Emission Spectrochemistry in Nutrition Research

The potential utility of spectrochemistry in mineral nutrition research is not yet fully exploited.

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"As well as can be judged from the literature, biological spectroscopy has not been troubled by too much imagination. In fact, one can briefly describe the cookbook recipe of experimental design: take a certain amount of urine, blood, or tissue of some animal or man. Destroy the organic parts with perchloric acid, fire, or sword until they have returned to ashes. Introduce the ash into a flame, an arc, or a spark, and photograph the spectrum. If unexpected metals are found, the literature has been enriched by two papers: the first one reporting the presence of an unusual metal, perhaps a rare earth; the second paper, a letter to the editor, contending that this new and startling finding represented contamination" (*1*).

Unfortunately the foregoing quotation seems to apply to a large amount of the mineral-nutrition research done in the past. This situation arose partly because much of the work was done by spectrochemists, whose primary purpose was to demonstrate the feasibility of applying spectrochemistry in this area of research. That this goal was achieved is evidenced by the large number of nutritionists presently interested in the technique as a means of obtaining useful data on mineral nutrients in various systems. The existing shortage of personnel thoroughly trained in spectrochemistry makes virtually inevitable a rapid increase in the number of people who will become "part-time spectrochemists" while remaining primarily interested in the elucidation of the fundamentals of mineral nutrition. It is important, therefore, that these people be thoroughly familiar with the basic concepts of spectrochemistry.

Emission spectrochemistry embraces all analytical methods based on the phenomenon that energized atoms, ions, and molecules emit electromagnetic radiation when they lose energy. In this article (*2*), attention is focused on optical emission spectrochemistry, the greatest emphasis being placed on the vaporization and excitation steps. In addition, I shall seek to clarify some misconceptions about the capabilities that this technique possesses, or that it may be expected to possess with further improvements in methodology. Some specific examples of the use of the technique in nutrition research are included.

Basic Principles

In 1944, Churchill (*3*) voiced the opinion that, "because of the almost unlimited number of combinations and permutations of electrical, optical, chemical, and physical variables possible in a spectrochemical analysis, and because of the interdependence of these variables on each other, there is no optimum value for any one of the variables except in relation to all of the others." Let us, then, first examine the over-all problem.

In qualitative analysis, the spectral lines emitted by an excited sample are generally photographed on a film or plate. When only a few specific elements are sought, their lines are usually identified by comparison with spectra of the pure elements, photographed in juxtaposition. In other instances it is often necessary to determine the wavelengths of lines by measuring precisely the distances that separate them from lines of known wavelength.

Quantitative analysis, on the other hand, is based on the fact that the intensity of a spectral line of an element is a function of the amount of that element in the source. To obtain a relative measure of intensity, photographic densities can be measured with a densitometer or microphotometer, these values being converted to relative intensities by means of an emulsion calibration curve relating these two variables. Strock (*4*) indicated some of the difficulties in this procedure and emphasized that photographic intensities are only relative measures of light-source intensities. Recently, direct-reading instruments, in which light intensities are recorded photoelectrically, have become very popular. Although these instruments are more expensive and less versatile than photographic ones, they eliminate many errors inherent in photographic procedures, thereby providing excellent precision for routine high-speed analyses. Apparently, then, quantitative analysis should involve only the construction of an analytical calibration curve relating the intensity of a line to the known amount of the element responsible for that line in a series of standards. This approach was employed with some success by Slavin (*5*) in his "total energy" method, but it has not gained general acceptance because of the multitude of factors that affect the total amount of light emitted by a given weight of an element. The interested reader can find numerous references to these factors in any one of several books—for example, Harvey (*6*).

Many of the difficulties of the "total energy" method can be overcome by using the principle of internal standardization, first introduced by Gerlach (*7*) in 1925. In this procedure, concentration of the element to be determined is measured in terms of the ratio of the intensity of the analysis line to the intensity of a "homologous" line of another element present in fixed concentration in all samples and standards. Uncontrollable fluctuations that affect the intensities of both lines to the same extent should not affect the intensity ratio between them. Unfortunately, complete success has never been attained in efforts to find line pairs whose intensity ratios are insensitive to changes in chemical and physical composition. Despite some limitations, the internal-standard principle placed quantitative analysis by optical emission spectrochemistry on a firm foundation.

There are four main steps involved in the technique: (i) vaporization and

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excitation, (ii) resolution of emitted radiation into its constituent wavelengths, (iii) recording of spectral lines, and (iv) interpretation. Each of these steps is considered separately below.

Vaporization and Excitation

Vaporization is generally accomplished by thermal means, as with flames and arcs, or by bombardment with positive ions and high-velocity electrons, as exemplified by the high-voltage spark source. But this process is never entirely thermal or entirely one of bombardment, and there is no sharp separation between arc and spark sources. Considerable effort has been expended in the development of "hybrid" sources that combine the more desirable features of both arc and spark (*8, 9*). Only three basic excitation sources will be considered here: flames, arcs, and high-voltage sparks.

Optical emission spectrochemistry is a technique for analyzing light emitted by atoms, by ions, and by some molecules that have been sufficiently excited to cause valence electrons to move to higher energy levels than they occupy in the stable state. The radiation emitted when an electron returns to a lower energy level appears as light of one or more discrete wavelengths that are characteristic of the atom of the element producing them. Each element is characterized by as many different spectra as the atom has electrons. Thus, the lines originating from electron transitions in the neutral atom are often called arc lines, whereas those from the singly ionized atom are called the first-spark spectra. Greater degrees of ionization do occur to a limited extent in conventional spectroscopic sources, but the lines originating from these ions are only occasionally of analytical importance.

Excitation of atoms in a discharge can be accomplished by (i) transfer of energy through collisions with atoms and ions that are already excited (collisions of the second kind); (ii) inelastic collisions with high-velocity electrons; and (iii) absorption of radiation (*10*). In addition, atomic spectra may be produced when the bonds in a molecule are ruptured (*11*). The process involves a change in an electron from a molecular orbit to an excited atomic orbit and, finally, a return to the ground state which produces the characteristic spectral line or lines. The relative activity of each of these processes depends on a multitude of factors and varies greatly in different types of sources.

All the elements can be excited, but gases and bromine and iodine are only infrequently determined in this way because they must be excited in sealed systems. These elements can sometimes be determined with conventional apparatus by measuring the band spectra of a compound such as calcium fluoride, but this approach has never been generally used. Carbon, phosphorus, and sulfur, whose sensitive lines lie below 2000 A, have been studied intensively only since the advent of vacuum spectrographs, during the last few years. Most elements that are readily studied by optical emission spectrochemistry produce useful lines (including their most sensitive ones) between 2000 and 10,000 A.

The energy required to excite the arc lines of most elements commonly studied by this technique ranges between 1 and 10 electron volts. To excite spark lines, the energy must be greater than the ionization potentials of the neutral atoms, which range from 3.89 electron volts for cesium to 24.48 electron volts for helium. Meggers (*12*) pointed out, however, that none of the elements commonly determined have ionization potentials greater than about 10 electron volts. Because of this relatively low energy requirement, several different sources of excitation have been used successfully; the most popular are flames, arcs, and sparks.

In flame sources the vaporization step is largely thermal. The minute droplets of solution dry to solid particles that vaporize and dissociate as gaseous atoms or molecules. These are excited by inelastic collisions with high-velocity molecules liberated by chemical reaction between the fuel gases. Commonly used gas mixtures yield temperatures ranging between 2000 and 4800°K, high enough to vaporize most materials. The relative proportions of the various excited species created depend to a great extent on the temperature of the flame. In general, low-temperature flames only excite lines whose excitation potential is low; for example, the oxyacetylene flame is not useful for exciting lines of excitation potential greater than about 5.5 electron volts. It is not difficult to understand why low-temperature flames are not very useful for exciting elements whose strongest lines have large excitation potentials. Until recently, therefore, the flame source was used mainly for the determination of alkalies and, to a more limited extent, of alkaline earths. The advantages of the flame are primarily the simplicity of the spectra and the stability of emission, which makes for high precision.

Recently there has been a pronounced renewal of interest in the flame source as a means of exciting refractory elements. One reason for this is the development of sources of much higher temperature, such as the oxycyanogen flame described by Baker and Vallee (*13*) and by Vallee and Bartholomay (*14*). This source is capable of providing satisfactory excitation of such elements as beryllium and molybdenum. Robinson (*11*) postulated that enhancement in such sources is not due entirely to temperature increase but is due in part to the ultraviolet light that is radiated by the reacting gases and absorbed by the elements, thereby causing excitation.

Perhaps of much greater importance was the discovery that organic solvents increase the emission intensity of many elements by a factor of 10 or more in such conventional flames as oxyacetylene. Gilbert (*15*) reported that line intensities of several elements were enhanced by a factor of approximately 1000 when alcohol was used in an air-hydrogen flame [a good review is given by Dean (*16*)]. The mode of enhancement by organic solvents is not completely understood. Reduction in surface tension gives rise in the mist to droplets of a smaller mean diameter and a greater emission intensity. Reduction in viscosity also tends to produce enhancement. To some extent, increase in temperature in the presence of organic solvents may be a factor, although Gibson and Cooke (*17*) indicated that organic solvents can actually lower the flame temperature. Robinson (*11*) suggested that efficiency in producing free atoms to be excited may increase in the presence of organic solvents, because the dissociation process is exothermic, rather than endothermic as in the case of aqueous systems.

Despite the pronounced increase in sensitivity that resulted from these studies, the flame source still cannot be considered ready for application to nutrition studies involving trace elements other than the alkalies, whereas optical emission spectrochemistry is most useful in the field of trace analysis. Nevertheless, flame sources have been and will continue to be used to good advantage in the determination of alkalies and alkaline earths in a great variety of materials involved in nutrition re-

search. It is not unreasonable to expect that, with increasing knowledge of the fundamentals of excitation in these sources, they may eventually become useful for trace determinations of many metals.

Traditionally, the direct-current arc has been the source chosen for most trace-element analyses of such nonconducting samples as the inorganic ashes of biological materials. It was the simplest and most versatile source available and provided maximum sensitivity in most cases, primarily because of the large quantity of sample consumed. This source was frequently criticized for its supposed inability to provide the precision required for quantitative work. But relative precision values in the range of ±5 to 15 percent are quite adequate for procedures used in the study of trace elements in biological systems. Methods in which the direct-current arc is employed are capable of this degree of precision when sufficient attention is given to such important aspects as packing of samples in electrodes and selection of analytical and internal standard lines. This is not to imply that we are satisfied with what we have, but only that we should not try to excuse very poor precision on the grounds that this is the best that can be done with this source of excitation. Failure to obtain adequate precision can generally be traced to faulty technique or methodology. But an operator often requires appreciably more training and experience to get precise results with a direct-current arc than with flame or spark sources, and this can be a serious limitation.

For direct-current arc excitation, the sample is generally placed in a crater in one of a pair of conducting electrodes (Fig. 1, *A* and *B*), with a low-voltage arc bridging the analytical gap between the electrodes. Graphite or carbon is the common electrode material, although such metals as copper and silver are used in a few instances. Temperatures produced in the gaseous arc column range between 4000° and 8000°K, depending on the conductivity, which in turn is a function of sample and electrode composition. The intensity of a spectral line depends on the number of atoms that occupy a given energy level and the efficiency with which they are excited. Since both these factors are greatly influenced by temperature, it is not surprising that much effort has been devoted to the measurement of arc temperature as a function of sample-matrix composition. Unfortunately the temperature in an arc column is not homogeneous, not even approximately so. Consequently, theoretical explanations for experimentally observed phenomena developed slowly, but the many papers that have been published on arc-temperature measurements provide a partial understanding of excitation phenomena. The works of Addink (*18*), Duffendack and LaRue (*19*), Leuchs (*20*) are typical of these.

Arc temperature is not the only important variable in direct-current discharges, nor is it the only one not completely understood. Mitteldorf (*21*) pointed out that basic information on functional relationships between such factors as arc current and sensitivity is relatively limited. He also suggested that further study be made of the results of making the sample-bearing electrode, in turn, the anode and the cathode. In most procedures the sample-bearing electrode is made the anode because the anode is much hotter than the cathode and a large amount of sample is thus rapidly volatilized. On this basis one might expect greater sensitivity with anode than with cathode excitation. The difficulty here is that the extremely hot anode radiates a large amount of continuous light that produces a heavy background, and sensitivity is determined by line-to-background ratio rather than by absolute line intensity. Furthermore, Mannkopff and Peters (*22*) showed that most elements emit most strongly in the immediate vicinity of the cathode. Strock (*23*) termed this phenomenon the "cathode layer effect." He found that sensitivity increased by a factor of as much as 100 for some elements in certain matrices if only the light from a 1- or 2-millimeter area directly adjacent to the cathode tip was admitted to the spectrograph. Enhancement was most pronounced for elements of low ionization potential present in trace amounts in small samples (of the order of 1 to 5 mg). According to Mitchell (*24*), who made extensive use of this technique in agricultural analyses, the presence of large amounts of the elements to be determined results in increased emission from the arc column, and the difference between it and the cathode layer decreases.

Mitteldorf (*21*) questioned the necessity of restricting sample size in view of the success achieved in the analysis of high-purity graphite and carbon electrodes by this method, because in such analyses the sample size is relatively unlimited. It might be rewarding to study the "cathode layer effect" for volatile trace elements in large samples (20 to 50 mg) where the volatilization of the matrix could be suppressed by some means. By preventing flooding of the discharge with the major components of the sample, the total number of atoms and ions in the discharge would remain small, since only the volatile elements would be present, and the "cathode layer effect" should therefore manifest itself. One possible method of achieving this result with certain

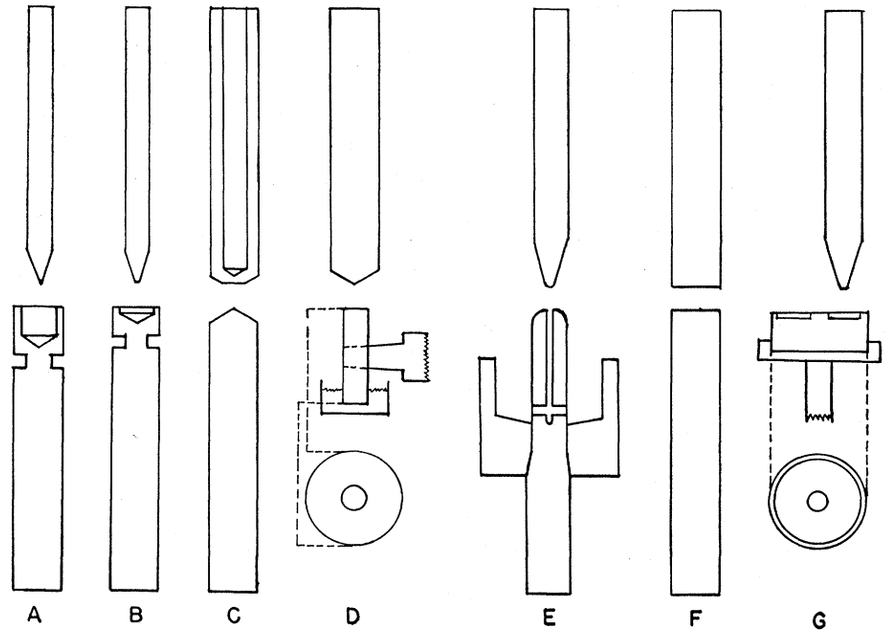


Fig. 1. Cross-sectional diagrams of a few common electrode combinations.

matrices might be to use argon or some other inert atmosphere around the discharge. Vallee and his associates (*25*) and Rupp, Klecak, and Morrison (*26*) reported large gains in sensitivity for volatile elements when inert atmospheres were used. Of the several factors operating in combination, prolongation of volatilization of volatile elements and suppression of matrix volatilization are probably the most important. Since light from the cathode tip was not isolated, the reduction in background as a result of the cooler anode very probably helped. Still another advantage of a gas such as argon is the elimination of troublesome cyanogen-band and metallic-oxide spectra. As a result of such elimination, several very sensitive lines that are normally useless because of the interfering band spectra become available for purposes of analysis.

As for atmospheres around the arc, Stallwood (*27*) demonstrated a marked increase in precision that resulted from blowing a curtain of air upward around the sample as it burned. Substantial reduction of matrix effects was also obtained. Many investigators now combine use of the jet and of inert gases quite advantageously.

A wide variety of electrode shapes have been devised to provide certain desirable properties for various types of sample matrices. Many of these are discussed by Mitteldorf (*28*). Recently much controversy has arisen over the choice between carbon and graphite electrodes. Graphite has been used predominantly in the United States whereas carbon is favored in other countries. The chief advantage of carbon is that it attains higher temperatures than graphite because its crystal structure makes it a poorer conductor of both heat and electricity. The main disadvantage of carbon is that it is difficult to machine because of hardness and brittleness. Spindler (*29*) reviewed recent comparisons of the two materials and reported his own observations. One of these was that although use of carbon craters speeded up vaporization of the sample, as expected, mixing of carbon powder with the sample materially slowed the vaporization process.

The problem of fractional distillation should be discussed more specifically. With the direct-current arc, thermal energy is used to vaporize a bulk sample from a crater, enabling low-boiling components to distill off first. The situation is complicated, however, by chemical reactions. For example, a normally volatile compound may react with the electrode material to form a refractory carbide. Usually, fractional distillation need not be too serious a problem, and it can often be used advantageously, as suggested earlier. Many of the difficulties relative to precision can be eliminated when data on the vaporization behavior of the material being analyzed are at hand (Fig. 2). In view of the ease with which such data can be obtained through jumping-plate studies, failure to obtain such information is inexcusable. Where fractional distillation affords no advantages, it can often be minimized by one of several methods, such as the use of the Stallwood jet. A concise introduction to fractional distillation is given by Ahrens (*30*).

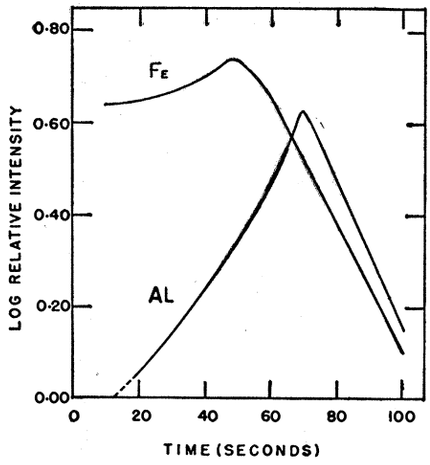


Fig. 2. Vaporization behavior of iron and aluminum from glass sand in a 10-ampere direct-current arc. Note that most of the iron but only a small portion of the aluminum was volatilized during the first 50 seconds. [J. F. Gamble, Rutgers University]

In contrast to the general use of the direct-current arc in agriculture and biology, the high-voltage alternating-current spark is preferred in the field of metallurgy. Four major generalizations account for this: (i) the spark provides better precision than the arc; (ii) metal samples, which are most easily analyzed as self-electrodes, are not melted by the spark; (iii) the sensitivity of the spark, although generally less than that of the arc because of the small amount of sample vaporized, is sufficient for determining most alloying constituents in metals; and (iv) matrix effects are minimized with the spark, although it must not be assumed that they are nonexistent. Recent research on the application of spark excitation to analysis of solutions resulted in significant increases in sensitivity, and this approach now seems very promising as a means of analyzing a great variety of samples. Samples from the area of nutrition research definitely fall within the scope of this technique.

Solution methods can be classified in two general categories: true solution and solution residue. The porous-cup technique developed by Feldman (*31*) is probably the foremost of the true-solution methods. In this technique, a hollow upper electrode with a thin porous bottom is used; the solution slowly percolates through the bottom (Fig. 1*C*). A very informative article on the excitation processes with this type of electrode was published by Feldman and Wittels (*32*). Mitchell and Scott (*33*) found this method very useful for the analysis of soil extracts, and Vallee (*1*) employed it for the analysis of biological tissue. Its major disadvantages are its failure to function well with solutions of high salt concentration and the variable porosity of its electrodes.

Another versatile true-solution method involves the use of a rotating graphite disk that dips into the solution and transports fresh sample on its periphery into the spark-excitation zone (Fig. 1*D*). This method was popularized by Applied Research Laboratories, Glendale, California (*34*). Of particular interest to the nutritionist is the application of this technique by Paolini and Kennedy (*35*), who determined five elements in food products directly, without ashing. An interesting study of the effects of basic variables on excitation was reported by Waggoner (*36*).

Flickinger, Polley, and Galletta (*37*) described a method in which the solution is held in a polyethylene vial cap fitted tightly around a center-post electrode. The solution feeds through a small hole in the outer electrode wall and bathes the center post, which conducts it to the analytical gap by capillary action. A modification of this technique, in which the electrode has a small hole drilled in the top for conduction of the solution, was recently described by Zink (*38*) (Fig. 1*E*). Though very promising, this system has not been adequately evaluated as yet. There are several other true-solution methods of less interest, which I shall not consider here.

In all probability the best-known solution-residue method in which spark excitation is employed is the copper-spark technique of Fred, Nachtrieb, and Tomkins (*39*). Hydrochloric acid solutions of the samples are dried on the ends of high-purity copper rods and excited. The sensitivity obtained, which is comparable to that obtained with the

direct-current arc, was attributed to the ease with which the residue can be excited, due to the fact that it does not penetrate into the copper. There is one major limitation: solvents that react with copper cannot be used. In an attempt to circumvent this limitation Pickett and Hankins (*40*) used graphite electrodes treated with paraffin dissolved in toluene (Fig. 1*F*), but this coating was not impervious to the perchloric acid solutions they wished to use. Morris and Pink (*41*) obtained sensitivities in the low millimicrogram region for several elements evaporated from aqueous solutions containing little or no acid by treating graphite electrodes with Apiezon N grease dissolved in ether. I have since shown (*42*) that good precision and accuracy can be obtained with several different acid solutions, but only when penetration into the electrode is completely eliminated. (Plicene and polyethylene were satisfactory as "acid proofing agents" in most cases.) As a result, the scope of this procedure has been considerably enlarged. Similar considerations apply to the rotating "platrode" developed by Rozsa and Zeeb (*43*), wherein a 0.25-inch graphite disk is substituted as the bottom electrode so that solution volumes up to 0.5 milliliter can be evaporated (Fig. 1*G*). An excellent comparative study of both solution-residue and true-solution methods was published by Baer and Hodge (*44*).

Another approach, somewhat analogous to residue methods, is to mix sample ash with briquetting graphite and mold a pellet under high pressure. Such a pellet becomes an electrical conductor that can be affixed to a supporting electrode. Muntz and Melsted (*45*) used this approach for unashed plant material, but the precision attained was less than is usually required.

Although true-solution methods are often more convenient, the laboratory of the department of soils at Rutgers has been primarily concerned with residue methods. W. J. Hanna of that department suggested that it would be of considerable value to be able to analyze plant tissue and related material containing low levels of radioactive tracers. Analytical data depicting the over-all mineral-nutrition status of a plant should materially aid in the interpretation of tracer behavior. Of the several methods discussed, the solution-residue method, with 0.25-inch electrodes, is best adapted to this problem because of its small heat production, its cleanliness and simplicity, and its utilization of samples of minimum size. Such a discharge can readily be enclosed, and the small amount of gaseous material produced can be scrubbed and monitored for radioactivity.

Jarrell (*8*) presented some simplified circuit diagrams of instruments that have been used to generate spark discharges and discussed the principles involved. Of special interest is the electronically controlled spark, by which Bardocz (*46*) was able to greatly reduce continuous background and air lines by preventing radiation from the first 10 microseconds of each gap breakdown from entering the spectrograph. In combination with those solution techniques that can be used over long exposure times, a sharp reduction in background might possibly result in appreciable increases in sensitivity.

Vaporization and excitation processes in spark discharges are extremely complex and are not completely understood. Feldman (*47*) described the mechanism of the spark as the ejection of material from the sample into the discharge, "shot-gun style." Braudo, Craggs, and Williams (*48*) and Feldman and Wittels (*32*), among others, reported on temperature studies, but the nature of the discharge was a complicating factor. Perhaps the most comprehensive report on the fundamentals of excitation in the spark was that of Mandelstam (*49*). Further breakthroughs may result when more work has been done on time-resolved spectra with the Bardocz or similar sources.

Resolution of Emitted Radiation

Many successful arrangements with prisms or gratings have been developed for resolving emitted light into its component wavelengths. This problem has too many aspects to be thoroughly discussed here, and it would be presumptuous of me to consider any one system to the exclusion of others. I shall present only a few points of special interest in biological analysis [for a description of fundamentals, see Sawyer (*50*)].

One of the most important requirements of a spectrograph is adequate dispersion, usually expressed as the reciprocal linear dispersion in angstroms per millimeter, on the photographic plate. This expression is unfortunate in that higher values represent less dispersion—that is, more crowding of lines. According to Mitchell (*24*), "It is in general not the elements to be determined, but the source to be used and the composition of the material to be examined, in so far as its major constituents are concerned, which decide the instrument to be used." As the number of lines produced by the major components increases, the reciprocal linear dispersion must decrease, so that lines from the matrix material will not interfere with analytically important lines. Since biological matrices generally give rise to spectra with only a moderate number of lines, most spectrographs have sufficient dispersion.

Sensitivity is directly related to dispersion. Line-to-background ratio is most often the factor limiting sensitivity. This ratio increases as the reciprocal linear dispersion decreases, because the same amount of background is spread over a larger area while the line remains unaffected. Jarrell (*50*) emphasized that this increase in sensitivity occurs only up to the critical dispersion at which the slit and line widths are equal. Further reduction in the reciprocal linear dispersion actually decreases line-to-background ratio. Figures 3 and 4 illustrate these relationships.

A second factor of particular interest is speed, which we can approximately define as light yield. Mitteldorf (*52*) pointed out that speed is the most important factor in the analysis of micro samples, whereas in the determination of trace elements in large samples, line-to-background ratio is the limiting factor. Speed is also limiting for very volatile elements. The relative speed of spectrographs is normally inversely proportional to the squares of their effective *f*-numbers. Unfortunately, low *f*-number and low reciprocal linear dispersion are mutually exclusive, since the small spectrograph required for low *f*-number results in a large reciprocal linear dispersion. Nevertheless, instruments of low *f*-number should be increasingly useful, particularly in combination with such external devices as inert atmospheres that lower the background, for determining such volatile elements as arsenic, mercury, and selenium. The use of gratings blazed for a specific wavelength can also increase speed appreciably.

Recording Spectral Lines

Of the problems attendant on the use of photographic emulsions, photographic speed merits special mention. Eastman 103-0 plates are very high

speed plates and are especially useful in those cases where background can be minimized. With large samples it is often better to use a plate of moderate speed, such as Spectrum Analysis No. 1, to take advantage of the fine grain and high contrast, which contribute to good precision.

Direct photoelectric recording of spectral intensities is much faster and considerably more precise than photographic recording. But direct readers are very expensive and lack the flexibility often required of a research instrument. Despite this, in the last 5 years several direct readers have been installed in laboratories responsible for the analysis of large numbers of biological and agricultural samples. Instruments that are interconvertible as either direct readers or photographic instruments are available and would seem to afford the best solution to the problem of analyzing both large numbers of routine, and lesser numbers of nonroutine, samples.

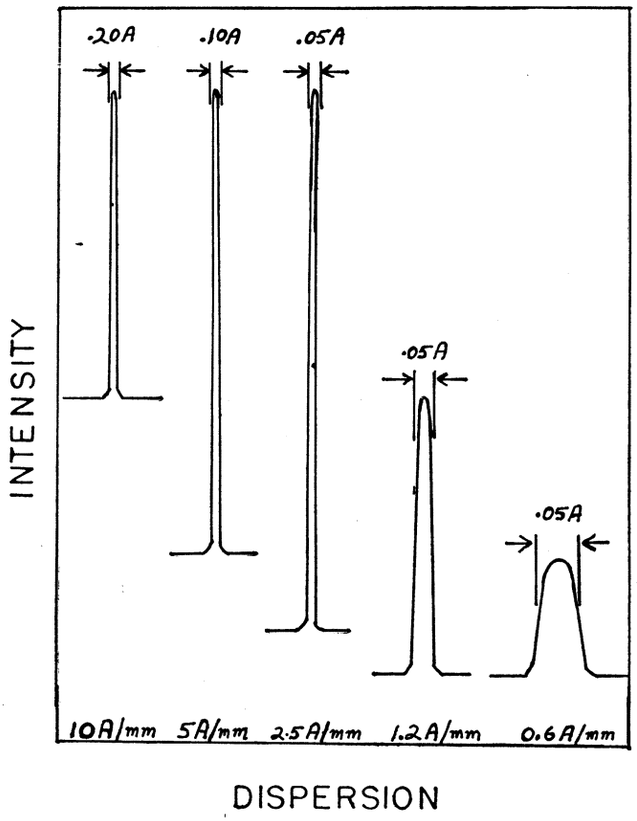
Interpretation

Quantitative optical emission spectrochemistry depends on the conversion of measured intensities or intensity ratios to concentrations by means of a calibration curve established from several standard samples. The lack of primary standards for biological and agricultural materials is, therefore, a problem of utmost concern. X-ray fluorescence, colorimetry, and many other techniques are plagued by this same difficulty. The use of secondary standards that have been analyzed by other methods presents many problems. If samples are analyzed by only one laboratory, the likelihood of serious bias exists, and when several laboratories participate in a standardization program, there is seldom good agreement in analytical data, particularly for trace elements. The heterogeneity of this type of sample material undoubtedly contributes heavily to this variability. As a result, most optical emission spectrochemistry laboratories synthesize their own standards from high-purity chemicals. In this connection, solution methods have the advantage of homogeneity, a property that is difficult to achieve with powder standards, which are likely to fractionate on standing because of differences in particle size and density and because of electrostatic effects.

If availability of satisfactory standards, is assumed, there still remains the necessity to maintain close control over calibration curves, which are subject to shifts caused by environmental and other factors. It is common practice in many laboratories to expose one or more standards along with samples on the same photographic plates. Original calibration curves are then shifted to fit these new but very limited numbers of points. I have for some time been employing a statistical-control approach that has distinct advantages over other methods.

Another important aspect of calibration is critical matching of analysis and internal standard-element lines. If possible, all lines selected should be free from self-reversal. In most cases, they must be free of interference by lines of other elements, although satisfactory corrections can occasionally be applied. Matched lines should (i) be similar in volatilization behavior, (ii) be as close as possible in excitation potential, (iii) be from elements of similar ionization potential, and (iv) be reasonably close in wavelength. Some compromises are necessary in general-purpose methods for analyzing several elements, but insofar as possible these criteria should be met.

One further problem in the interpretation of trace-element analyses is contamination from reagents, electrodes, air, and miscellaneous sources. Contamination can never be completely eliminated, but complete elimination is



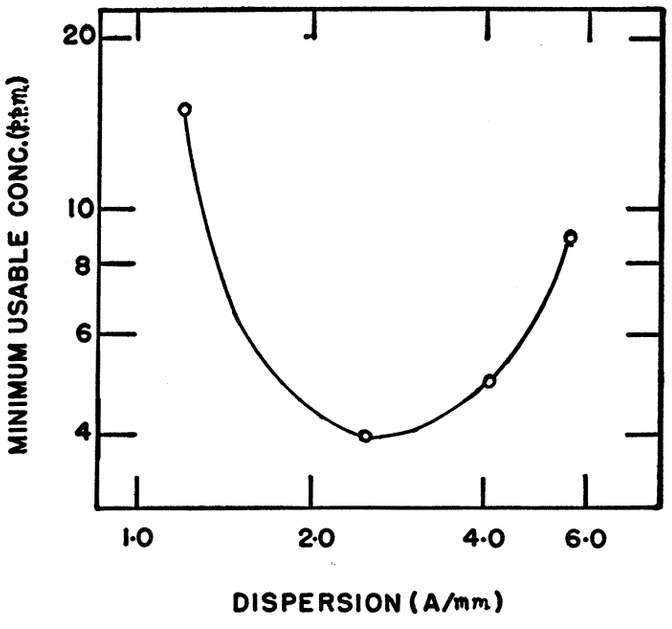


Fig. 3 (left). Variations of spectral line and background intensities with dispersion, for a slit width of 20 microns. Fig. 4 (above). Sensitivity-dispersion relationships for the determination of cadmium in zinc. [Both figures courtesy Jarrell-Ash Co., Newtonville, Mass.]

the goal the investigator should strive for. For this purpose, direct procedures, involving a minimum of reagents and the shortest possible time lapse from start to finish, are most favorable. Contamination problems start at the moment of sample collection. Too frequently the information sought from a sample has been invalidated before the sample ever reaches the laboratory. Disposable polyethylene gloves and containers are quite helpful in this regard.

Application in Nutrition Research

Both plants and animals require many different nutrient elements, a number of them in trace amounts. Since the concentration of each of these elements is in part dependent on the levels of one or several of the others, thorough diagnosis of nutritional status cannot be made on the basis of information on three or four elements. The value of an analytical scheme providing data on many elements in a small sample becomes obvious.

In the area of plant nutrition, many data have been accumulated on trace-element concentrations in various plants grown on a variety of soils. These data are far too extensive for detailed discussion here, but they have afforded a basis for detecting deficiencies or toxicities, or both, of certain elements in some plants, and they often make it possible to demonstrate lack of balance between elements. Much of this work has been concerned with forage crops, because the requirements of many animals for such elements as cobalt are fairly well established. Nutrient-element composition of fruit-tree leaves has been studied with optical emission spectrochemistry by Kenworthy (*53*) and others in an attempt to establish reliable concentration ranges for many elements in trees not showing evidence of a deficiency or an excess. Influences of climate, age of plant, season, location, and many other factors greatly complicate this situation. Bradford and Harding (*54*) reported variations of as much as 50-fold in the minor-element contents of orange-tree leaves sampled from high-yielding orchards. They suggest that sampling be carried out annually in order that any long-term trends may be detected, and this may be the only workable solution to this problem. At present, optical emission spectrochemistry is the method best adapted to the handling of such a program.

So far, little progress has been made toward evolving analytical procedures for estimating the supply of minor elements on the basis of amounts of "available" nutrients in the soil. The work of Mitchell and Scott (*24, 33*) and that of Pickett and Dinius (*55*) give an idea of the degree of success achieved. Despite the slow rate of progress, expansion of this work is justifiable in view of the need for better control of crop production to optimize economic return. It is conceivable that soils not now deficient in trace elements may become so in the near future. To carry out preventive fertilization, deficiencies of these elements must be detected during the incipient stages. For success in this endeavor, periodic analyses of many samples for many elements must be made.

The mineral-nutrient content of foods consumed by human beings is also of interest. Hopkins and Eisen (*56*) have demonstrated the feasibility of applying optical emission spectrochemistry to the analysis of fresh vegetables from urban markets. Such information may eventually be related to the geographical distribution of conditions of malnutrition and to distribution of certain diseases, although the varied diet of human beings probably makes such a connection less likely in man than in domestic animals. These studies also provide an index of contamination by spray residues containing copper, zinc, arsenic, or other elements.

Metal-binding in medicine is being studied quite intensively (*57*), and optical emission spectrochemistry is being applied to a considerable extent in this research. Patterns of excretion of trace metals and the change in these patterns upon infusion of chelating agents has been investigated. The work of Perry and Perry (*58*) with ethylenediaminetetra-acetic acid is an example of a study in which optical emission spectrochemistry was advantageously employed.

Vallee (*1*) has investigated subcellular fractions of animal organs by this method, in studies wherein ability to obtain a maximum amount of information from a sample of minimum size was essential. Far more insight into basic biological functions was obtained from a study of individual fractions than would have resulted from analyses of the total material. As biochemical separations are further refined, ever-smaller samples will be available for analysis. Optical emission spectrochemistry should make a major contribution to the furtherance of this research.

In conclusion, it appears that intelligent and imaginative application of this technique to nutrition research can be most rewarding, although other techniques offer greater advantages in the study of some problems. But any laboratory performing multiple trace-element determinations will find that a good spectrograph is a tool of extreme versatility and capability when manned by a competent spectrochemist.

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INSTRUMENTS AND TECHNIQUES

New Method for Heart Studies

Continuous electrocardiography of active subjects over long periods is now practical.

Norman J. Holter

Electrocardiography today is an indispensable tool for physiologist and physician. Cardiac electrophysiology began in 1887 when Ludwig and Waller first noted changing chest potentials, and practical electrocardiography began in 1893 with Einthoven's string galvanometer work. Then followed the body of classic work in this field, but the electrocardiograph did not find wide use until the advent of modern direct-writing instruments. Today's clinical instrument is convenient and dependable and will remain an important tool in research and in examinations of established heart conditions. It is still only a hit-or-miss affair for studying long-period heart action or detecting transient heart aberrations.

Until recently, electrocardiography required connecting leads from subject to instrument. This was no handicap in building present-day principles but has been a handicap in studying active subjects. Leads can be detached during exercise and reconnected later, and with special electrodes some exercise is feasible during recording. However, considerably more physical freedom is desirable if one is to learn more about the heart under realistic conditions of daily life.

This article reports a series of concepts and developments concerned with obtaining long-period continuous electrocardiographic records from active subjects in order to obtain data which constitute a statistically valid sample of heart action under conditions that give the subject the greatest possible freedom of activity. This goal automatically generates the problem of handling, in a convenient and practical way, the very voluminous data acquired. No one can adequately examine 100,000 continuous ordinary electrocardiograms (24-hour recording at a pulse rate of 70). A number of early ideas have led to the concept of breaking away from the limitations of orthodox electrocardiography to solve the scientific problem of adequate sampling and the medical problem of obtaining electrocardiograms in situations other than the highly artificial and unrealistic situation of resting quietly on a comfortable pad after a good sleep, with no breakfast, and with calm confidence in one's physician.

In 1939 J. A. Gengerelli and I became interested in remote stimulation of physiological systems as means for minimizing interference with the system. By modifying a classic experiment, we produced contractions of frog muscle by stimulating its nerve supply by means of a changing electric field without electrodes or connecting wires (*1*). This raised the converse question of whether an external field is created by a nerve impulse. From these two basic and converse ideas developed a series of studies leading, on the one hand, to the remote stimulation of the brain of the intact animal and a study of corresponding behavior (*2, 3*) and, on the other hand, to the use of radio for the accurate transmission of electroencephalograms and electrocardiograms from freely exercising subjects (*4, 5*). With the electronics of 1942, a nerve impulse field was not detected (*2*), but recently we obtained evidence for the existence of such a field (*6*). Radioelectrocardiography as a practical and convenient technique is now becoming relatively routine; its first clinical application was by MacInnis in 1954 (*7*).

Steps toward Freedom

Up to this point there has been developed only what I would call an initial step toward freedom—the elimination of entangling wires. Moreover, while telemetering per se does provide greater freedom of action, it does not provide practical long-period continuous electrocardiography. It also requires an in-

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