ANALYTICAL CONSIDERATION AND METHODOLOGIES FOR ELEMENTAL DETERMINATIONS IN BIOLOGICAL SAMPLES

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ABSTRACT

Available analytic methods for elemental determinations of biological samples are reviewed and discussed. Preparation of samples (including wet digestions and dry ashing techniques), analytical standards, and analytical blanks are considered. Current practices of elemental marking techniques and of the use of inherent elemental composition as a marker in itself are presented. Specifically, atomic absorption spectroscopy, inductively-coupled plasma atomic emission, X-ray fluorescent spectroscopy, neutron-activation analysis, laser-enhanced flame ionization, and mass spectrometry are considered in respect to elemental analysis of plant and arthropod samples. Emphasis is placed on atomic absorption and prerequisite sample preparation because of the practicality of the technique and its present widespread use.

INTRODUCTION

Today there are many biological applications that require elemental determinations of samples. Many of these are in the fields of health, food, and environmental safety. A rather unique application is an ecological one, the use of elementally-marked arthropods to measure dispersal which requires elemental determinations of arthropods and of the marked flora that serves as the source of the marker (*e.g.*, articles in this supplement and references cited in them). Well-designed studies require that the field investigators (usually nonchemists) have a solid knowledge of the analytical procedures for the elements available for selection in a particular experiment, methodologies associated with them, and ability to do the data analysis that must accompany each of them. Often such investigations involve a team approach with cooperation between biologists, analytical chemists, and statisticians. Chemists must be able to recommend procedures suitable for appropriate analysis of the biological samples. Also, they must help determine if the analytical procedures available are sensitive enough to resolve the elements in question, given the volume limitations often present with arthropod samples. Plant samples seldom

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have the same limitations of sample volume but on the other hand may require a more elaborate preparation procedure.

Here, general analytical considerations, sample preparation, and instrumental methods of analyses will be discussed. Also, cost, labor factors, and other practical considerations will be addressed. Information and comparisons (*e.g.*, Parson et al. 1983) useful for the actual analysis of various elements will also be presented.

GENERAL ANALYTICAL CONSIDERATIONS

Sample. Initially, we would like to discuss some basic, general analytical considerations which are important in any analysis. First, one should insure that the sample being analyzed is one that represents the process being studied. Without a representative sample, results are often difficult if not impossible to interpret. Once samples are collected, the method of storage should preserve sample integrity. Generally, freezing samples works well; but prior to processing, samples should be freeze-dried or oven dried and then stored in desiccators prior to preparation.

Analytical Blank. An important parameter often forgotten is the analytical blank. The analytical blank can be defined as any response which is not due to the sample and arises from contamination from all sources external to the sample. The importance of the analytical blank is that it ultimately determines the limit of quantification that a method is able to achieve. Because the accuracy in discriminating two numbers of similar magnitude is poor, the blank should be as low as possible. In addition, the variability of the blank must be reduced to achieve optimum detection limits. Many sources external to the sample can contribute to contamination of the blank (and also the sample). Dust and particulates from the environment need to be reduced to a minimum, and clean air hoods and rooms may be required for ultra-trace analysis. High purity acids and reagents may be needed depending upon the sample of interest. Generally, distilled, deionized water provides a clean, reliable source for most applications. A good procedure for cleaning and storage of glassware needs to be followed rigorously. Finally, the analyst should be aware of personal contributions (sweat, skin, hair, cosmetics, jewelry, etc.) to contamination of the analytical blank (see Cornelis 1986).

Reference Material and Control Procedures. During and after development of an analytical procedure, it is advisable to use reference materials to determine the accuracy and precision of the method. Certified standards can be used for individual elements and mixtures of them can be run to determine interactive (matrix) effects (Becker 1986). Since a certified reference insect standard is not available, bulk digestions and spikes (blanks made up from pure water and/or arthropod or plant material that have known quantities of elements added to them) may be helpful for evaluating rates of recovery and for determining matrix effects. A quality assurance/quality control (QA/QC) program should be established to monitor the analysis through time (Becker 1986). Documentation of calibrations and instrument stability needs to be performed.

BIOLOGICAL SAMPLE PREPARATION

Preparation of biological samples for elemental analyses generally requires the destruction of the sample matrix. This can be accomplished in a number of ways. The most common means are wet digestions in an oxidizing medium or dry ashing

at an elevated temperature (500°C or greater) and dissolving the ash in dilute acid. Other approaches include the use of oxygen and acid bombs and fusions.

Wet Chemical Oxidation and Dry Ashing. Determination of a digestion method appropriate for the sample involves consideration of a number of parameters: volatility of target elements, possible addition of elements as contaminants from a digestion acid, number of samples to run, time involved for the preparation method chosen, and facility and budget constraints. (See Iyengar and Sansoni 1980, Katz and Jenniss 1983). Most of our experience involves the use of wet oxidation.

Generally, wet oxidation requires less time, but more supervision and expertise is required as compared to dry ashing. Because lower temperatures are used, wet oxidations have less problems with volatilization and retention (sample loss) as compared to dry ashing. Retention of elements by the ashing container or acid insoluble fraction can be a problem in dry ashing. With wet oxidations, the nature of the sample is of less concern while with dry ashing, the sample's nature can be important. In dry ashing for example, samples with high levels of chloride can cause problems with volatilization of certain elements. Usually larger sample sizes can be used with dry ashing. Elemental concentration in the reagent blank is generally high with wet oxidations because of the relatively large volumes of acid required, but depending on the elements of concern and the purity of the acid this may or may not be a problem. High purity acids can be purchased to reduce elemental levels of blanks (*e.g.*, ULTREX™). To increase the speed of dry ashing, ashing aids are often used (nitric acid, sulfuric acid, magnesium nitrate).

For some wet digestions, nitric acid alone is sufficient, but generally more complete digestions can be performed with acid mixtures. For example, nitric:perchloric, nitric:sulfuric, nitric:hydrogen peroxide, nitric:perchloric:sulfuric and sulfuric:hydrogen peroxide are some common acid mixtures used. The use of perchloric acid has been common for several years (Smith 1953) but requires special precautions (see below). But there is no set "right way", and the choice is usually dependent upon the analyst's preference, facilities and convenience.

A wet digestion scheme (Burns 1984, Murphy 1982) that was suitable for preparation of pink bollworm, *Pectinophora gossypiella* (Saunders), boll weevil, *Anthonomus grandis* Boheman, and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) is as follows: Insects were dried at 100°C for 2 h, weighed to ± 0.1 mg, digested in nitric acid followed by 30% hydrogen peroxide (0.1 ml of each) or digested in a 1:1 nitric:perchloric acid mixture, diluted to 1 ml with distilled-deionized (DDI) water, mixed with a vortex stirrer, and transferred to autosampler cups. These digestions were performed in aluminum heating blocks and about 40 to 50 insects could be digested simultaneously. For digestion using the nitric:perchloric acid mixture, two heating blocks were used with one at 90°C for the first 20-25 min. and the other at 150°C for the second 20-25 min. Also, the wet digestion method described above has been modified to utilize ultrasonic baths in conjunction with or in place of the heating blocks (Akey 1988). This is another means of reducing the heat required for digestion.

Common laboratory safety precautions (Nat. Res. Council 1981, Steere 1971) should always be followed when using any acid for digestion, as an ashing aid, or even for glassware cleaning. In particular, safe use of perchloric acid requires special precautions (Everett and Graf 1971, Nat. Res. Council 1981, 1983) and may require a fume hood certified for perchloric acid (Am. Soc. Heating, Refrig. and Air-Condi-

tioning Eng., Inc,. 1971, Nat. Res. Council 1981, refer to ref. in sec. I. H. 7, and Hamilton 1980).

Microwave Heating. Another approach to wet digestions is use of microwave heating as opposed to thermal heating. Microwave digestion offers several advantages with time savings being the primary one. Microwave digestions in closed vessels can be performed at elevated pressures and temperatures, and more efficient and complete digestions can be realized resulting in reduced dissolution times. Also, with closed digestion vessels, less acid volumes are required and therefore lower reagent blanks are obtained since less acid volumes are required. In contrast, use of microwave digestion with open vessels is still usually quicker than with heat blocks, permits many samples to be processed simultaneously, and low vessel cost results in a relatively inexpensive method. A number of commercial microwave digestion system are available (see Am. Lab., Buyers Guide by Inter. Sc. Communications, P.O. Box 870, Shelton, CT 06484). Some investigators have successfully used conventional (home use) microwave ovens. Such ovens should be at least 600 watts and have an internal rotating base to prevent the sample from being exposed to "hot spots" of energy within the oven (*e.g.*, Carousel™ II, Sharp Electronics Corp., Sharp Plaza, Mahwah, NJ 07430). The cost ratio of conventional ovens to commercial ovens (designed to withstand acid fumes and have even energy dispersal) may be an important factor for some research budgets. The conventional oven is simply discarded if it wears out and another purchased. One consideration would be to occasionally monitor the door seals of conventional microwave ovens that are used with acids to be sure the unit is not leaking radiation. Meters are available for this purpose.

Pressure Decomposition (Bombs). A distinct advantage of wet digestions is complete dissolution of the sample in a liquid acid at temperatures low enough that sample volatiles do not form. A disadvantage is the time involved for digestion and the scrutiny required to avoid overheating (boiling) the sample if the heating blocks are not perfectly set to the proper temperature. Pressurization of the digestion vessel can reduce digestion time and prevent escape of any sample volatiles that form. Commercial bombs are available (Paus 1972); other investigators have used plastic (Adrian 1971), screwtop thick-walled glass culture tubes with teflon™ gaskets, or capped polypropylene centrifuge tubes (Pearson et al. 1989).

Oxygen Plasma Decomposition. A relatively novel technique for organic decomposition is the use of a low temperature oxygen plasma. The sample is oxidized by reactive oxygen species, which are generated and activated by a radio frequency (RF) field (500 W) to form a plasma at a low pressure (1 torr) (Kaiser et al. 1971, Lutz et al. 1977). The plasma temperature is generally about 130°C. This approach has the advantage of low potential for volatilization and lower elemental concentration in analytical blanks. It is a fairly complicated technique with several parameters to control, long decomposition times, and limited sample throughput.

INSTRUMENTAL METHODS FOR ELEMENTAL ANALYSIS OF BIOLOGICAL SAMPLES

AAS. By far, the most common means of metals analyses in the last 30 years has been atomic absorption spectrometry (AAS)(see Schrenk 1975, Katz and Jenniss 1983). AAS (in the absorption mode) has been concisely defined by Welz (1985) as "the measurement of the absorption of optical radiation by atoms in the gaseous state." Numerous improvements in AAS include the development of dual-beam

instruments, slit and grating resolution and accuracy, Zeeman or similar correction, multiple lamp turrets (Welz 1985), and the use of the graphite furnace with pyrolytically-coated tubes and platforms or other methods that act to produce similar sensitivity (Schrenk 1975, Katz and Jenniss 1983). As with almost all analytical instrumentation today, AAS instruments are often computer interfaced and usually have digital input and output. Automatic dilution autosamplers have taken much of the labor out of overcoming one of the AAS shortcomings, that of a relatively small analytical range. Also, changing between flame operation (relatively easy to use, less sensitive, and often requiring 0.5 ml of sample per burn) to graphite furnace operation (relatively more difficult to use, very sensitive, and requiring as little as 20 μl of sample) is usually time-consuming, but some instruments now handle this problem by manipulation of optical paths or use of other technology to expedite beam alignment. In general, AAS is very useful for single-element determinations and in some cases for sequential determinations. AAS is comparatively moderate in equipment cost, and the skills required to use the instrumentation are relatively straightforward and not difficult.

AAS has been the technique used most in elemental marking studies. Berry et al. (1972) first applied AAS for rubidium determinations in marking work, and it is still much used today (Hayes and Hopper 1987, Cohen and Jackson 1989, Hayes 1989, Bridges et al. 1990). The other papers published in this supplement have numerous references to studies conducted with AAS as the analytical tool, and the reader is referred to them for additional reference sources for use of AAS in marking studies. In addition, the use of AAS in the emission mode for the detection of rubidium has been reported to be easy and sensitive (see Fleischer, this supplement).

ICP-AES. In the late 1970's, inductively-coupled plasma atomic emission spectrometry (ICP-AES) became the analytical method of choice for a wide range of elements, particularly if analysis of several elements was desirable (Jones et al. 1982, Barnes et al. 1983). Briefly, ICP-AES is the use of high frequency electromagnetic fields (*e.g.*, 27.12 MHz) to produce an inductively-coupled argon plasma at about 8000° K into which the sample is injected (Greenfield et al. 1964, Wendt and Fassel 1965, Haas and Fassel 1980). Under these conditions, the sample is vaporized and atomically excited to higher energies. Emission of energy at discrete frequencies occurs as the atoms return to lower energy states and this emission energy is quantified to measure elemental concentrations in the sample. While AAS is a single element approach, in contrast ICP-AES offers the advantage of multi-elemental (simultaneous) analysis. Depending upon the element, low detection limits (sub-ppm to ppb) are obtained with both techniques. ICP-AES offers the advantage of linear calibration curves of 4-6 orders of magnitude. The ICP-AES is much less susceptible to matrix effects than AAS, but spectral interferences can be a problem. For routine ICP-AES analysis, compromise operating conditions can be used (see Kornblum et al. 1983 for an example of determining compromise operating conditions), while with AAS, each element requires unique experimental conditions. Finally, the initial cost and maintenance expenses are higher with the ICP-AES as compared to AAS. One limitation in the use of ICP-AES is that it is not very sensitive to the alkali metals K, Rb, and Cs. Comparatively, AAS with graphite furnace is very sensitive to these metals. A particular strong point of ICP-AES is that it is very sensitive to the alkaline-earth metals Ca and Sr.

Murphy (1982) was the first to use ICP-AES for analysis of insects, and the technique has been applied to investigations of elemental markers for sterile mass-reared pink bollworm (Burns et al. 1983a) and Mediterranean fruit fly (Burns et al.

1983b) and for studies performed to evaluate elemental composition for native pink bollworm (Burns et al. 1983a) and boll weevil (Burns et al. 1985). The latter work demonstrates how multi-elemental composition was used to discriminate different collection sites using elemental data and multivariate analysis.

XRS, XRFS, and PIXES. In addition to AAS and ICP-AES, other techniques are available that utilize X-rays for elemental determinations (Russ 1984, Williams 1987). There are two types of X-Ray spectrometers (XRS): wavelength dispersive (WD) and energy dispersive (ED) (Russ 1984). The two differ in sensitivity and application choice, WD XRS uses a dispersion grating to separate the signal into a spectrum whereas ED XRS uses a solid state detector and measures signal amplitude. WD XRS has considerably higher resolution than ED XRS (both in theory and practice) but requires specific parameter setups that are difficult to change; WD XRS generally is not as versatile as ED XRS. ED XRS was first used for qualitative multi-elemental analysis, but present instruments have been improved considerably to approach their theoretical limits (Russ 1984). An example of modern ED XRS is microprobe analysis (Williams 1987).

The use of XRS for trace element analysis was greatly improved by the development of X-ray fluorescence spectroscopy (XRFS). It involves exposing the sample to an X-ray source followed by monitoring the induced X-ray emission from the elements in the sample and is a nondestructive technique (Williams 1987). XRFS has been incorporated in both WD and ED XRS systems but now is most common in ED XRS systems although WD XRFS is more appropriate for quantitative analysis; *e.g.*, Chaudhri et al. (1983) reported elemental levels in liver samples as low as 0.08 ppm for strontium. With ED XRFS, quantitation is still generally more difficult but qualitative information can be readily obtained. Although the sensitivity of XRFS depends on the elements chosen for analysis (light elements are more difficult), it can be very useful as a screening tool to determine if an element is present. On the other hand, biological discrimination of differences between groups (populations) or individuals has often been based on groupings of major or minor elements (Bowden et al. 1979, Bowden et al. 1985, Burns et al. 1985, Demster et al. 1986, and see discussion of elemental composition of insects using XRS, by Akey this supplement), and the statistical methodology is tightly linked to the analytical method used (Williams 1987). The most recent advance in XRS is particle-induced X-ray emission spectrometry (PIXES), and quantitative work has been reported in ppms by Minqian et al. (1983) and to 0.1 ppm by Järnström et al. (1983).

NAA. Neutron activation analysis (NAA) is one of the very first elemental marking techniques (Jahn et al. 1966). It is very sensitive for a number of elements (*e.g.*, detection of: dysprosium and europium to 1 picogram (10^{-12}g), strontium to 100 picograms, and rubidium to 1 nanogram (10^{-9}g) (Amiel and Feldstein 1981). A NAA experiment involves irradiating the sample (presumably containing the marker elements, usually stable isotopes, *i.e.*, nonradioactive elements) with a neutron source for sufficient time to give an adequate induced activity (production of radionuclides by nuclear transformation) for the elements of interest to emit gamma radioactivity which is then detected (Ross 1964, Valković 1977). The use of NAA is limited by the availability of a neutron source of which there are several types (Valković 1977). Advantages of the method include high sensitivity, low opportunity for the sample to become contaminated, and few matrix effects. However, the cost of the instrumentation is high, and most investigators use existing facilities. The detection method for qualitative analysis can be simplified by using autoradiography (*e.g.*, Costa

and Byrne 1988) and for quantitative analysis by using a crystal detector (*e.g.*, Richardson et al. 1969, Monro 1968, Curtis et al. 1973). As with many analytical techniques today, NAA is sometimes combined with XRS to achieve even lower detection limits for certain applications (Mantel and Amiel 1981).

Several investigators have successfully used NAA in elemental marking studies. Haisch et al. (1975) used the technique with dysprosium and samarium for marking the cherry fruit fly, *Ragoletis cerasi* L. Costa and Byrne (1988) marked the sweet potato whitefly, *Bemisia tabaci* (Gennadius), with dysprosium. Europium and dysprosium (as chlorides, offered in blood meals) were used to mark adults of the tsetse fly, *Glossina morsitans morsitans* Westwood (Curtis et al. 1973). Lanthanum was successfully used for the bark beetle, *Ips typographus* L., (Naumann-Etienne et al. 1977). Hamann and Iwannek (1979) labeled pupae of *Glossina palpalis palpalis* Robineau-Desvoidy with europium, dysprosium and lanthanum (each as single labels); and they investigated blood meals, dipping, and aerosols as methods of application, and dimethylsulfoxide (DMSO) as a "wetting agent" [Authors' note: DMSO is an extremely active carrier in biological systems, investigators working with this compound must exercise caution].

AFS. A very sensitive and selective method for elemental analysis is atomic fluorescence spectroscopy (AFS) (Schrenk 1975, Welz 1985). In AFS, the sample is introduced as an aerosol into a flame or ICP. Radiation from a hollow cathode lamp, laser, continuum source or ICP source is used to excite the element of interest (Schrenk 1975). The excited atoms decay to lower energy level and emitted radiation or fluorescence is measured. General use of AFS has been limited; but depending upon the application, it can be a very useful approach (Schrenk 1975, Welz 1985).

LEI and Other Laser Techniques. A rather novel technique which is currently being investigated is laser-enhanced (flame or plasma) ionization (LEI) (also known as laser assisted flame ionization-LAFI) (Travis et al. 1982, 1984; Turk et al. 1983, Turk 1987). A sample is aspirated into a flame, plasma, or other atomic reservoir, and a tunable dye laser is used to promote the target atom to a discrete excited state. From the excited state, thermal ionization occurs at an enhanced rate as compared to the ground state. This results in an increase in the flame's (or other atomic reservoir) conductivity which is electrically detected by applying a voltage across it and monitoring the resultant current. Numerous other applications of lasers are in use. Some of these include spectroscopic laser systems, resonance photoionization techniques (LEI may be considered a type of this technique, Turk 1987), laser absorption, molecular dissociation, laser raman techniques, and laser-induced fluorescence spectroscopy (Radziemski et al. 1987).

ICP-MS. Finally, one of the most promising recent developments for elemental analyses is ICP mass spectroscopy (MS) (Houk 1986, Houk and Thompson 1988). In addition to being an excellent excitation source for thermal atomic emission, the ICP is also an efficient ionization source. About 54 elements are ionized with efficiency of 90% or better. With ICP-MS, the plasma is directed into a water-cooled sampling cone of a quadrupole mass analyzer. Through a system of skimmers, mechanical vacuum pumps and ion lenses, the ions are collected, focused and transmitted into the quadrupole where ions of the selected m/z value leave the mass analyzer and are deflected into an electron multiplier for detection. The detection limits for most elements (about 70) are in the sub-ppb range. As with the other techniques discussed previously, efforts are being made to combine ICP-MS with other analytical methods (Houk 1986). Due to its extreme sensitivity, ICP-MS will

probably become the future method of choice for trace multi-elemental determinations.

CONCLUSION

Clearly, the most popular method for detection of elemental markers of arthropods has been the use of Rb with detection by AAS. The simplicity of instrumentation and recent advances has made the method suitable for even multi-elemental determinations in marking experiments. However, the goals of the investigations (*e.g.*, use of elemental composition as a biological marker in itself) and the availability of analytical instrumentation have often determined which method(s) and elements have been used. The need for careful sample preparation and sound analytical procedures underlie all of the methods discussed herein.

One of the most pressing problems in previous investigations has been the need to process large numbers of samples. Logically, simpler sample preparation will result in the more samples being processed. Methods to reduce sample preparation time will contribute to more widespread use of elemental marking of biological samples. The methods discussed above detail those commonly in use today but also introduce some techniques that offer promise for greater analytical sensitivity and reduced sample preparation.

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NOTE

Since this presentation in 1988, three texts have been published that the authors suggest for additional reading: Iyengar 1989, Valković 1989, and Varma 1990 (see literature cited).

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