukemia and Hodgkin's disease.

It has become obvious that a knowledge of the functions of trace metals in metabolism should help both in the diagnosis and treatment of human diseases. In a healthy person the concentrations of essential trace elements are probably kept within narrow limits by homeostatic mechanisms, and deviations from these limits may reflect abnormal metabolism. Although there are, at this time, many unanswered questions regarding the correlation of metal concentrations and disease, the analysis of body fluids and tissues for trace metals is becoming an important clinical diagnostic test (49). A large part of the difficulty in interpreting changes in mineral metabolism is due to the fact that metals in the body do not act alone, but rather they interact with each other. Several workers have discussed the importance of interactions among the trace metals and the need for further study in this area (48, 62-64). The interactions among the trace metals in the body are so important that studies with individual metals can be seriously misleading unless their quantitative relationships to other interacting metals are known and considered (64). A few examples will illustrate these interactions.

Gross disturbances in dietary mineral balance can greatly affect the absorption of iron. Very high levels of phosphate reduce iron absorption (65), and absorption tends to increase with low phosphate diets (66,67). High intakes of zinc, cadmium, copper, and manganese also interfere with iron absorption, apparently through competition for protein binding sites

in the intestinal mucosa, and have been shown to raise iron requirements. Another illustration of such interelement competition is provided by the work of Förth (68), who demonstrated increased uptake of cobalt, manganese, zinc, and iron in iron-deficient rats. Settlemire and Matrone (69,70), obtained evidence that zinc reduced iron absorption in rats by interfering with the incorporation of iron into, or the release from, ferritin. The increase in iron requirement brought about by the high zinc intakes was enhanced by a shortening of the life span of the erythrocytes, resulting in a faster turnover of iron. In animals, a common expression of copper deficiency is an anemia indistinguishable from that due to iron deficiency, even when the tissues contain abundant iron (48,69). Copper is necessary for the formation of erythrocytes. Increased copper allows the release of iron from the tissues into the plasma. The relationship between iron and copper must be an indirect one; copper is not a part of the hemoglobin molecule, nor does it form a complex with iron.

Although the examples given above illustrate the importance of trace metal interactions, it is quite probable that they may be less important in the overall metabolic scheme than interactions about which little or nothing is known at present. According to Underwood (64) further study of such interactions, with a view toward understanding and quantitating their effects, constitutes one of the most significant areas of trace element research. Such studies would undoubtedly be facilitated by the development of a sensitive method for the simultaneous determination of many metals in biological samples.

In order to relate trace metal concentrations to the biochemistry,

physiology, and pathology of living systems, one must have data on normative distributions of the elements. Although several such investigations have been conducted (36-44), there is much more work to be done. In view of the large number of elements present in the human body, a system capable of performing multielement analyses at ultratrace levels is particularly valuable.

B. The Analysis of Biological Materials

An ideal analytical system for the ultratrace determination of the metals and metalloids in biological systems should provide for the rapid, simultaneous multielement determination of many elements in the concentration range of one part in 10^6 to 10^9. The capability of performing these analyses on small samples (~ 1 ml) is also important since biological fluids and tissues are often available only in small quantities. The method should involve minimum or preferably no sample preparation prior to analysis.

Among the methods that have been used for the determination of trace metals in body fluids and tissues are neutron activation analysis (71), X-ray fluorescence spectroscopy (36,37), atomic absorption spectroscopy (72-108) and dc carbon arc emission spectroscopy (38-40, 42-44, 109,110). Even though each of these systems satisfies some of the above criteria, none satisfies all of them. For example, neutron activation analysis is sensitive and is capable of multielement determinations; however, the period of irradiation alone can be several hours and the availability of a nuclear reactor is generally required. Furthermore, in the case of biological samples it is usually best to perform chemical separations prior

to counting. X-ray fluorescence is very sensitive for many elements but with small ("non-infinite") samples quantitative measurements are difficult and the determination of trace quantities is often slow. Giauque et al. (111) have recently described an X-ray fluorescence instrument for the analysis of non-infinite samples (air particulates) which overcomes many of these disadvantages. However, several different X-ray excitation sources are required to cover all of the elements of interest and the counting times for biological samples is ~ 30 minutes with each source.

The technique most commonly used for trace element analysis is atomic absorption spectroscopy, and its applications to biology and medicine have been reviewed by Christian and Feldman (72). The major limitation of this technique is that it is not generally adaptable to simultaneous multielement determinations, except in a few special cases where the appropriate multielement hollow cathode exists. Also, many of the procedures described in the literature requires considerable sample treatment (ashing, chemical separations, etc.) prior to analysis with the attendant risk of contamination or loss of trace constituents. The Delves-cup technique has been successfully used for blood analysis but the application has been essentially limited to Pb determinations (80,107,108).

Several authors have described the use of flameless atomization devices for the determination of trace elements in biological fluids (73,79,83,102-106). In some instances prior separation of the metal is required while, in other cases, the samples are run directly. In most instances, an automatic background corrector is necessary to reduce the

"absorption" error due to the scattering of radiation from the primary source (106). Again, since these are atomic absorption techniques, only one element can be determined at a time.

Optical emission spectroscopy is inherently a multielement technique and the use of direct-reading spectrometers, instead of photographic instruments, has made this a very rapid method of analysis. However, dc arc excitation, which is usually necessary to obtain adequate sensitivity, produces "indifferent reproducibility" (109). Ashing of the sample is usually necessary and often concentration techniques are required to obtain the required detection limits.

The inductively coupled plasma has been shown to be an excellent excitation source for emission spectrometry (1-22) and coupled with a direct reading spectrometer, it should provide an excellent system for the analysis of biomedical samples. As a result, this portion of the investigation was undertaken to determine the applicability of such a plasma source to the analysis of biological fluids. Whole blood and serum samples are used as typical sample materials. The elements chosen for study were Al, Co, Cr, Cu, Mn, Ni, P, Pb and Zn since all of these are of biological importance and some of them represent the most difficult elements to determine because of their low concentrations in biological fluids.

C. Experimental Procedure

Heparin was used as an anticoagulant in the whole blood samples and serum was separated from the unheparinized blood in the normal manner (112). The whole blood samples were diluted tenfold with 0.1 M HCl and serum samples two-fold with deionized water.

Two different calibration procedures were used for the analyses. The standard additions method (113) was used for all of the analyses. In addition, standards for blood serum were prepared by incremental additions of the appropriate elements to a synthetic blood electrolyte solution (110) as a base material. The latter standards were diluted with deionized water in the same ratio as the samples. In many instances deionized water can be used for the blank but for a few elements, whose concentrations are very low and thus the signal/background ratio less favorable, a 2000 µgK/ml or an unspiked blood electrolyte solution was used instead. These latter blank solutions were used to compensate for the small but significant changes in the background levels which sometimes occur when solutions containing high concentrations of easily ionizable elements are introduced into the plasma.

D. Results and Discussion

Actual wavelength scan recordings of the emission signals obtained for three elements in diluted whole blood samples are shown in Figures 4 and 5. These recordings are typical of the traces obtained for all of the elements studied.

A more rapid method for performing the analysis involves parking the spectrometer on the spectral line and alternately recording the signals from the samples and the blank. This method approximates the procedures which would be used for multielement analysis using a direct-reading spectrometer. When this technique is used, the requirement that the blank accurately represents the background level is more rigid. In Figure 6 a typical standard addition curve is shown with the recorder traces of the signals vs. time obtained while the spectrometer was set on the

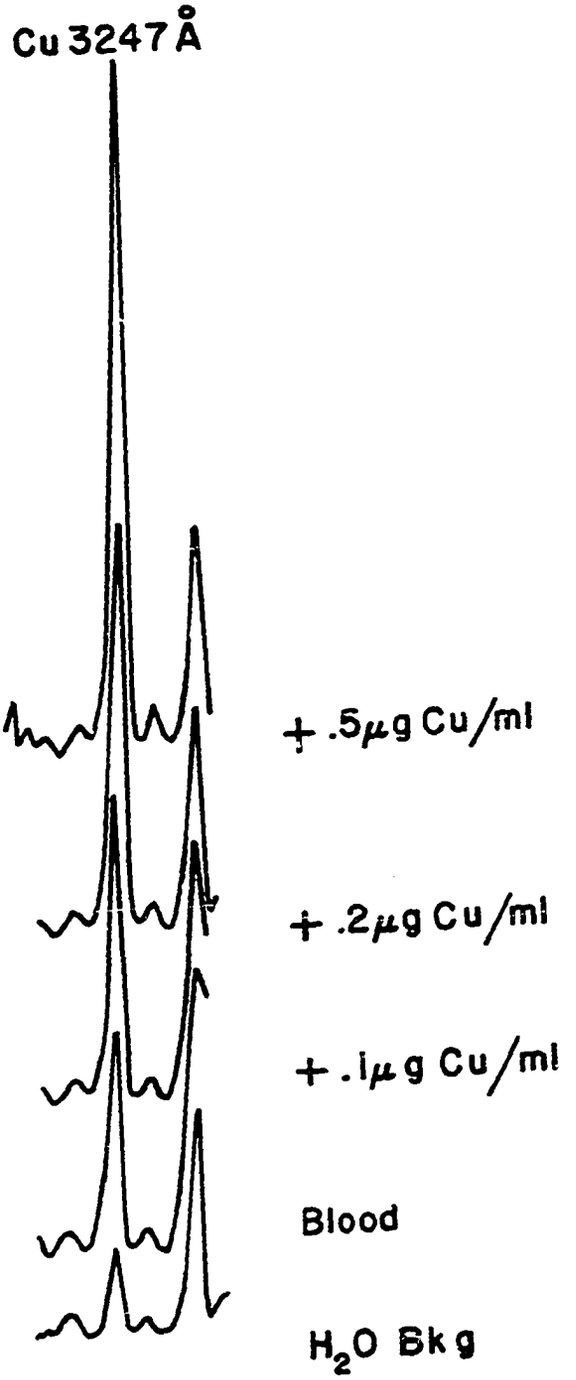

Figure 4. Wavelength scans of the Cu 3247 Å line in blood addition standards (ten-fold dilution)

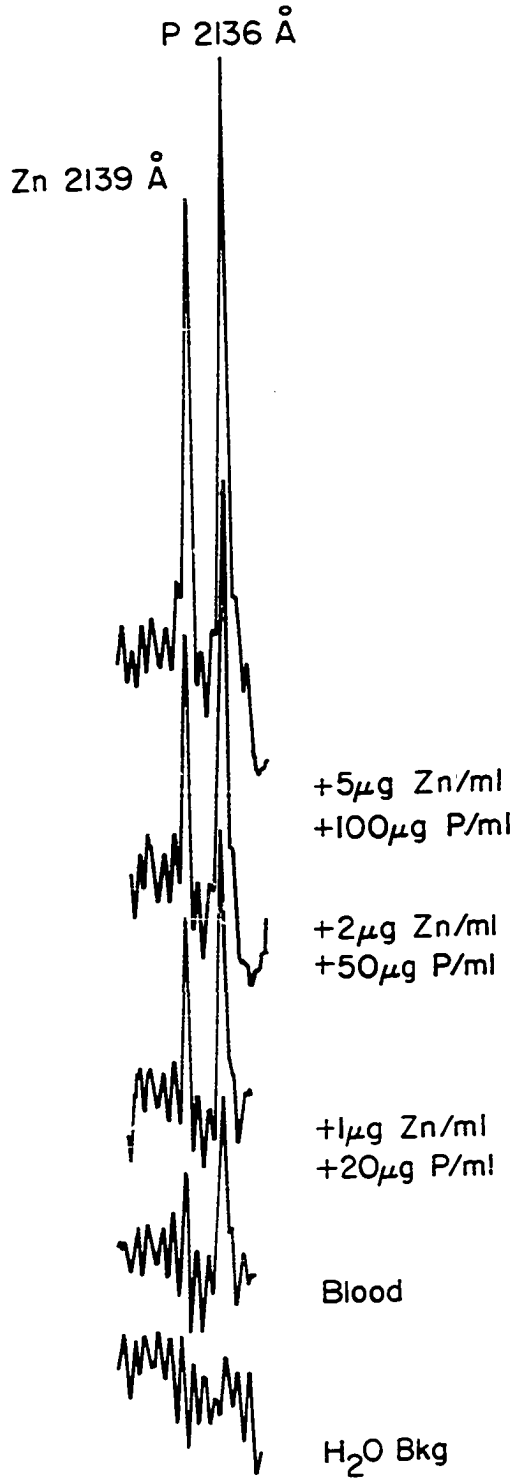

Figure 5. Wavelength scans of the Zn 2139 Å and P 2136 Å lines in blood addition standards (ten-fold dilution)

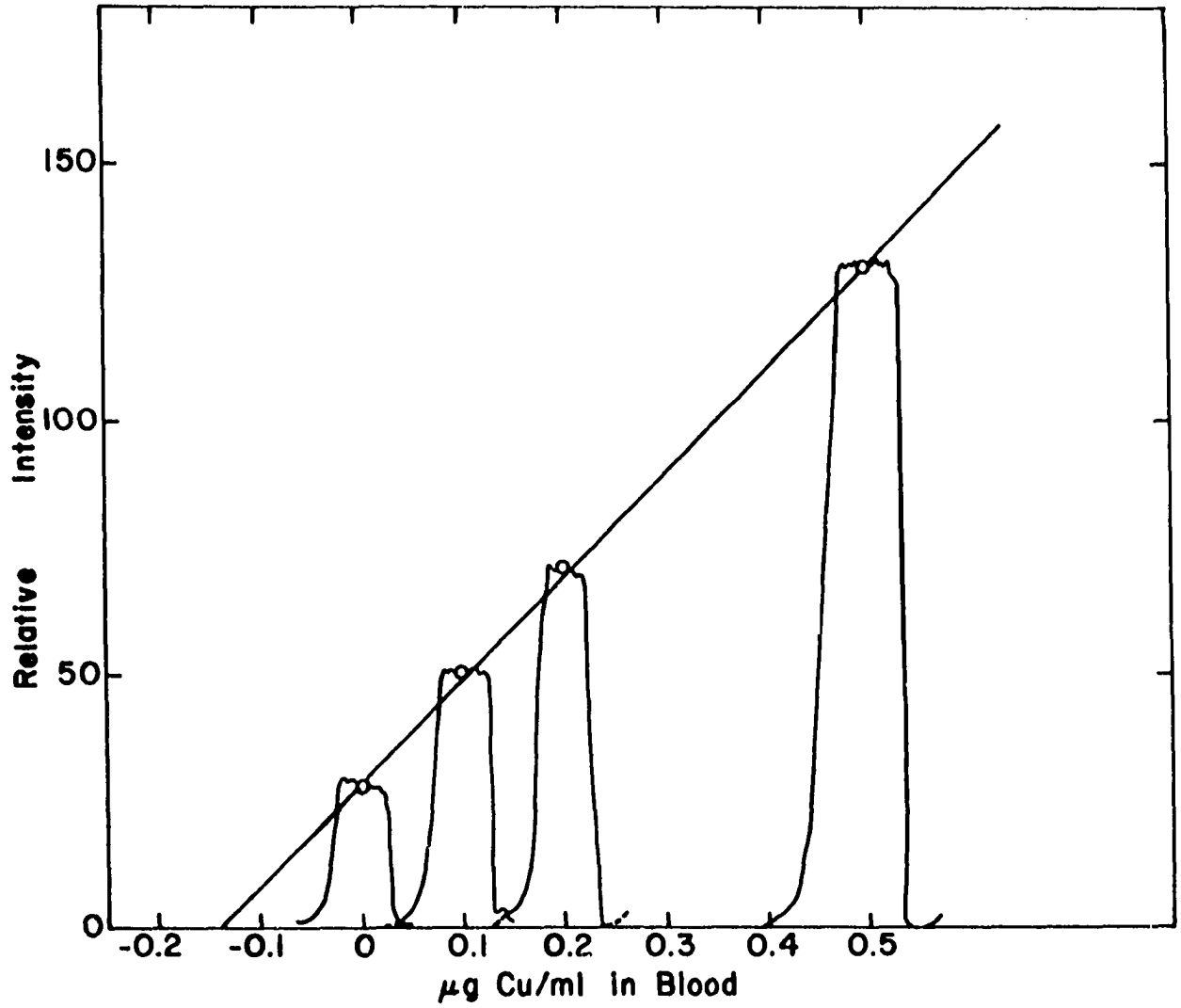

Figure 6. Standard additions analytical curve for Cu in blood (ten-fold dilution)

appropriate spectral line.

It should be emphasized that all of the data were obtained without any prior chemical treatment other than dilution of the original sample. Also all of the analyses were run using the net signals above background, and no internal standardization was used.

Table 5 summarizes the data obtained for 9 elements in serum and/or whole blood. For six of these elements, the determinations in the serum were run by both the standard additions method and by direct analysis using synthetic blood electrolyte standards. Several general conclusions can be drawn from this table. First, the inductively coupled plasma source is capable of measuring these elements at the levels at which they occur in whole blood and serum. Second, the agreement between the determined concentrations and the "normal" values is generally good considering that the serum and blood samples were from horses while most of the listed "normals" are for human blood and serum. Third, the synthetic standards appear to be valid for many elements. An exception is the case of Cr for which the synthetic standards provide a reasonable result for Cr at normal serum levels but the slope of the analytical curve obtained from these synthetic standards is slightly lower and thus deviates considerably from the standard additions curve at high concentrations. As a result there is some question as to the validity of the synthetic standards for Cr. The reasons for this deviation are not clear at the present time.

The detection limits for some of the trace elements in whole blood and serum are given in Table 6. The values are the detection limits for

Table 5. Analytical results obtained for trace elements in horse blood and serum

Element	Concentration (ug/ml)				
	Serum			Whole Blood	
	Standard Additions	Electrolyte Standards	Normal	Standard Additions	Normal
Al				0.22	0.13-0.17[b]
Co	0.11	0.11	?		
Cr	0.017	0.019	0.01-0.38[a]		
Cu	1.00	0.94	0.70-1.4[a]	1.38	0.5-1.5[b]
Mn	0.040	0.042	0.01-0.07[a]	0.045	0.04[c]
Ni	0.076	0.076	0.01-0.06[a]		
P				390	350[d]
Pb				0.38	0.30-0.40[a]
Zn	1.20		1.21[a]	8.3	6.6-8.8[a]

[a]From reference (72).

[b]From reference (48).

[c]From reference (114).

[d]From reference (115).

Table 6. Estimated detection limits for trace elements in whole blood and serum

Element	Detection limit (ug/ml)	
	Serum	Whole Blood
Al	0.001	0.005
Co	0.005	--
Cr	0.001	--
Cu	0.002	0.008
Mn	0.002	0.006
Ni	0.004	--
P	--	0.05
Pb	0.002	--
Zn	0.008	0.03

these elements in the original blood and serum samples.

For some elements the detection limits could not be directly determined using blood or serum samples because the lowest "standard" was the original sample and the concentration levels at which these elements occurred was so high in relation to the detection limits that the signal/noise ratio could not be accurately measured. In these cases the detection limits were estimated from electrolyte standards.

E. Application to the Determination of Trace Elements in Microliter Sample Volumes

Although the technique described above requires only a small amount of sample (~ 1 ml), sample volumes often are more limited. For example, blood samples from new-born infants are very limited in volume and if several different tests are to be performed, the quantity available for elemental analysis may be in the microliter range. In mass screening projects, it is preferable to obtain blood samples via finger punctures rather than using venous punctures since the former is faster, less expensive and requires much less skill. However, the volume of blood obtained from a finger puncture is small and is normally taken in capillary tubes. The ideal method for use in conjunction with mass screening should use these samples directly, without any sample preparation, in order to handle the samples rapidly and minimize the chances of contamination.

The "continuous" nebulization technique described above requires a minimum of ~ 1 ml of solution per analysis. Microliter samples could be run by diluting them to a 1 ml volume. For example, a 25 µl sample could be diluted 40-fold to provide 1 ml of solution but this high dilution

would adversely affect the ultimate sensitivity of the method. As a result the sample introduction system was modified to permit nebulization of samples directly from microliter pipets.

A schematic diagram of the microliter sample handling system is shown in Figure 7. A short piece of gum rubber (or Tygon) tubing (1.18 mm i.d.) was attached to the polyethylene sample uptake tubing (.77 mm i.d. x 1.23 mm o.d.) from the pneumatic nebulizer. A simple pressure or pinch-clamp was placed around the length of larger tubing in order to control the sample flow. This system is rather crude in its present form and minor modifications should be made to make it more convenient to operate or to provide more automatic operation.

The sequence of operations when microliter samples are handled is as follows:

1. The sample uptake tube is filled with "blank" solution by opening the clamp and allowing normal nebulizer action;
2. The flow is stopped by closing the clamp and then a small air bubble is introduced by removing the tubing from the solution and momentarily opening the clamp;
3. The microliter pipette containing the sample is inserted into the open end of the rubber tubing with the "extra volume" end (above the calibration mark) open to the atmosphere; in this way an air bubble is left in the "extra volume" end;
4. The end of the pipette is then placed in a beaker containing the blank solution and the clamp is opened to allow nebulization to proceed.

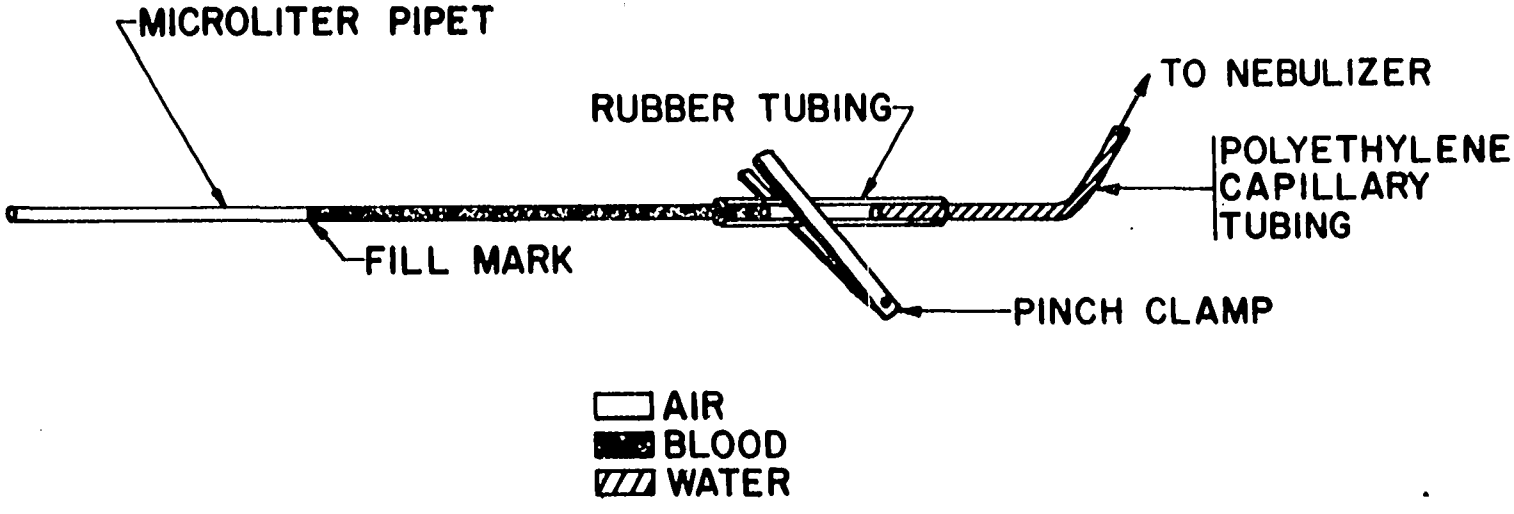

Figure 7. Sampling system for microliter-size samples

In this procedure the air bubbles isolate the sample solution from the blank solution, hence the sample aerosol travels through the plasma as a separate cloud. The emission signal from this sample cloud is measured with the spectrometer wavelength set on the appropriate spectral line. Typical signals are shown in Figure 8. The blank background is observed first and then a sample peak occurs followed by the background signal from the blank solution. Although integration of the peak signal would probably provide better results, the use of peak height measurements was satisfactory and the latter method was used in all of these studies because of its simplicity.

Disposable glass micro-sampling pipets (Corning #7099-S, 25 µl) were used for sampling. Whole blood samples were treated with Heparin as an anticoagulent. Although whole blood samples could be examined directly, ten-fold dilutions with ~ .1 N HCl were employed when addition standards were prepared. Serum samples were either nebulized directly or diluted two-fold with water.

A typical set of data observed on 25 µl aliquots of appropriately diluted whole blood addition standards is shown in Figure 8. The peak intensities were used to construct the analytical curve shown in Figure 9. This figure also shows an analytical curve for Mn in plasma obtained using 25 µl samples. These curves are typical of those obtained for the other elements studied. The results of the analysis of a blood sample from a horse for six elements shown in Table 7, compare very favorably with the values listed as normal. The detection limits for the direct nebulization of 25 µl of undiluted blood samples are also included in

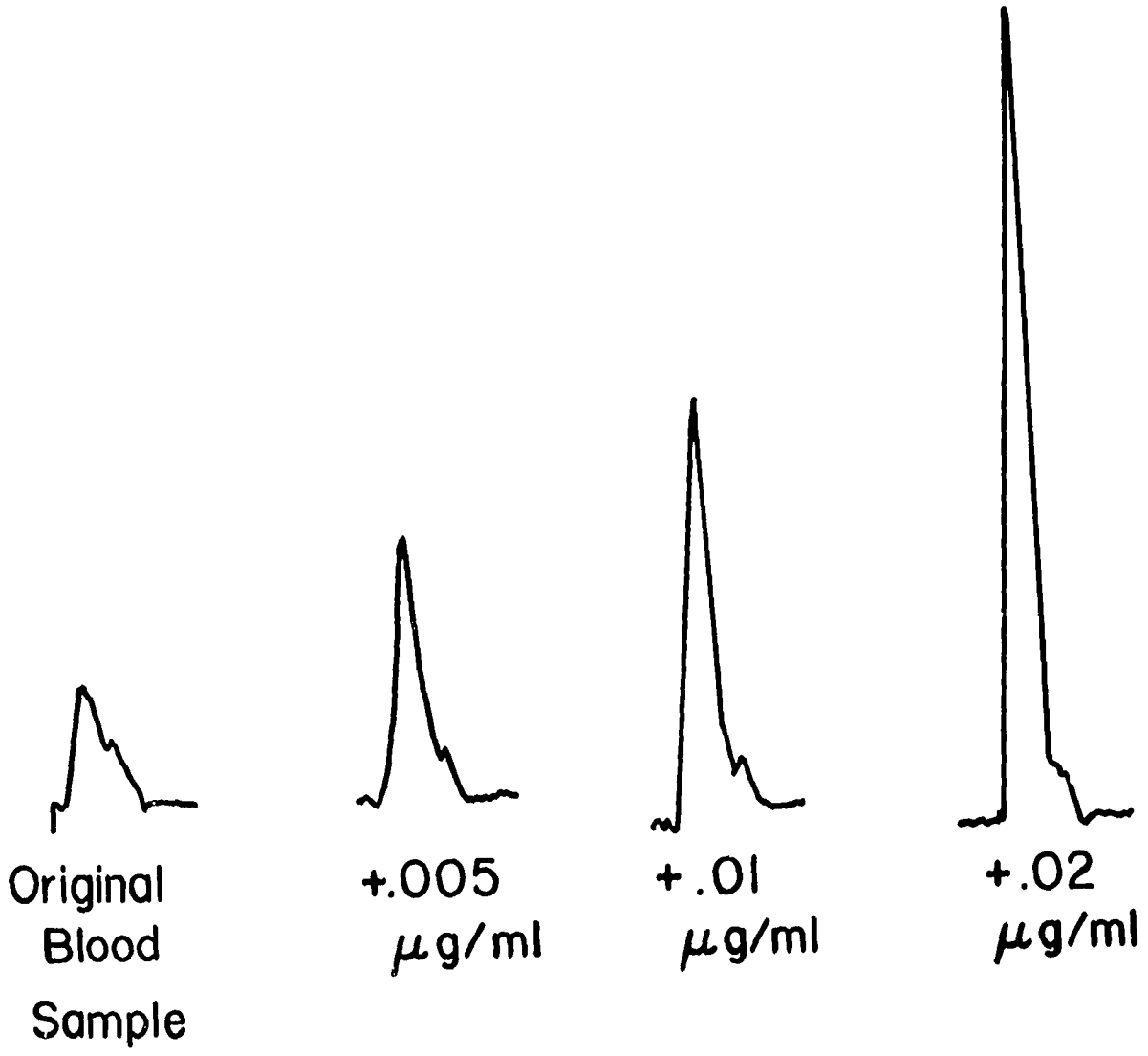

Figure 8. Signals obtained from the Mn 4030 Å line using 25 μl aliquots of blood addition standards (ten-fold dilution)

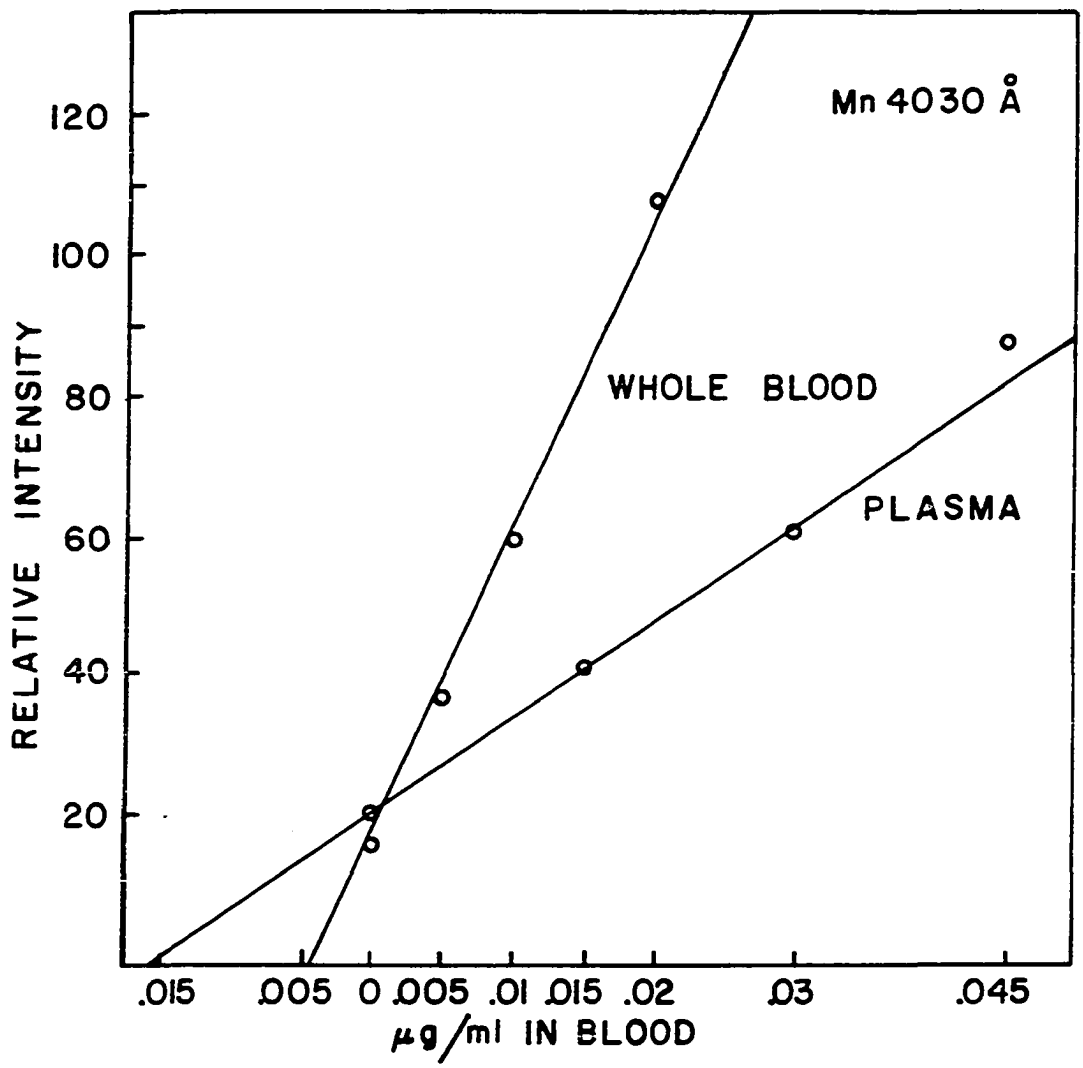

Figure 9. Analytical curves obtained from 25 µl aliquots of addition standards for Mn in human whole blood and plasma (whole blood diluted ten-fold and plasma two-fold)

Table 7. Typical analytical results obtained from 25 µl aliquots of horse blood and serum

Element	Wavelength (nm)	Concentration† mg/l		Detection Limit†† mg/l
		Found	Normal	
Al*	396.1	0.20	0.2[a]	0.007
Ca**	422.7	110	101[a]	not determined
Cu*	324.7	1.4	.7-1.6[b]	0.006
Fe*	371.9	450	420-480[c]	not determined
Mg**	279.5	20	21[d]	not determined
Mn*	403.0	0.070	0.04[a]	0.002

†Reported values are concentrations in the original blood samples.

††Detection limits are for direct nebulization of 20 µl of undiluted blood samples. Blood samples previousiy analyzed by the standard additions method were used for determining these values.

*Whole blood samples diluted ten-fold prior to taking 25 µl aliquots for analysis.

**Serum sample diluted two-fold prior to taking 25 µl aliquots for analysis.

[a]From reference (39).

[b]From reference (48).

[c]From reference (116).

[d]From reference (114).

Table 7. When 150-200 µl samples are nebulized directly, the detection limits are improved by a factor of ~ 5.

Figure 10 illustrates the determination of Cu in whole blood using 25 µl aliquot of a blood sample which had been diluted ten-fold. The copper concentration in the sample of normal blood was determined to be 0.92 µg/ml while the concentration in the abnormal blood sample, taken from a leukemic patient whose leucocyte count was ~ 85,000, was 1.92 µg/ml.

The peak height recordings shown in Figure 11 represent typical reproducibilities obtained for the direct nebulization of 25 µl volumes of undiluted blood. For these seven replicates the coefficient of variation was ~ 5%. For a similar study involving the direct nebulization of 100 µl volumes, the coefficient of variation was reduced to ~ 3%.

The high electrolyte concentrations present in blood tend to produce a signal enhancement. This effect is illustrated in Figure 12. From standard addition experiments the original blood sample (recording in the center of the Figure) was determined to be 0.017 µg Al/ml. Thus the total Al content in the 0.020 µg/ml addition standard was 0.037 µg Al/ml. The signal observed for this addition standard is almost twice the signal obtained from a water solution containing 0.05 µg Al/ml, even though its Al content is 26% less. For the analyses of diluted whole blood and serum, this enhancement effect is accommodated in the calibrations if synthetic standards containing a total electrolyte concentration approximating that found in normal serum are employed. This approach has not been found acceptable for the direct analysis of undiluted whole blood,

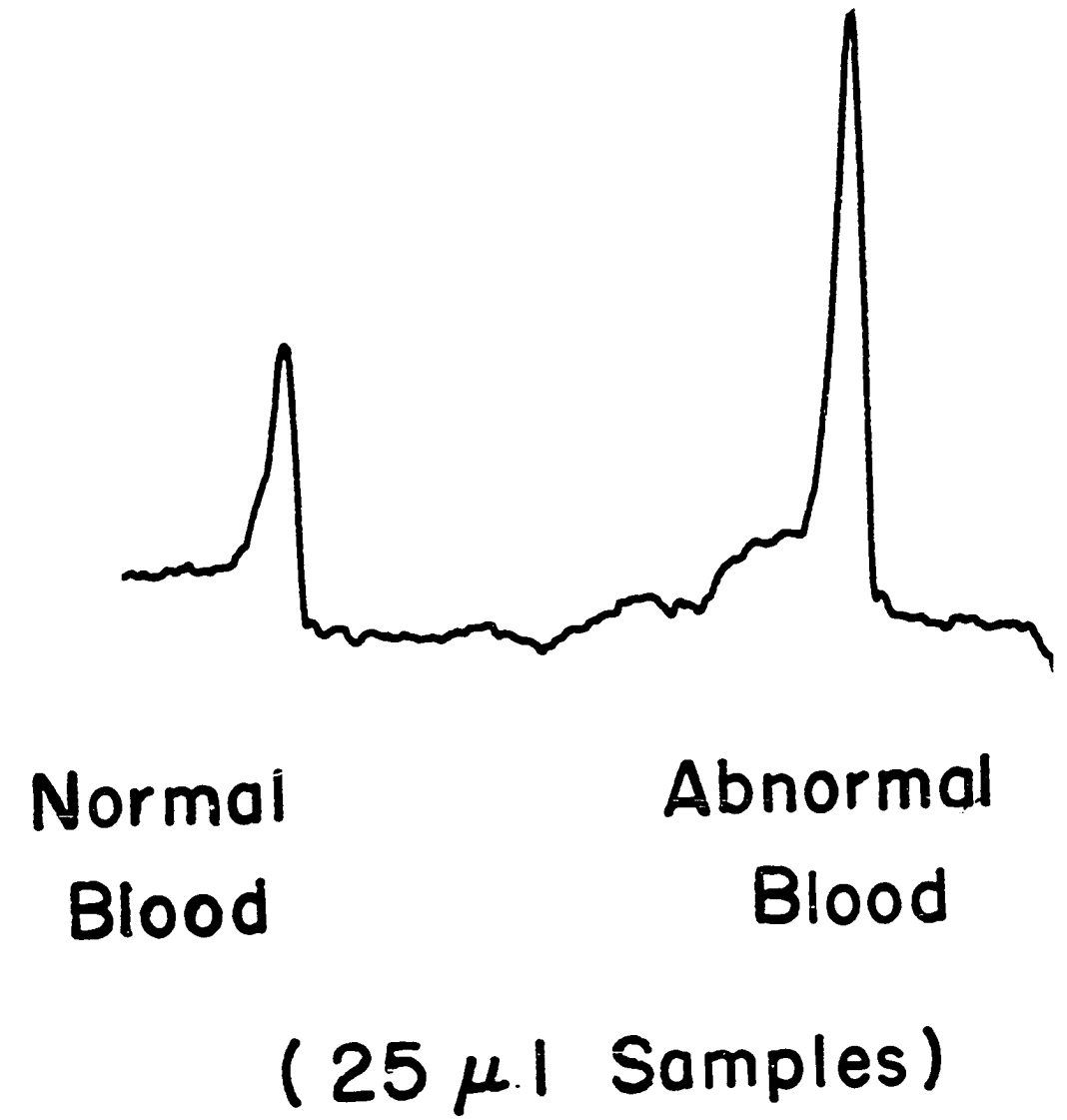

Figure 10. Signal obtained for Cu in 25 μl aliquots of whole blood samples (ten-fold dilution). The abnormal sample came from a leukemic patient

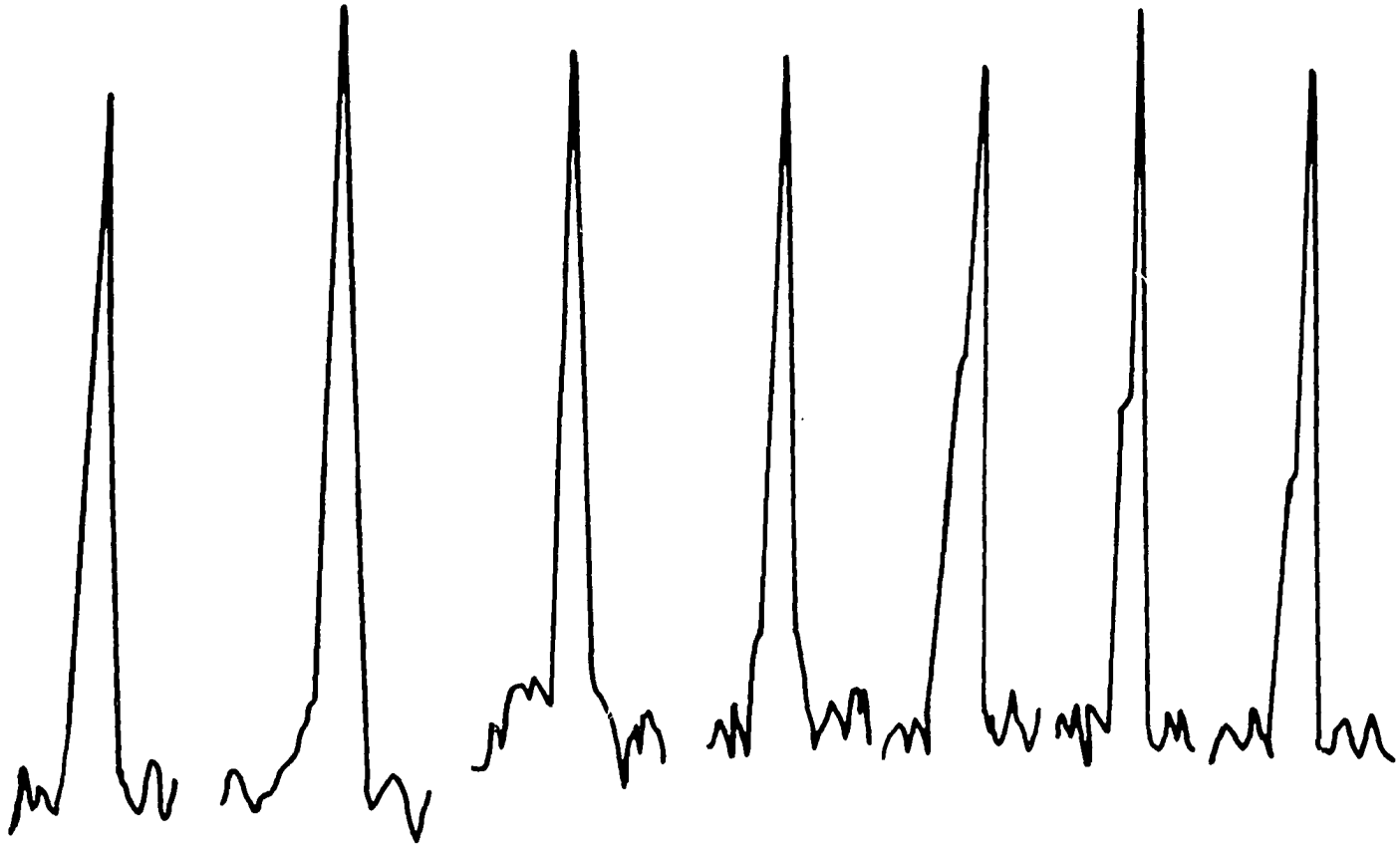

Figure 11. Reproducibility of signals for Mn from 25 µl aliquots of whole blood (undiluted). $\sigma = \pm 5\ \%$

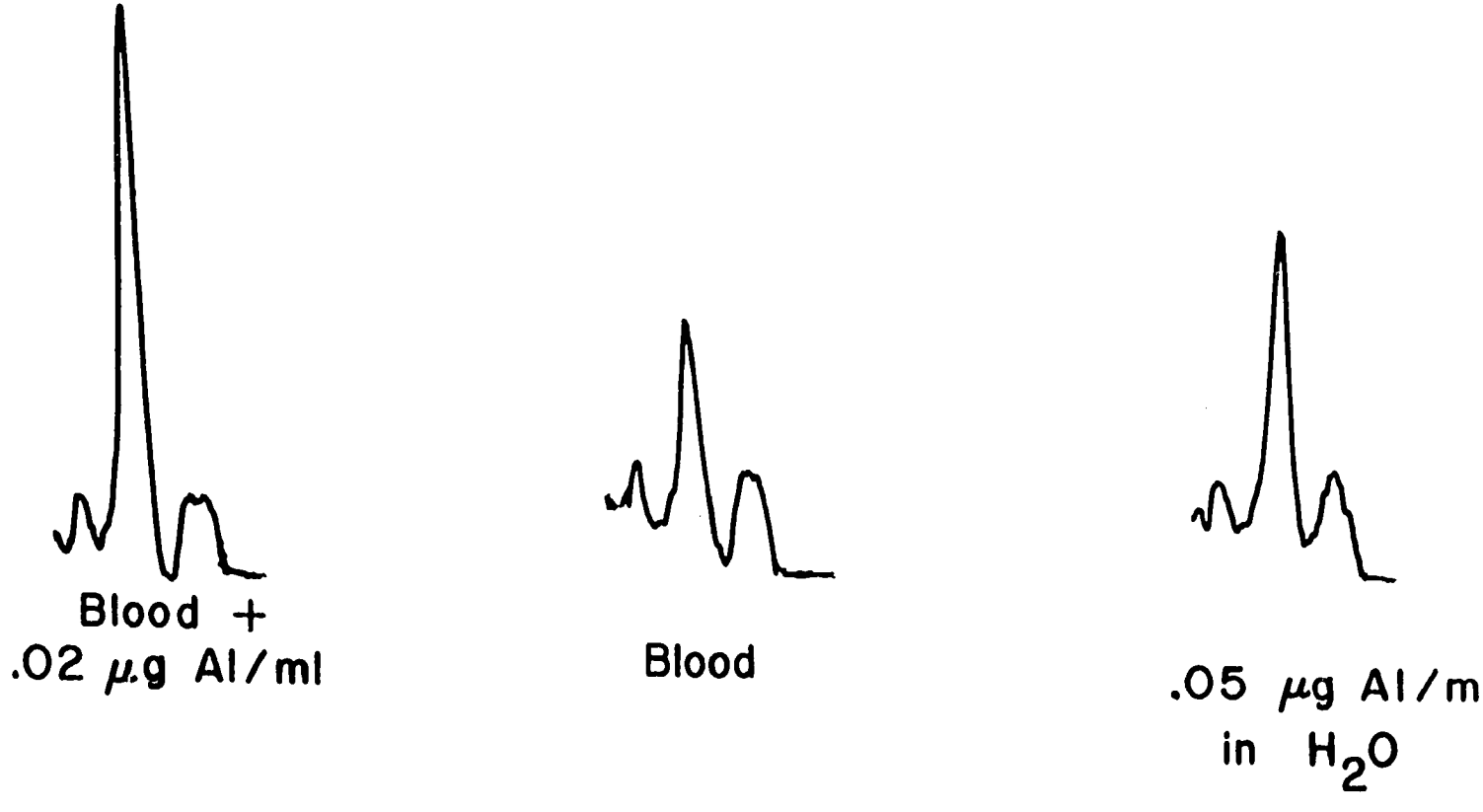

Figure 12. Comparison of signals obtained for Al in blood and Al in a water standard (25 μl volumes nebulized)

probably because of differences in the physical properties, such as viscosity and surface tension, between water and blood.

F. Summary

The data presented above show that emission spectrometric techniques combined with an inductively coupled plasma excitation source can provide rapid analytical results for major and trace concentrations of metals in biological fluids even though only a very limited volume of sample is available. Although all of the data presented were obtained with a single-channel spectrometer, the extension of the technique to simultaneous multielement analysis by using a multichannel direct-reading spectrometer is obvious. With an automated sample-handling capability, such a system would permit the determination of 20 or more major and trace elements on microliter volumes at the rate of one sample every 15 to 30 seconds.

Although the above discussion deals only with the analysis for trace elements in blood, it is highly probable that the method could easily be extended to other biological samples such as urine, tissues, solubilized foodstuffs, pharmaceutical products, etc. Only minor changes in the methods of sample handling and standardization should be required.

V. APPLICATION OF AN INDUCTIVELY COUPLED PLASMA-EMISSION SPECTROMETRIC SYSTEM TO THE DIRECT DETERMINATION OF ALLOYING AND IMPURITY ELEMENTS IN STEELS

A. General Considerations

Metallurgical samples, as represented by high and low alloy steels, present somewhat different analytical problems than those encountered in the analysis of biological materials. Blood and other biological fluids have a relatively constant matrix composed principally of alkali and alkaline earth elements. The analyte elements in biological samples usually occur within a somewhat narrow range and analytical curves covering one order of magnitude are usually sufficient. This prior knowledge of the composition of the samples provides a basis for the preparation of standards which have elemental concentrations approximating those of the samples and thereby minimizing any matrix effects which may be present.

Metallurgical samples often have highly variable matrices and require that the analytes be determined over a very wide concentration range. In low alloy steels the Fe concentration is generally > 95% while in some high alloy steels, such as the Cr-Ni series, the matrix may contain < 5% Fe. These wide matrix variations often cause analytical problems. For example, Alkemade (117) has briefly described the various matrix effects which occur in flame atomic emission and absorption spectrometry. Although Becker (118) showed that some of these could be eliminated by careful choice of flame and flame conditions, some of the matrix effects persist. Ramirez-Munoz (113) has discussed the occurrence of these matrix effects

in the analysis of metallurgical materials. In the most commonly used method for steel analysis, i.e., optical emission spectrometry utilizing a high voltage spark for sample excitation, both the matrix compositions and the previous metallurgical histories of the samples and standards must be closely matched (119).

The determination of trace quantities of metals is also important in the analysis of steels. Some of these trace metals are alloying elements added to enhance certain physical characteristics of the steel. In other cases there are residual impurities which have a detrimental effect on the properties of the alloy. The analytical requirements are often very stringent since a small change in composition can have profound influence on the characteristics of the alloy.

B. Determination of Common Alloying Elements in Steel

The determination of common alloying elements in steel is typical of an analytical problem in which elements are determined over a wide concentration range in the presence of a high concentration of a matrix of variable composition. The elements chosen for this portion of the study were Al, Cu, Cr, Mn, and Ni since they are representative of the elements normally determined in steels.

The samples were dissolved in a mixture of 30 ml of 1:1 HCl and 5 ml of HNO_3 following the procedure described by Fassel et al. (120). The resultant solutions were diluted to yield a solution containing 5.0 mg of sample per milliliter. The standards were prepared by additions of the analyte elements to pure Fe solutions: no attempt was made to match the total composition of the samples and standards.

Wavelength scans of the analysis lines are shown in Figure 13. The background tracings were obtained using a 5000 µg Fe/ml as a blank solution. No spectral interferences from Fe were observed but the Ni line lies adjacent to a moderately strong Ar line. However, this represents a stable background and Ni could be quantitatively measured at the 0.001% concentration level (approximately the residual level in the Fe used to prepare the standards) which is more than adequate for steel analysis.

Typical analytical curves are shown in Figures 14 and 15. The data for these curves was collected over a period of one year and the vertical bars represent the total spread of data points. Data from day to day were normalized to a single analytical curve by using one of the standards as a reference intensity. It is apparent from this figure that the analytical curves are linear over a wide concentration range. This is shown dramatically in Figure 15 where the analytical curves for Cr and Ni extend over a range of four or more orders of magnitude without any apparent departure from linearity. As a result both the major and trace constituents in a sample can be determined using a single dilution. In contrast, analytical curves obtained by flame atomic emission and absorption are linear only over a 1½ to 2 orders of magnitude range (121) and thus several sample dilutions are often necessary if both major and minor constituents are determined.

Data points from some other analytical determinations are also plotted on the Cr analytical curve. These data points were obtained for Cr in several different matrices, e.g. deionized water, Mn, blood serum and solubilized food composites. The fact that data from Cr in all of

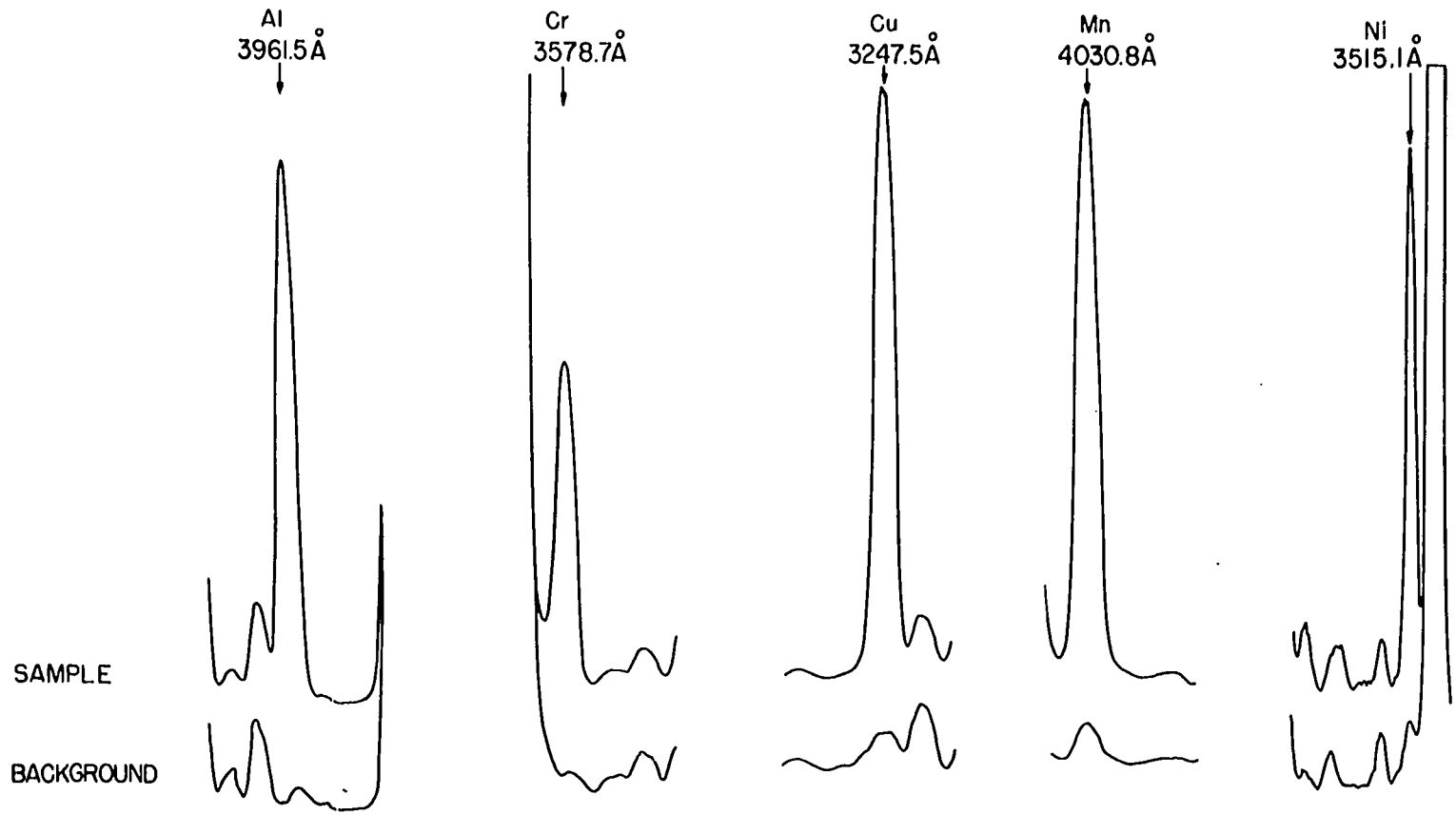

Figure 13. Wavelength scans of the analytical lines used for the determination of common alloying elements in steel

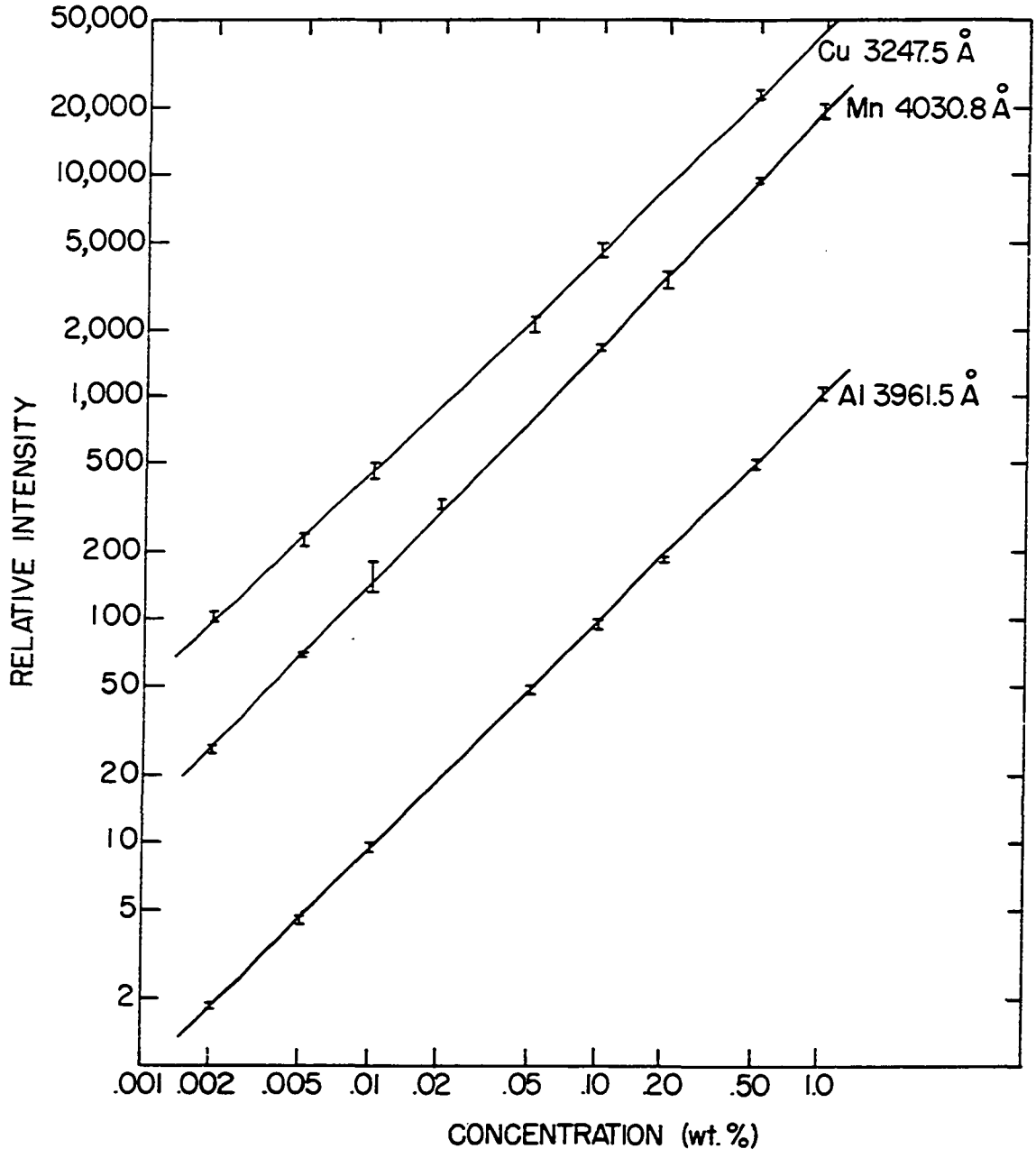

Figure 14. Analytical curves for the determination of Cu, Mn and Al in steel

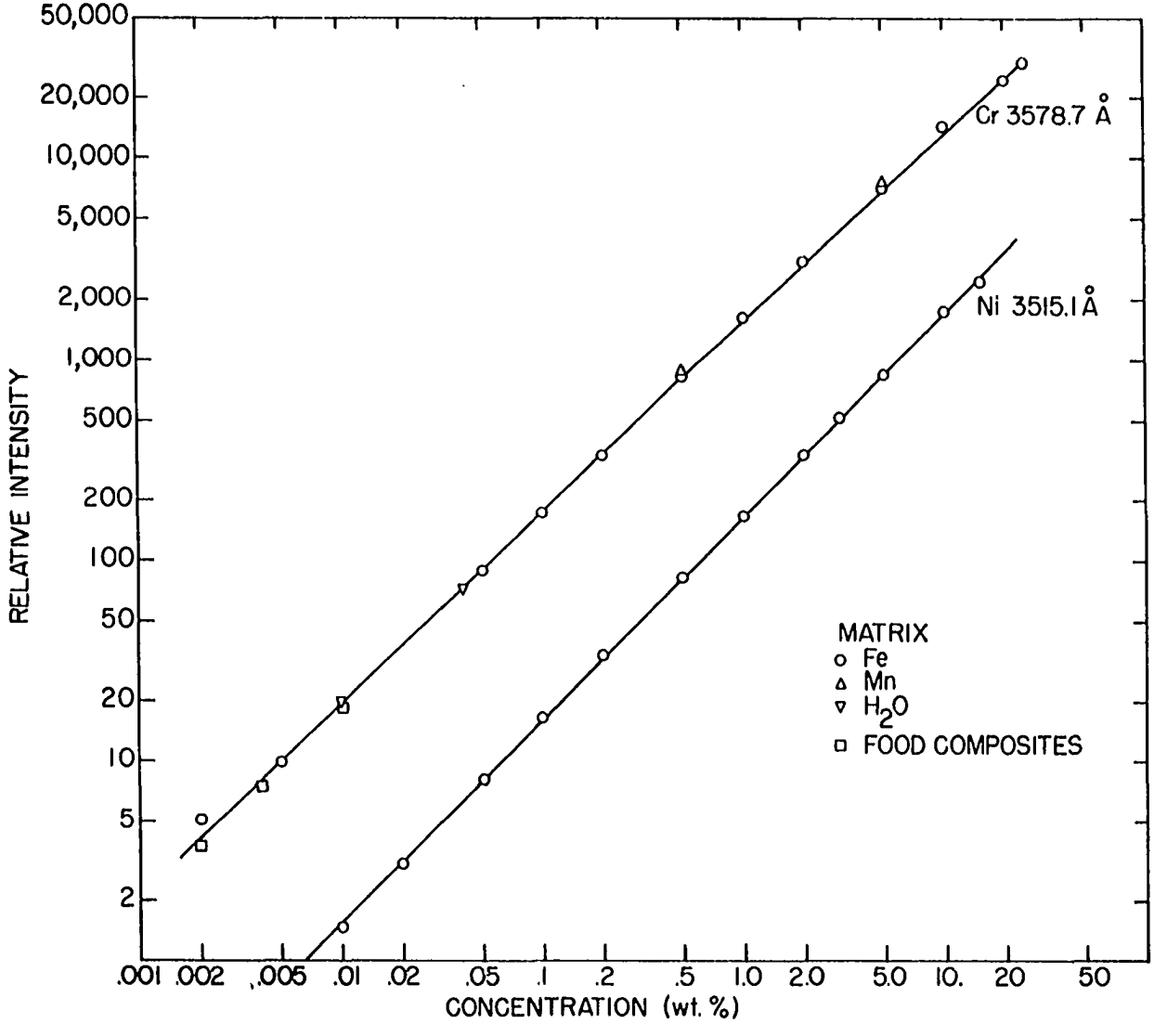

Figure 15. Analytical curves for the determination of Cr and Ni

these matrices fall on a single congruent analytical curve illustrates the validity of the background corrections and the absence of any measurable matrix effects.

Table 8 shows the results of a precision study conducted over a period of four months. This study reflects the influence of normal changes in the analytical system (cleaning and replacement of torches, repair of nebulizers, etc.). All of the data were net emission signals; no internal standardization was used. Preliminary studies indicate that the use of suitable internal standard lines can generally improve the precision by a factor of 3 to 5. Further improvement could also be expected if integration techniques were used.

The accuracy of the technique can be assessed from the results for the analysis of the National Bureau of Standards standard samples presented in Table 9. All of the concentrations reported are within the range of analytical values reported by NBS even though the Fe content of the matrix varies from > 99% (NBS-19g) to < 1% (NBS-169). The results for Al are particularly interesting since the determination of Al by atomic absorption spectrometry requires that the reference blank solution contain approximately the same Fe content as the test solution (122). It is apparent from Table 9 that this is not a necessary requirement when an inductively coupled plasma source is used. Thus the use of a pure Fe solution as a matrix for preparing standards as well as a blank reference solution for background correction is apparently valid.

Table 8. Precision data

Element	Coefficient of variation (%)[a]
Al	1.4
Cr	2.4
Cu	1.8
Mn	2.5
Ni	3.2

[a]Coefficients of variation were determined at the 0.02% concentration level in steel.

Table 9. Results of the analyses of National Bureau of Standards steel samples

Element	NBS Sample Number	Matrix[a]	Concentration (Wt.%)		
			NBS Range	NBS Ave	This Work
Al	33c	3 Ni	0.030-0.034	0.032	0.033
Al	19g	Open hearth (9.2%C)	0.027-0.033	0.031	0.031
Al	169	77 Ni-20 Cr	0.087-0.105	0.095	0.095
Cr	129b	Bessemer (0.1%C)	0.014-0.019	0.016	0.018
Cr	19g	Open hearth (0.2%C)	0.369-0.380	0.374	0.370
Cr	33c	3 Ni	0.049-0.056	0.052	0.055
Cu	160	19 Cr-9 Ni-3 Mo	0.047-0.06	0.053	0.051
Cu	169	77 Ni-20 Cr	0.013-0.02	0.015	0.016
Cu	341	20 Ni-2 Cr	0.145-0.159	0.152	0.153
Ni	73c	Stainless steel, 13 Cr	0.241-0.251	0.246	0.255
Ni	111b	1 Mn-2 Ni	1.80-1.83	1.81	1.79
Mn	19g	Open hearth (0.2%C)	0.550-0.559	0.544	0.56
Mn	33c	3 Ni	0.733-0.735	0.733	0.73
Mn	73c	Stainless steel, 13 Cr	0.325-0.34	0.33	0.33

[a]The elements listed are the major constituents other than Fe and the numbers refer to their nominal concentrations as wt.%.

C. Determination of Rare Earth and Refractory Metals in Steel

A more difficult problem in the analysis of steels is the determination of those elements which form highly stable monoxide molecules in combustion flames particularly since some of these elements occur at very low concentration levels. Typical examples are rare earth elements and refractory metals which are added, often in small quantities, to steel in order to obtain certain desirable properties. Normal spark-excitation emission spectrometric and flame atomic emission and absorption techniques are generally adequate when these metals are present in high concentrations but preliminary chemical concentration procedures must be employed when the concentrations are in the ppm range.

Dickenson and Fassel (5) found that the inductively coupled plasma source is capable of detecting elements which form very stable monoxide molecules at much lower concentration levels than can be detected by other common analytical methods. Dickenson (123) also showed that Ti, which forms a very stable metal monoxide, could easily be determined at the ppm level in steel, without resorting to any preconcentration procedures. Three rare earth elements, Ce, La, and Pr, are major constituents of the rare earth mixtures ordinarily used for additions to steel. The concentrations of Ce, La, and Pr in these special steels range down to 0.008%, 0.005%, and < 0.005% respectively (124). Two recent review articles (125,126) on ferrous metallurgy have summarized the methods proposed for the determination of these rare earths in steels. Most of the methods use optical emission or X-ray fluorescence spectrometry to determine the individual elements. Prior chemical separation of the rare earths is

necessary in order to attain adequate sensitivity and eliminate interferences. Koch and Bosch (127) have recently developed an X-ray fluorescence method which provides absolute detection limits for Ce, La, and Nd in the range of 0.1 to 0.2 µg. However, this method requires chemical separation of the rare earths as hydroxides and ignition of the precipitate to the oxides. Also, two different X-ray tubes (Cr target and W target) must be used to determine these three elements.

The analytical curves shown in Figure 16 illustrate that the inductively coupled plasma source can be used for the direct determination of Ce, La, and Pr in steels down to the ppm level. The vertical bars on the curves represent the total spread of data points obtained over a period of approximately two months. At the .01% concentration levels the coefficients of variation (as %) were 3.3, 4.1, and 3.0 for Ce, La, and Pr, respectively, based on precision data collected over a two-week period. The ability to directly determine these elements in an Fe matrix at very low concentrations and without prior chemical separation, greatly simplifies this analytical problem and again illustrates the general analytical utility of the inductively coupled plasma source.

The refractory metals Nb, W, and Zr are present in certain modified low alloy steels at very low concentration levels and represent some of the most difficult elements to determine in steel. Thomerson and Price (128) have described an atomic absorption method for the direct determination of W in steels but the lowest determinable concentration is approximately 0.1%. For many applications this is not satisfactory as illustrated by the fact that only two standards in the National Bureau of Standards

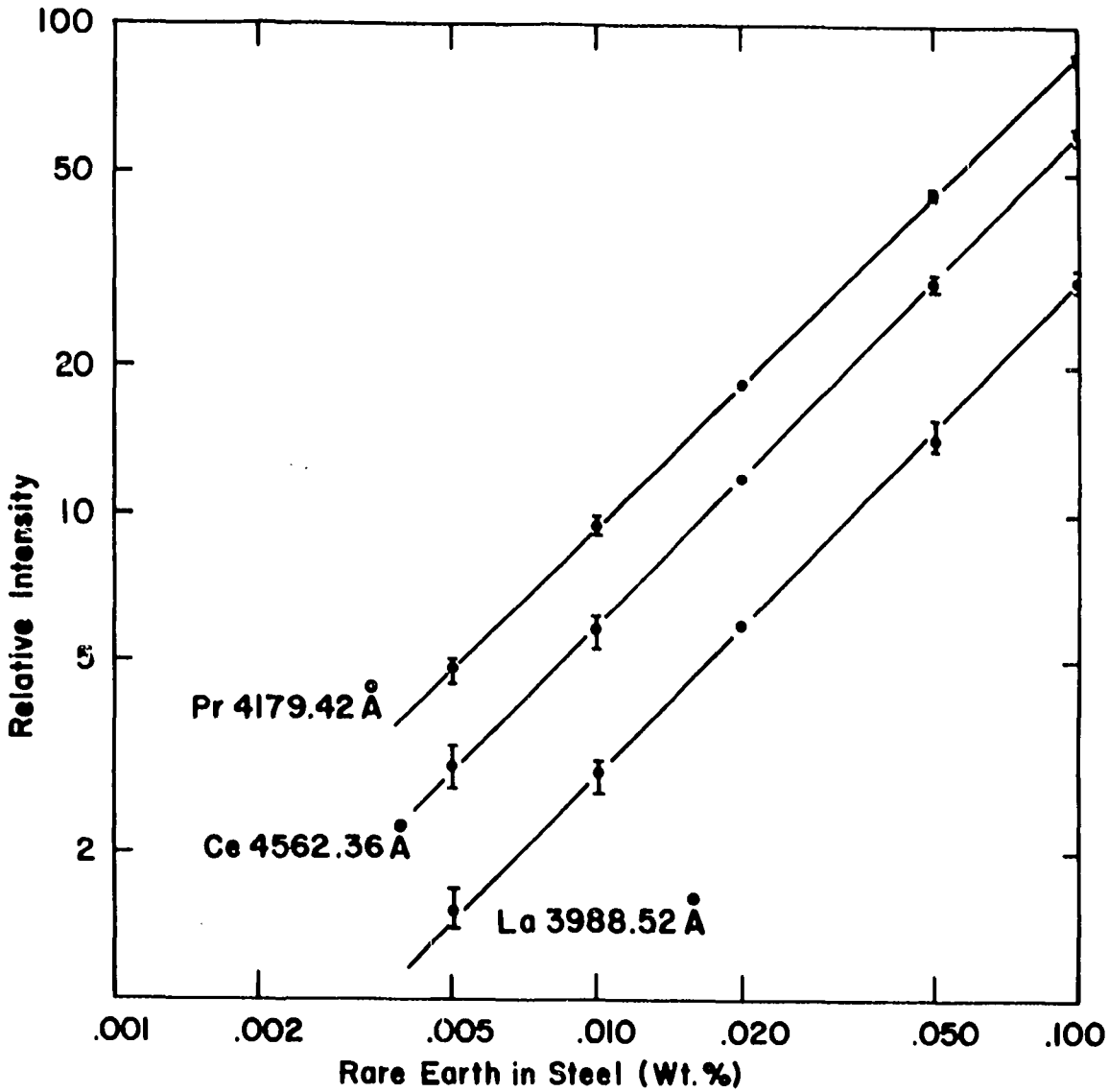

Figure 16. Analytical curves for the determination of La, Ce and Pr in steel

460 series of steel standards contain W concentrations in excess of 0.1%.

Schiller (129) reported that atomic absorption techniques could not directly determine Nb in steel at concentrations below 0.3%; a preconcentration method involving the removal of the Nb from the sample as the carbide was used to bring the Nb concentration into the determinable range. This procedure requires a 60 g sample in order to determine Nb at the 0.01% level and prior knowledge of the steel type is necessary since the method is only applicable to very low alloy steels. A flame emission method (130) has been described for the determination of Nb in steels but preconcentration of the Nb by solvent extraction is necessary. A 100 g sample is required to determine Nb at the 0.02% level, which is common in steels.

By contrast, the inductively coupled plasma source can be applied to the direct determination of these elements. Figure 17 shows the analytical curves for the determination of Nb, W, and Ta down to concentrations of 0.005%, which are approximately the lowest values normally encountered in steel analyses. The major problem with this determination is dissolution of the samples since the normal acid mixtures used do not completely dissolve steels containing Nb, W, Zr, and other refractory metals. The National Bureau of Standards (131) has recommended dissolution in a HNO_3-HCl mixture and filtering to remove the residue. This residue is then dissolved in an HF-HNO_3 mixture in a Teflon bomb (132) and the resulting solution is combined with the original one prior to analysis. The procedure is lengthy and the resulting solution contains considerable fluoride ion which is not compatible with the glass needles

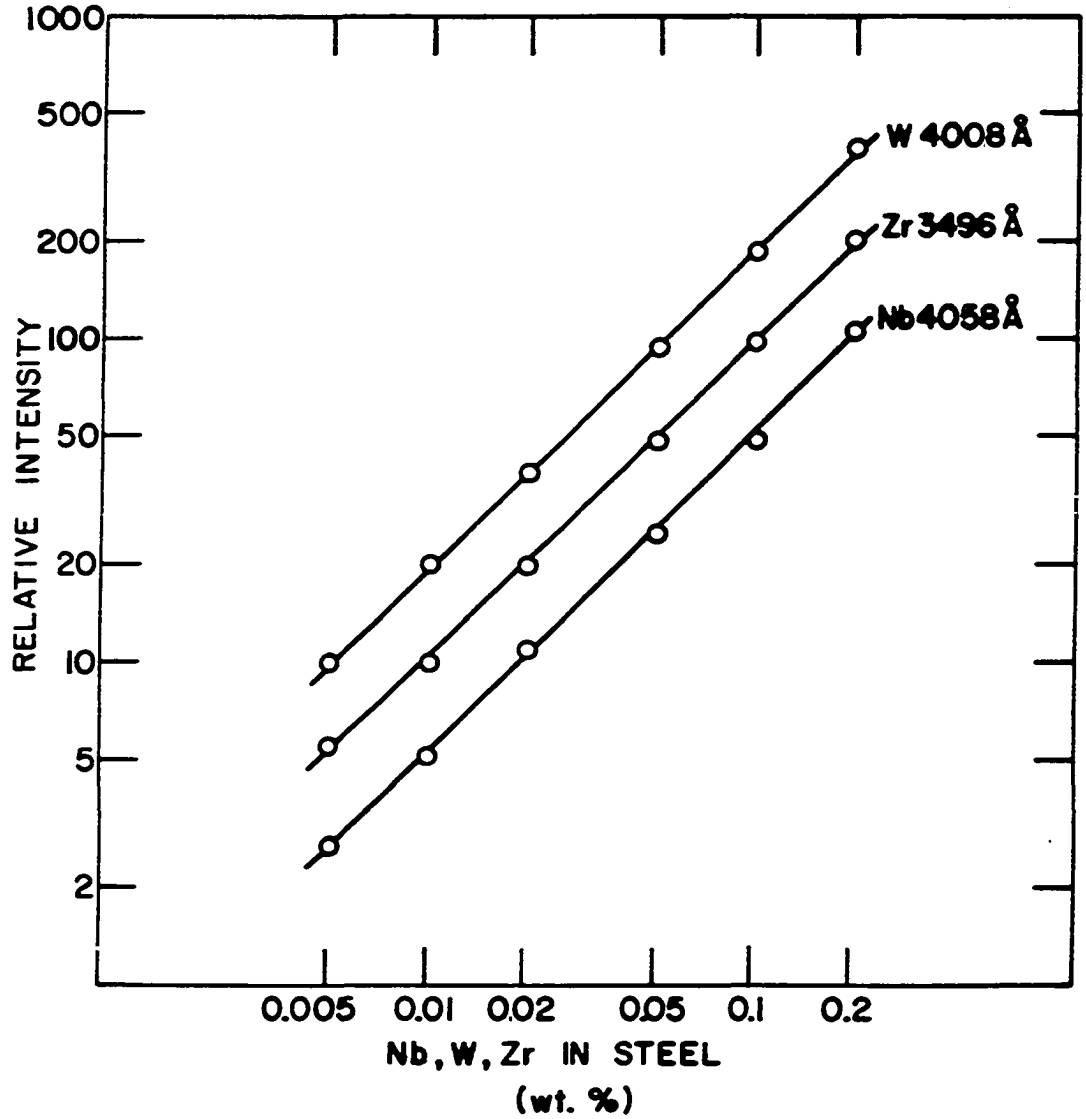

Figure 17. Analytical curves for the determination of Nb, W, and Zr in steel

used in the pneumatic nebulizer. Thus improvements in the nebulizing system or in the dissolution procedures are necessary to make these analyses practical. However, the analytical curves clearly show the potential of the plasma excitation source.

D. Detection Limits

The detection limits obtainable using atomic emission, absorption and fluorescence techniques are generally reported under ideal conditions and in the absence of a matrix material. A question that commonly arises is "can these elements be detected at the same concentration levels in the presence of a very high concentration of matrix material?" Most authors avoid this question but some general conclusions can be drawn from the literature. In a discussion of detection limits in atomic absorption spectrometry, Ramirez-Munoz (113) states that the values in the literature "are almost always determined under ideal conditions with a complete absence of other components in the solutions: thus, no other component can interfere and so contribute to losses of sensitivity, such as may occur in solutions prepared from actual samples." Herrmann (133) states that "it should be remembered that the detection limits for flame atomic emission and absorption quoted by the manufacturer generally apply to simple solutions of one single salt. The presence of a large number of other substances, as in biological materials, increases the detection limit." These statements certainly indicate that the presence of a matrix element adversely affects detection limits in flame atomic emission and absorption methods.

Other information in the literature tends to support the statements

made by the above authors. As mentioned previously, with atomic absorption techniques the lowest determinable concentrations for W (128) and Nb (129) in steels are 0.1% and 0.3% respectively, The detection limit for both of these elements is reported as 3 µg/ml (72). If the sample solutions contain 1% steel (the concentration used by Thomerson and Price (128) and normally used by other workers) the calculated minimum determinable concentration (five times the detection limit) should be 0.015% for both elements. This calculated value is an order of magnitude below the minimum detectable concentrations reported, indicating the deleterious influence from the presence of a steel matrix in the solution to be analyzed.

Ramirez-Munoz (134) studied the effects of varying Fe concentrations on the atomic absorption sensitivities (concentration required for 1% absorption) for various elements. The effects due to the presence of an Fe matrix were highly dependent on the burner used and the height of observation in the flame. However, in most instances the presence of 0.5-1.0 mg Fe/ml caused a loss of sensitivity and thereby adversely affected the detection limits. In some cases the effects were small but in others the losses in sensitivity were as much as a factor of 6.

Do the same matrix effects occur in the inductively coupled plasma source? The data collected during the study on steels provides at least a tentative answer to this question. Table 10 shows the detection limits obtained with the system described in Chapter III for the various elements in an Fe matrix (0.5 mg fe/ml) as compared with those reported for dilute aqueous solutions with no matrix elements present. This table clearly

Table 10. Comparison of detection limits obtained in the presence and absence of a matrix material

Element	Wavelength (Å)	In H_2O solution (μg/ml)	Detection limits in 0.5% Fe solution (μg/ml)	In steel samples (Wt.%)	Lowest quantitatively determinable concentrations in steel (Wt.%)
Al	3961	0.002	0.004	0.00008	0.0004
Ce	4562	0.007	0.013	0.0003	0.0014
Cr	3578	0.001	0.002	0.00004	0.0002
Cu	3247	0.001	0.0003	0.000006	0.00003
La	3988	0.003	0.003	0.00007	0.0003
Mn	4030	0.003	0.003	0.00007	0.0004
Ni	3515	0.06	0.07	0.001	0.007
Nb	4058	0.01	0.02	0.0004	0.002
Pb	4057	0.008	0.006	0.0001	0.0005
Pr	4179	0.008	0.01	0.0002	0.001
W	4008	0.002	0.003	0.00006	0.0003
Zr	3496	0.02	0.02	0.0005	0.002

illustrates that the presence of a high concentration of a matrix element such as Fe has essentially no influence on the detection limits. The tendency for the detection limits to be slightly poorer ($\lesssim 2x$) in the Fe solutions is not significant since this is within the normal day-to-day fluctuations. These data strongly indicate that the detection limits for elements in dilute aqueous solutions are directly transferable to the analysis of real samples when an inductively coupled plasma analytical system is used.

Table 10 also lists the calculated minimum determinable concentration (5 times the detection limit) in a matrix element present in solution at a concentration of 0.5 mg/ml. This concentration represents that which should produce a signal level that is 10 times higher than the background noise. These determination limits are very low and it is instructive to inquire as to the practicality of analyzing samples at these levels.

In steel analysis the most sensitive Zr line cannot be used because of a spectral interference from Fe. A less sensitive line (3496 Å) is used instead and the minimum determinable concentration of Zr, using the latter line is 0.0023%. Figure 18 shows the signals obtained for 0.008% Zr in steel (NBS Standard Sample No. 1261) and for 0.005% Zr in a Pb-Bi alloy. These signals are easily measurable and demonstrate that the calculated value for the determination limit is perhaps conservative.

The detection limit for Pb is 0.008 µg/ml, hence the lowest concentration determinable should be 0.0016% in Fe if a solution containing a sample concentration of 0.5 mg/ml is used for the analysis. Figure 19 shows an analytical curve for Pb in Fe extending down to 0.001%. The

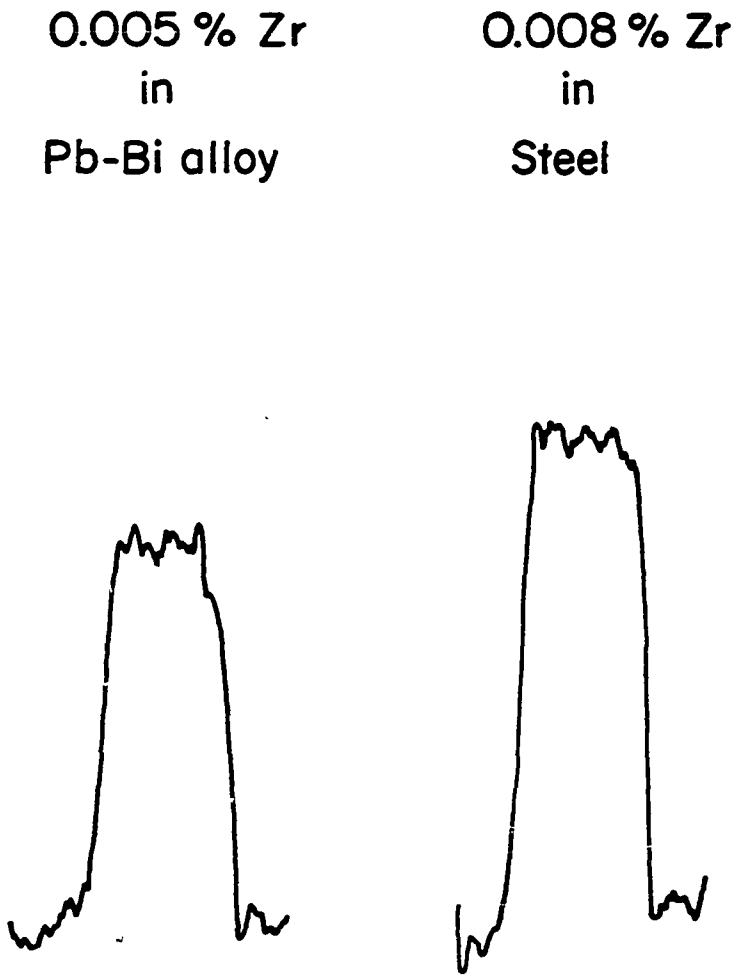

Figure 18. Signals obtained for Zr in two different matrices

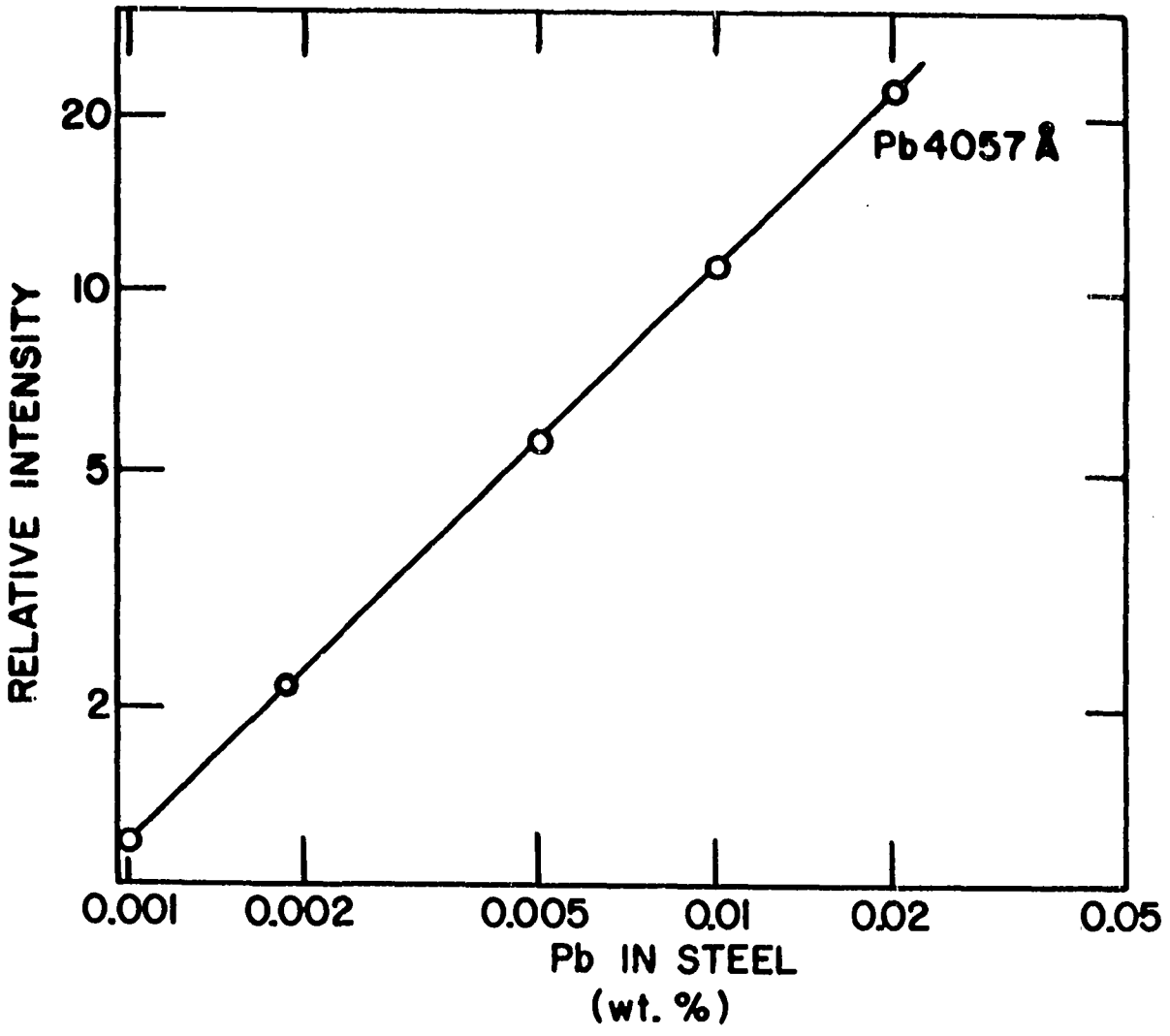

Figure 19. Analytical curve for the determination of Pb in steel

signal obtained from the 0.005% standard is shown in Figure 20. It is interesting to note that the flame atomic absorption detection limit for Pb is essentially the same (0.01 μg/ml) but the minimum determinable concentration in a 0.5 mg Fe/ml solution is 6 to 10 times higher (0.006-0.010%) (133) than that obtained with the inductively coupled plasma system.

The extremely low detection limits shown in Table 10 for this group of elements in an Fe matrix again illustrates the analytical capabilities of an inductively coupled plasma source. These detection limits are equal to or far superior to those reported for other commonly-used techniques for steel analysis.

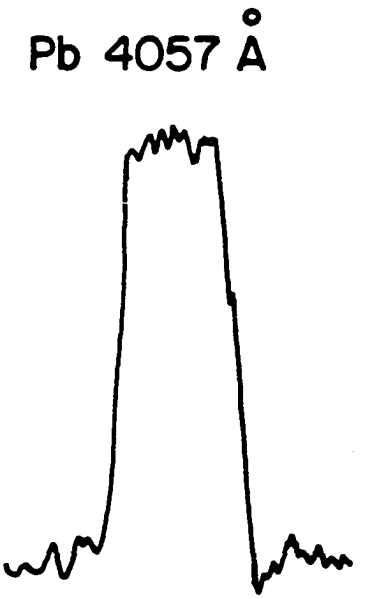

Figure 20. Signal obtained for 0.005 % Pb in steel

VI. CONCLUSIONS

The literature contains adequate documentation of the ability of the inductively coupled plasma optical emission spectroscopy to detect the metallic elements and metalloids at the fractional µg/ml down to the fractional ng/ml level. The data presented in this thesis show that these low detection limits can still be achieved in the pesence of a high concentration of a matrix material. Matrix effects appear to be minimal and a single set of standards suffices for the analysis of samples of widely varying compositions. The analytical curves are linear over four or more orders of magnitude and one sample dilution allows the determination of both major and trace constituents. This is in sharp contrast to other atomic emission and absorption techniques for which the linearity of the analytical curves is usually restricted to 1½ to 2 orders of magnitude and several dilutions of the sample are necessary to determine both the major and minor elements. Sample volumes in the microliter range can be easily and quickly analyzed and the detection limits achievable are only slightly inferior to those reported for "infinite" sample volumes.

The studies on the analysis of blood opens many avenues of investigation on the application of the inductively coupled plasma source. The extention to other biological fluids such as urine and cerebrospinal fluid is obvious. As pointed out in Chapter IV, these applications could have an important impact on human health problems.

Less obvious are applications to other systems with organic matrices. The determination of trace toxic and essential elements in solubilized

foodstuffs is an important problem which needs immediate attention. The drug industry is in need of a sensitive and rapid method for the determination of trace heavy metals in drugs. The concentration of trace elements in lubricating oils from engines can provide information on engine wear but this is a difficult analytical problem. An equally difficult problem is the analysis of crude oil for trace elements which poison catalysts used in catalytic-crackers.

The analytical work on steels also indicates many areas of application to difficult analytical problems. If further investigations continue to show that this source is relatively free from any major matrix effects then perhaps a wide range of materials can be analyzed using a single set of standards. Natural waters and industrial effluents represent samples of variable matrix composition which must be analyzed for a large number of elements at trace level. Soils, ores and rock are often analyzed for both major and minor constituents. Analysis of these samples with an inductively coupled plasma source would probably require only a single sample dilution since the analytical curves should be linear over several orders of magnitude. Atmospheric particulates offer a special challenge because trace elements must be determined in a very limited sample (50-1000 μg).

The list of possible applications could be greatly extended but those mentioned suffice to illustrate the potential utility of the inductively coupled plasma source in analytical spectroscopy. The ability to perform simultaneous multielement analyses makes this source even more exciting. All of the work in this thesis was done with a single-channel

spectrometer but the substitution of a conventional direct-reading spectrometer is all that is needed to provide this multielement capability. However, modification of a conventional multichannel spectrometer to provide automatic background corrections and the capability of integrating pulse signals (such as those from the microliter samples) would be the most desirable approach. Other improvements in the inductively coupled plasma optical emission analytical system will undoubtedly occur as the technique becomes more widely used as a routine analytical tool.

VII. LITERATURE CITED

1. R. H. Wendt and V. A. Fassel, Anal. Chem. 37, 920 (1965).
2. R. H. Wendt and V. A. Fassel, Anal. Chem. 38, 337 (1966).
3. W. Barnett and V. A. Fassel, Spectrochim. Acta 23B, 643 (1968).
4. V. A. Fassel and G. W. Dickenson, Anal. Chem. 40, 247 (1968).
5. G. W. Dickenson and V. A. Fassel, Anal. Chem. 41, 1021 (1969).
6. S. Greenfield, I. L. Jones, and C. T. Berry, Analyst 89, 713 (1964).
7. S. Greenfield, Proc. Soc. Anal. Chem. 2, 111 (1965).
8. S. Greenfield, P. B. Smith, A. E. Breeze, and N. M. D. Chilton, Anal. Chim. Acta. 41, 385 (1968).
9. H. Dunken and G. Pforr, Z. Chem. 6, 278 (1966).
10. G. Pforr, in Proceedings XIV Colloquium Spectroscopicum Internationale Adam Hilger Ltd., London, 1968, p. 687.
11. G. Pforr and O. Aribot, Z. Chem. 10, 78 (1970).
12. M. E. Britske, V. M. Borisov, and Y. S. Sukach, Zavod. Lab. 33, 252 (1967).
13. H. C. Hoare and R. A. Mostyn, Anal. Chem. 39, 1153 (1967).
14. C. Bordonali and M. A. Biancifiori, Met. Ital. No. 8, 631 (1967).
15. C. Bordonali and M. A. Biancifiori, in Proceedings XIV Colloquium Spectroscopicum Internationale, Adam Hilger Ltd., London, 1968, p. 1153.
16. C. Veillon and M. Margoshes, Spectrochim. Acta 23B, 503 (1968).
17. J. M. Mermet and J. Robin, in Proceedings XIV Colloquium Spectroscopicum Internationale, Adam Hilger Ltd., London, 1968, p. 715.
18. V. M. Goldfarb and V. J. Goikhman, Zh. Prikl. Spektrosk. 1968, 193 (1968).
19. I Kleinmann and V. Svoboda, Anal. Chem. 41, 1029 (1969).
20. G. H. Morrison and Y. Talmi, Anal. Chem. 42, 809 (1970).

21. D. Truitt and J. W. Robinson, Anal. Chem. Acta 49, 401 (1970); 51, 61 (1970).

22. P. W. J. M. Boumans and F. J. DeBoer, Spectrochim. Acta 27B, 391 (1972).

23. W. B. Barnett, V. A. Fassel, and R. N. Kniseley, Spectrochim. Acta 25B, 139 (1970).

24. A. D. Stokes, J. Phys. D: Appl. Phys. 4, 916 (1971).

25. T. B. Reed, Intern. Sci. Technol. 1962, No. 6, 42 (1962).

26. T. B. Reed, J. Appl. Phys. 32, 821 (1961); 32, 2534 (1961); 34, 2266 (1963).

27. T. B. Reed, Proc. Nat'l. Electron. Conf. 19, 654 (1963).

28. M. Riemann, in Emissionspectroskopie, Akademie Verlag, Berlin, 1964, p. 173.

29. R. Woodriff, Appl. Spectrosc. 22, 207 (1968).

30. G. D. Christian and F. J. Feldman, Appl. Spectrosc. 25, 660 (1971).

31. J. D. Winefordner, V. Svoboda, and L. J. Cline, Crit. Rev. Anal. Chem. 1970 (August), 233-274.

32. R. N. Kniseley, C. C. Butler, and V. A. Fassel, unpublished research results, Iowa State University, 1971.

33. R. N. Kniseley, H. Amenson, C. C. Butler, and V. A. Fassel, paper submitted to Appl. Spectros., 1973.

34. S. Greenfield, I. Ll. W. Jones, C. T. Berry, and D. J. Spash, British Patent No. 1,109,602, April 10, 1968.

35. V. A. Fassel, in Proceedings XVI Colloquium Spectroscopium Internationale, Adam Hilger, London, 1972, p. 63.

36. J. W. Gofman, Advan. Biol. Med. Phys. 8, 1 (1962).

37. J. W. Gofman, O. F. de Lalla, E. L. Kovich, O. Lowe, W. Martin, D. L. Piiuso, R. K. Tandy, and F. Upham, Arch. Environ. Health 8, 105 (1964).

38. R. E. Nusbaum, E. M. Butt, T. C. Gilmour, and S. L. DiDio, Amer. J. Clin. Path. 35, 44 (1961).

39. E. M. Butt, R. E. Nusbaum, T. C. Gilmour, S. L. Didio, and S. Mariano, Arch. Environ. Health 8, 52 (1964).

40. W. Niedermeier, J. H. Griggs, and R. S. Johnson, Appl. Spectrosc. 25, 53 (1971).

41. H. M. J. Bowen, Trace Elements in Biology, Academic Press, London and New York, 1966, Chapter 5.

42. I. Tipton, in Metal Binding in Medicine, M. J. Seven and L. A. Johnson, Eds., J. B. Lippincott Co., Philadelphia, Pa., 1960, Chapter 3.

43. I. Tipton and M. J. Cook, Health Phys., 9, 103 (1963).

44. I. Tipton, M. J. Cook, R. L. Steiner, C. A. Boyce, H. M. Perry, Jr., and H. A. Schroeder, Health Phys. 9, 89 (1963).

45. D. Keilin and T. Mann, Nature 144, 442 (1939).

46. J. M. Orten, in Zinc Metabolism, A. S. Prasad, ed., Thomas and Co., Springfield, Ill., 1966, p. 38.

47. B. L. Vallee, Physiol. Rev. 39, 443 (1959).

48. E. J. Underwood, Trace Elements in Human and Animal Nutrition, 3rd ed., Academic Press, New York and London, 1971, Chapters 1 and 3.

49. J. T. McCall, N. P. Goldstein, and L. H. Smith, Fed. Proc. 30, 1011 (1971).

50. M. Hrgovcic, C. F. Tessmer, T. M. Minckler, B. Mosier, and G. H. Taylor, Cancer 21, 743 (1965).

51. C. F. Tessmer, M. Hrgovcic, and J. Wilbur, Cancer 31, 303 (1973).

52. S. H. Mortazavi, A. Bani-Hashemie, M. Mozafari, and A. Raffi, Cancer 29, 1193 (1972).

53. C. F. Tessmer, M. Hrgovcic, B. W. Brown, J. Wilbur, and F. B. Thomas, Cancer 29, 173 (1972).

54. C. F. Tessmer, M. Hrgovcic, F. B. Thomas, J. Wilbur, and D. M. Mumford, Cancer 30, 358 (1972).

55. N. W. H. Addink, Nature 186, 253 (1960).

56. N. W. H. Addink and L. J. P. Frank, Cancer 12, 544 (1959).

57. I. Vikbladh, Scand. J. Clin. Lab. Invest. 2, 143 (1950).

58. S. K. Nuryagdyev, Vop. Onkol. 17, 7 (1971); Chem. Abstr. 75, 6127j (1971).

59. S. Szmigielski and J. Litwin, Cancer 17, 1381 (1964).

60. J. M. Morgan, Cancer 25, 1394 (1970).

61. N. W. Teitz, E. F. Hirsch, and B. Neyman, J. Amer. Med. Assn. 165, 2187 (1957).

62. G. K. David, in Proceedings of the University of Missouri's 3rd Annual Conference on Trace Substances in Environmental Health, D. D. Hemphill, ed., Missouri University Press, Columbia, Mo., 1969, pp. 135-148.

63. W. J. Pories, W. H. Strain, C. G. Rob, J. Henzel, J. A. Hennessen, and F. R. Plecha, in Proceedings of the University of Missouri's 1st Annual Conference on Trace Substances in Environmental Health, D. D. Hemphill, ed., Missouri University Press, Columbia, Mo. 1967, pp. 114-133.

64. E. J. Underwood, in Trace Element Metabolism in Animals, Proceedings of WAAP/IBP International Symposium, Aberdeen, Scotland, July, 1969, C. F. Mills, ed., E. and S. Livingstone, Edinburg and London, 1970, pp. 5-21.

65. H. J. van Peenen and A. Patel, Arch. Path. 77, 53 (1964).

66. P. O. Wester, Acta Med. Scand. 178, 765 (1965).

67. P. O. Wester, Acta Med. Scand. Suppl. 439, (1965).

68. W. Förth, in Trace Element Metabolism in Animals, Proceedings of WAAP/IBP International Symposium, Aberdeen, Scotland, July, 1969, C. F. Mills, ed., E. and S. Livingstone, Edinburg and London, 1970, pp. 298-310.

69. C. T. Settlemire and G. Matrone, J. Nutr., 92, 153 (1967).

70. C. T. Settlemire and G. Matrone, J. Nutr. 92, 159 (1967).

71. Kwan Hsu, Advan. Biol. Med. Phys. 8, 41 (1962).

72. G. D. Christian and F. J. Feldman, Atomic Absorption Spectroscopy, Wiley-Interscience, New York, 1970.

73. M. D. Amos, P. A. Bennett, K. G. Brodie, P. W. Y. Lung, and J. P. Matousek, Anal. Chem. 43, 211 (1971).

74. J. Y. Hwang, P. A. Ullucci, S. B. Smith, and A. L. Malenfant, Anal. Chem. 43, 1319 (1971).

75. F. J. Feldman, E. C. Knoblock, and W. C. Purdy, Anal. Chim. Acta 38, 489 (1967).

76. M. M. Joselow and J. D. Bogden, At. Absorption Newslett. 10, 65 (1971).

77. I. W. F. Davidson and W. L. Secrest, Anal. Chem. 44, 1808 (1972).

78. A. A. Cernik and M. H. P. Sayers, Brit. J. Ind. Med. 28, 392 (1971).

79. E. Norval and L. R. P. Butler, Anal. Chim. Acta 58, 47 (1972).

80. W. F. Barthel, A. L. Smrek, G. P. Angel, J. A. Liddle, P. J. Landrigan, S. H. Gehlbach, and J. J. Chisholm, paper presented at the 164th American Chemical Society National Meeting, New York, New York, August, 1972.

81. J. Bloomfield and R. A. MacMahon, J. Clin. Path. 22, 136 (1969).

82. E. T. Backer, Clin. Chem. Acta 24, 233 (1969).

83. E. Berman, At. Absorption Newslett. 6, 57 (1967).

84. I. J. T. Davies, M. Misa, and T. L. Dormandy, J. Clin. Path. 21, 359 (1968).

85. J. B. Dawson and B. E. Walker, Clin. Chim. Acta 26, 465 (1969).

86. R. O. Farrelly and J. Pybus, J. Clin. Chem. 15, 573 (1969).

87. F. Feldman and E. C. Knoblock, Anal. Chim. Acta 38, 489 (1967).

88. B. M. Hackley, J. C. Smith, and J. A. Halsted, Clin. Chem. 14, 1 (1968).

89. T. R. Hauser, T. A. Hinners and J. L. Kent, Anal. Chem. 44, 1819 (1972).

90. S. Meret and R. I. Henkin, Clin. Chem. 17, 369 (1971).

91. D. G. Mitchell, F. J. Ryan, and K. M. Aldos, At. Absorption Newslett. 11, 120 (1972).

92. J. B. Willis, Anal. Chem. 34, 614 (1962).

93. K. Fuwa, P. Pulido, R. McKay, and B. Vallee, Anal. Chem. 34, 614 (1962).

94. S. Sprague and W. Slavin, At. Absorption Newslett. 4, 228 (1965).

95. J. B. Dawson, D. J. Ellis, and H. Newton-John, Clin. Chim. Acta 21, 33 (1968).

96. R. S. Pekerek, W. R. Beisel, P. J. Bartelloni, and K. A. Bostian, Amer. Journ. Clin. Path. 57, 506 (1972).

97. J. Pybus, Clin. Chim. Acta 23, 309 (1968).

98. N. G. Sellers, Anal. Chem. 44, 410 (1972).

99. F. W. Sunderman, Jr. and N. O. Roszel, Amer. Journ. Clin. Chem. 48, 286 (1967).

100. C. G. Thin and P. A. Thomson, J. Clin. Path. 20, 280 (1967).

101. A. Zettner, L. C. Sylvia, and L. Capacho-Delgado, J. Clin. Path. 45, 533 (1966).

102. J. P. Matousek and B. J. Stevens, Clin. Chem. 17, 363 (1971).

103. M. Glenn, J. Savory, L. Hart, T. Glenn, and J. Winefordner, Anal. Chim. Acta 57, 263 (1971).

104. H. L. Kahn, Amer. Lab. 35, (1971).

105. B. Welz and E. Wiedeking, Z. Anal. Chem. 252, 111 (1970).

106. I. Kubota, V. A. Lozar, and F. Losee, Arch. Environ. Health 16, 788 (1968).

107. H. Delves, Analyst (London) 95, 431 (1970).

108. F. J. Fernandez and H. L. Kahn, At. Absorption Newslett. 10, 1 (1971).

109. K. M. Hambridge, Anal. Chem. 43, 103 (1971).

110. R. L. Steiner and D. H. Anderson, Appl. Spectros. 26, 41 (1971).

111. R. D. Giauque, F. S. Goulding, J. M. Jaklevic, and R. H. Pehl, Anal. Chem. 45, 671 (1973).

112. A. Sonnerwirth, in Grandwohl's Clinical Laboratory Methods and Diagnosis, S. Frankel, S. Reitman and A. Sonnenwirth, eds., C. V. Mosby Co., St. Louis, 1970, p. 1478.

113. J. Ramírez-Munoz, Atomic Absorption Spectroscopy, Elsevier Publishing Co., Amsterdam, 1968.

114. H. O. Kunkel, P. B. Pearson, and B. S. Schweigert, J. Lab. Clin. Med. 32, 1027 (1947).

115. K. B. Sehra and B. Ahmad, Ann. Biochem. Exp. Med. (Calcutta) 5, 145 (1945).

116. C. Birdsong, J. Amer. Med. Tech. 1964, September-October, 5 (1964).

117. C. Th. J. Alkemade, Anal. Chem. 38, 1252 (1966).

118. D. A. Becker, Ph.D. thesis, Iowa State University (1970) (unpublished).

119. H. F. Beeghly, in Standard Methods of Chemical Analysis, Vol. IIIA, Instrumental Analysis, F. J. Welcher, Ed., D. Van Nostrand Co. Inc., New York, 1966, pp. 853-863.

120. V. A. Fassel, R. W. Slack, and R. N. Kniseley, Anal. Chem. 43, 186 (1971).

121. P. J. T. Zeegers, R. Smith, and J. D. Winefordner, Anal. Chem. 40, No. 13, 27A (1968).

122. P. Konig, K. H. Schmitz, and E. Thiemann, Z. Anal. Chem. 244, 232 (1969).

123. G. W. Dickenson, Ph.D. thesis, Iowa State University (1969) (unpublished).

124. H. A. Tucker, R. T. Coulehan, and W. G. Wilson, Department of the Interior, Bureau of Mines, Report of Investigations No. 7153 (1968) (unpublished).

125. L. C. Pasztor and C. R. Hines, Anal. Chem. 41, No. 5, 91R (1969).

126. C. R. Hines and D. R. Dulski, Anal. Chem. 43, No. 5, 100R (1971).

127. W. Koch and H. Bosch, Mikrochim. Acta 1971, 593 (1971).

128. D. R. Thomerson and W. J. Price, Analyst (London) 96, 825 (1971).

129. R. Schiller, At. Absorption Newslett. 9, 111 (1970).

130. J. B. Eskew, E. A. Jennings, and J. A. Dean, Analyt. Lett. 1, 947 (1968).

131. T. C. Rains, National Bureau of Standards, Washington, D.C., private communication, (1971).

132. B. Bernas, Anal. Chem. 40, 1682 (1968).

133. R. Herrmann, in Analytical Flame Spectroscopy, R. Mavrodineanu, Ed., Macmillan and Co. Ltd., London, 1970, p. 483.

134. J. Ramirez-Munoz, Flame Notes 3, No. 2, 17 (1968). Published by Beckman Instruments Inc., Fullerton, Calif.

VIII. ACKNOWLEDGMENTS

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IX. APPENDIX

The work described in this thesis encompasses studies on the analytical applications of inductively coupled plasma sources. The author has also participated in other investigations involving inductively coupled plasma sources and these studies are summarized in the abstracts presented in this Appendix.

1. W. B. Barnett, V. A. Fassel, and R. N. Kniseley, "Theoretical Principles of Internal Standardization in Analytical Emission Spectroscopy", Spectrochim. Acta 23B, 643 (1968).

   The principles which affect the choice of analysis and internal standard lines in analytical atomic spectroscopy are explored theoretically using a computer-based model. The spectral source chosen for the model was the induction-coupled argon plasma fed with a water solution of metallic salts. The effect of excitation energy, ionization energy, partition functions and electron density on the analytical line pair intensity ratio is explored. Examples are presented which illustrate the contribution of each variable to the intensity ratio over the 3000 to 15000 K temperature range, allowing the reader to assess the importance of each based on a knowledge of the properties of his own source. Finally, some of the problems encountered in the course of this study are discussed.

2. W. B. Barnett, V. A. Fassel, and R. N. Kniseley, "An Experimental Study of Internal Standardization in Analytical Emission Spectroscopy", Spectrochim. Acta, 25B, 139 (1970).

   Several of the important principles for choosing a good internal standard element and line were examined experimentally, utilizing the induction-soupled plasma as the spectral source. This discharge was chosen because it avoided some of the problems presented by more conventional sources and could be easily profiled spatially to study intensity ratio behavior as temperature, sample density, and electron density varied. In addition, some attention was given to sample distribution in the plasma and its temperature characteristics. Comparisons are presented which demonstrate the relationship between experimental results and those calculated using a computer model developed in an earlier paper.

3. R. H. Scott, V. A. Fassel, R. N. Kniseley, and D. E. Nixon, "Inductively Coupled Plasma-Optical Emission Analytical Spectroscopy: A Compact Facility for Trace Analysis of Solutions", Anal. Chem., Accepted for publication, 1973.

This paper describes a compact inductively coupled plasma-optical emission system for the trace determination of metallic elements in solution. Calculations to determine the operating parameters are presented. The aerosol desolvation system commonly used with this type of source has been eliminated, and pneumatic nebulization is employed in place of the more elaborate ultrasonic method. Some characteristics of the plasma are reported. Detection limits are in the range 0.1 - 10 parts per billion for most elements studied. The present facility is readily adaptable to simultaneous multielement trace analysis.

4. D. E. Nixon, V. A. Fassel, and R. N. Kniseley, "inductively Coupled Plasma-Optical Emission Analytical Spectroscopy: Tantalum Filament Vaporization of Microliter Samples", Anal. Chem., Submitted.

The adaptation of a tantalum filament vaporization system as a sample introduction device for the inductively coupled plasma is described. The potential advantages of this analytical system for simultaneous multielement determinations of elements at the ng/ml level are discussed and a comparison of the plasma system with the filament techniques utilized in flame atomic absorption or fluorescence spectroscopy is presented. One set of operating conditions is sufficient to obtain detection limits for many elements at the ng/ml level for microliter sized samples. Typical precision data and an analytical curve for the determination of Be in the range of 0.001 to 10 µg/ml are included.

5. R. N. Kniseley, V. A. Fassel, and C. C. Butler, "Application of Inductively Coupled Plasma Excitation Sources to the Simultaneous Multielement Determination of Trace Metals in Microliter Sample Volumes of Biological Fluids", Clin. Chem., Accepted for publication, 1973.

It is becoming increasingly important in health-care programs to have a knowledge of the concentrations of biologically essential elements in normal subjects and to be able to measure accurately the smallest deviation which is significant with respect to diseases. Experiments in our laboratory have shown that the inductively coupled plasma is

an excellent excitation source for the simultaneous multi-element determination of trace elements in biological fluids at ng/ml concentration levels. Sample volumes as low as 25 µl of fluid have been used for the analysis of whole blood, serum and plasma with essentially no sample preparation. This technique offers significant advantages over other methods requiring considerable sample handling that may greatly increase the danger of contamination or loss of trace constituents. Since this is an emission spectroscopic technique, a multichannel direct-reading spectrometer provides simultaneous multielement analysis capabilities on microliter size samples.