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Atomic Absorption Determination of Zinc and Copper in a Multivitamin

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Background

Atomic absorption spectroscopy (AAS) is an important analytical technique based upon the absorption of radiation by free atoms. Virtually all metallic elements can be directly detected with excellent accuracy, precise quantitation, and very sensitive detection limits. This technique can also be used to indirectly detect and measure the amounts of some nonmetallic elements.

Atoms of each element in the ground state have a specific electron configuration. These atoms can absorb quanta of light energy when that energy exactly matches the energy associated with any of the allowed electronic transitions within that atom. (See Figure 1.) Atoms that absorb energy are said to be in an excited state. Their electrons absorb exact quanta of energy, elevate to higher energy levels, and then fall back to the ground state, emitting photons of light. This emitted light has a specific wavelength, since it corresponds to the energy released when the electrons fall back down to the ground state. This wavelength corresponds to the energy of one of the allowed transitions within the atom. The light emitted, therefore, produces an emission spectrum consisting of specific wavelengths visualized as Frauenhofer Lines.

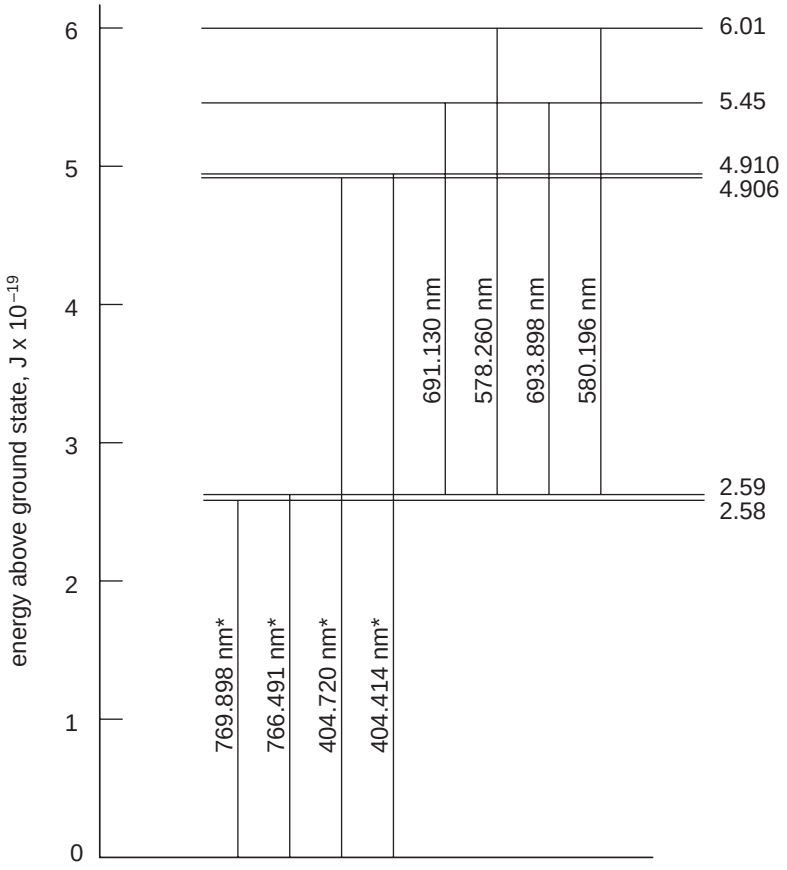

Figure 1: The major energetic levels in a potassium atom. Adapted from Braun, R.D. Instrumental Analysis; *McGraw-Hill: New York. 1987; p 176. The transitions with asterisks are those that are normally used for AAS.*

Two closely related spectroscopic techniques are commonly used in analytical laboratories to measure the light absorbed and emitted by atoms. These are emission spectroscopy and absorption spectroscopy. Emission spectroscopy measures the light emitted by the electrons as they descend back to the ground state. On the other hand, absorption spectroscopy measures the original absorption of energy by ground-state electrons. Since many more atoms are in the ground state than in an excited state in a sample at any one time, absorption measurements are generally more sensitive than emission measurements.

In addition, relatively few factors interfere with atomic absorption measurements, and it can be used to quantitate elements in many types of samples. AAS has been widely used in medicine for the analysis of trace metals, metal toxicology determinations, and even in forensic pathology studies. Modern medical concerns over environmental mercury and lead poisoning, as well the ingestion of nutritionally required metals at toxic levels, are reflected in the effective use of atomic absorption analysis.

Coupled with x-ray electron microscope analysis of metals in solid samples, atomic absorption has been a useful tool in metallurgy, especially with the advent of highly complex modern metal alloys. Since atomic absorption is relatively inexpensive and fast and requires only a minimum of proper laboratory training, it is an excellent technique for performing first-run analyses of industrial metals.

Similarly, soils analysis has benefitted greatly from the use of this technique. Farmers, homeowners, and horticulturists desire soils containing optimum levels of primary nutrients, macronutrient metals, and trace elements. Soils are ideal for atomic absorption since the particles are already in a finely ground state and often are composed of water-soluble metal salts that lend themselves readily to atomization and analysis. Soil additives can be analyzed using these same techniques with a precision that allows the person in the field, whether a farmer, landscape architect (or designer), or homeowner, to apply the exact amounts of the nutrients necessary to obtain a balanced soil composition for the specific plants to be grown.

Atomic absorption is applied just as easily to industrial process analysis. It is effective in detecting contaminants in petroleum refining and distribution so as to prevent the inclusion of unwanted metals in exhausts that would pollute the environment. In the past, this technique was widely used in the quantitation of tetraethyl lead antiknock agents in gasoline. The same procedures are now used with great success to detect the by-products of burning this gasoline, in the form of lead residues on the shoulders of highways, in adjacent plant and animal tissues, and in the ecosystems contaminated by this formerly useful material. This example is just one of many where industrial chemical processes have used and, in many cases still do use, atomic absorption on a broad scale. It is also an example of how the same techniques can be just as easily applied to detect many environmental pollutants. The results of these analyses often impact our lives in terms of the environmental effects, the medical toxicology, and even the political machinery generated to tackle problems generated by metal contamination.

Food in the United States and in much of the world is commonly analyzed for the presence or absence of a variety of substances—both required nutrients and undesired contaminants. AAS plays a role in many of these analyses because of its relative ease and relatively low cost. As in soils analysis, marketers desire agricultural produce that have the correct levels of elements that are nutritional requirements and do not have elements that may be toxic or unnecessary. For business as well as health reasons, farmers and grocers have specific concerns that can be addressed effectively using atomic absorption analysis.

Forensic pathology uses a tremendous variety of analytical techniques, many of which are unfamiliar or unknown to the typical citizen. It is often necessary to analyze samples that are taken from the most unsanitary of sources so as to determine the presence of toxic metals. Even the examination of bullets, gunpowder, and crime residues frequently involves the analysis for specific metals in order to determine sources of these materials or to match them with similar materials obtained in criminal investigations. Coupled with other analytical techniques, AAS can help a modern forensic pathologist determine exactly where a specific bullet, cartridge, or metal blade was produced, even to the point of the specific date or industrial run that produced the material. Atomic absorption has continually proven to be one of the most reliable and effective of these crime-fighting tools.

Atomic absorption requires a surprisingly simple apparatus. (See Figure 2.) The critical components are a hollow cathode lamp capable of emitting the line spectrum of the element to be analyzed, an atomizer connected to a flame where sample ions are converted to free atoms, a monochromator with the capability of wavelength selection, and a detector with an amplifier and readout device. Highly reliable commercial atomic absorption units are routinely available equipped with readily changeable lamps so that a variety of elements can be sequentially detected. The electronics required are minimal for a typical piece of lab equipment. A source of gas for the flame and a vent for the exhaust gas (for safety) are also required.

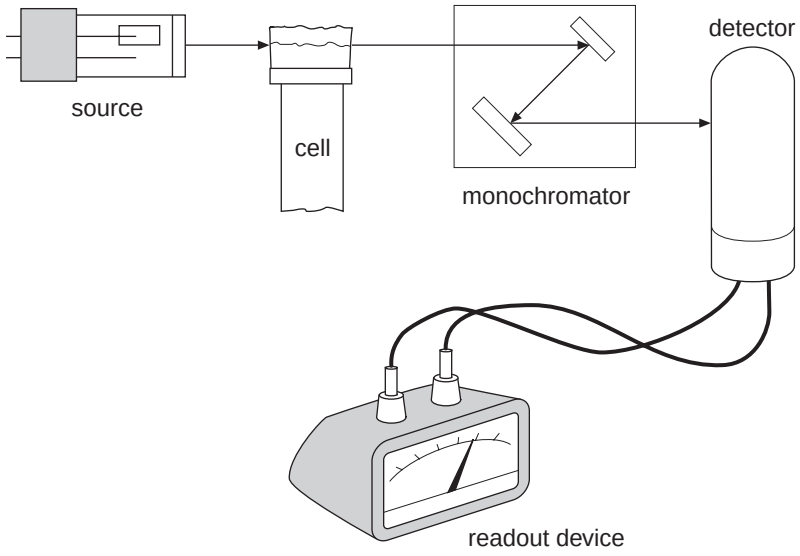

Figure 2: Diagram of a single-beam atomic absorption spectrophotometer.

Hollow cathode lamps are used as the source on an atomic absorption spectrophotometer. The lamp has a cylindrical metal cathode sealed in a glass tube containing argon or neon. When a voltage is applied, the metal atoms of the cathode become electrically excited and then emit photons of light when they return to the ground state. This emitted energy is the same energy that can be absorbed by the free atoms of the element in the flame. A different lamp is needed for each element to be measured. Multielement lamps can be made from alloys or mixtures of metals; however, only certain combinations of metals work well together.

The first atomizer used was a flame. When placed in the flame, the solvent evaporates from a solution of metal ions, leaving crystals of the metal salt. The metal ions are then transformed into free atoms. The flame requires both an oxidant and a fuel. The most common is air-acetylene, which typically gives a flame temperature of 2,300 K. Nitrous oxide-acetylene produces a hotter flame with

temperatures approaching 2,900 K. The choice of flame depends upon which metals are to be analyzed. Air-acetylene works well for both the copper and the zinc to be analyzed in this experiment.

Other methods of atomization are now in use. A graphite furnace can be used to convert metal ions into free atoms. The graphite furnace is an electrothermal atomizer. The graphite furnace can provide greater sensitivity than flame atomizers and is one of the most sensitive techniques for trace (ppb) metal determination.

A premix burner is commonly used with the flame. The sample is aspirated through a plastic tube, sprayed into a spray chamber in a fine mist (nebulized), and mixed with oxidant and fuel prior to being introduced into the flame. (See Figure 3.) Large droplets of sample flow to waste when they come in contact with flow spoilers in the spray chamber, leaving only the smallest droplets. The premixed sample is introduced into the burner head, which usually is rectangular with a 4- to 6-inch slot, and the oxidant, fuel, and sample are ignited. The nebulizer rate can be adjusted to obtain the optimum readout.

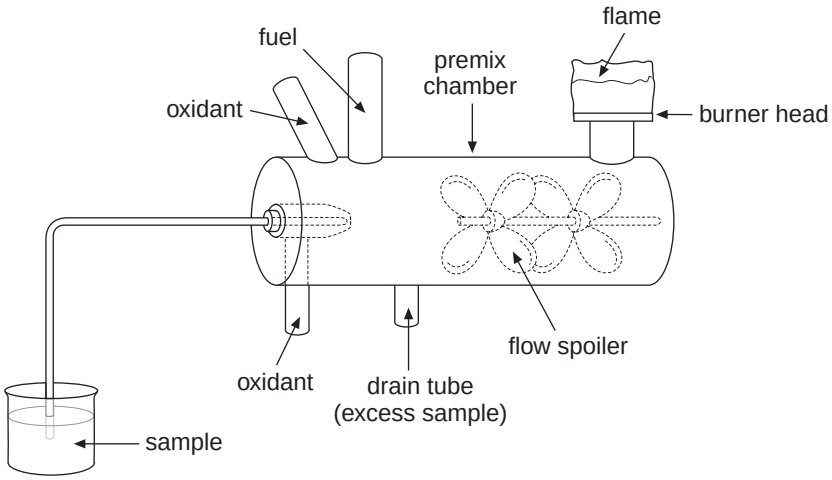

Figure 3: A premix burner.

Even though line sources are used, a monochromator is still necessary to eliminate unwanted radiation stemming from various sample components and from the flame itself. The monochromator isolates the desired wavelength needed to reach the detector. Atomic absorption instruments are single beam, since it would be extremely difficult to produce a reference beam with its own nebulizer and flame identical to the sample beam. Most of the extraneous radiation has a wavelength different from that of the wavelength to be measured and can be removed by the monochromator. To eliminate any flame radiation of the same wavelength, the source energy is modulated. The intensity of the hollow cathode lamp radiation fluctuates at a constant frequency. Only light with that frequency is sent to the amplifier and detector. The detector consists of a photomultiplier tube.

The analysis of metals within vitamins and foodstuffs always intrigues students and consumers as a result of the immediate use of the data in everyday life. Copper and zinc in multivitamin tablets lend themselves readily to this type of analysis since the elements can be extracted easily and then quantitatively analyzed in a matter of minutes. The procedure for this analysis follows.

Materials

- copper metal ribbon
- mossy zinc metal
- 6 M hydrochloric acid
- 0.1 M hydrochloric acid
- 7.9 M nitric acid
- 0.16 M nitric acid
- distilled water
- volumetric flasks (1 L, 100 mL)
- atomic absorption spectrophotometer with air-acetylene flame
- copper and zinc hollow cathode lamps or a multielement lamp containing copper and zinc
- multivitamins (containing 15 mg Zn and 2 mg Cu)
- mortar and pestle
- filter paper and funnel

Safety

It is your responsibility to specifically follow your institution's standard operating procedures (SOPs) and all local, state, and national guidelines on safe handling and storage of all chemicals and equipment you may use in this activity. This includes determining and using the appropriate personal protective equipment (e.g., goggles, gloves, apron). If you are at any time unsure about an SOP or other regulation, check with your instructor.

Methods

A series of standards for zinc and another series of standards for copper must be prepared. The zinc metal is dissolved in dilute acid to prepare a concentrated stock solution. The stock solution is diluted to prepare the analytical standards. The same procedure is followed for copper.

Zinc stock solution, 500 μg/mL

Dissolve 0.500 g zinc metal in a minimum volume of 6 M HCl, and then dilute to 1 L with 0.1 M HCl. To prepare the series of standards for zinc analysis, select five concentrations to set up a standard curve. Normally a spectrophotometer is linear within the range of 0.05 to 1.0 absorbance units. Standards should be prepared that will give an absorbance within this range. A standard containing 0.5 μg/mL Zn will typically give an absorbance of about 0.15. The five following standard concentrations can be conveniently prepared from the stock solution: 0.5 μg/mL, 1.0 μg/mL, 1.5 μg/mL, 2.0 μg/mL, and 2.5 μg/mL. Make three replications of each concentration (in order to get an average reading) using 100-mL volumetric flasks.

Preparation of the vitamin for zinc analysis

Thoroughly crush a multivitamin tablet (such as Centrum® from A to Zinc or any multivitamin that contains both copper and zinc) in a mortar and pestle. Add 20 mL 6 M HCl, mix thoroughly, and allow to stand for five minutes. Quantitatively transfer the solution to a funnel fitted with Whatman #1 filter paper and filter into a 100-mL volumetric flask. Repeat the extraction two more times with 20 mL dilute HCl each time. Rinse the filter with 0.1 M HCl and bring the volume to 100 mL with 0.1 M HCl.

Copper stock solution, 1,000 μg/mL

Dissolve 1.000 g copper metal in a minimum volume of 7.9 M HNO_3, and then dilute to 1 L with 0.16 M HNO_3. To prepare the series of standards for copper analysis, select five concentrations to set up a standard curve. Normally a spectrophotometer is linear within the range of 0.05 to 1.0 absorbance units. A standard containing 5.0 μg/mL Cu will give an absorbance of about 0.20.

The following five standard concentrations can be conveniently prepared from the stock solution and will give absorbances within the linear range of the spectrophotometer: 5 μg/mL, 10 μg/mL, 15 μg/mL, 20 μg/mL, and 25 μg/mL. Make three replications of each concentration using 100-mL volumetric flasks.

Preparation of the vitamin for copper analysis

Thoroughly crush a second multivitamin tablet (use the same brand as in the zinc analysis) in a mortar and pestle. Add 20 mL 7.9 M HNO_3, mix thoroughly, and allow to stand for five minutes. Quantitatively transfer the solution to a funnel fitted with Whatman #1 filter paper and filter into a 100-mL volumetric flask. Repeat the extraction two more times with 20 mL dilute HNO_3 each time. Rinse the filter with 0.16 M HNO_3 and bring the volume to 100 mL with 0.16 M HNO_3.

Setting up the atomic absorption spectrophotometer

Consult the operation manual for the specific instrument being used to make sure all adjustments are properly made.

It is important to ensure that the instrument is properly adjusted to provide the optimum conditions for analysis of each element. Make sure that the instrument is equipped with a zinc hollow cathode lamp. Turn on the instrument. Adjust the wavelength to 2,138.6 Å (the first resonance line for zinc). Prepare to light the flame by turning on the regulators for the air and the acetylene fuel. Adjustments must be made for the particular instrument being used. Turn on the air and fuel flows and ignite the flame. Aspirate distilled water through the nebulizer for about 10 minutes to allow the instrument to warm up. Start aspirating one solution of the highest concentration of zinc. Adjust the aspiration rate to optimize the flow for the zinc determination. (Watch the readout while adjusting the aspiration rate until the reading reaches the maximum.) Adjust the oxidant and fuel flow to provide the optimum fuel:oxidant ratio for the analysis of zinc. Make sure the flame is positioned properly to provide for the maximum readout.

Analysis of zinc

It will be necessary to dilute the vitamin extract 1:100.

Begin with the highest concentration of standard. Aspirate into the flame and record its reading. Some instrument models can be calibrated to display in concentration units. Adjust the readout according to the operation manual. Obtain a reading on all of the standards by aspirating each individually into the flame. Proceed from highest to lowest concentration. Organize the readings into a table or spreadsheet. Aspirate the vitamin tablet extracted with hydrochloric acid. Prepare three replications of the dilution. Aspirate each dilution and obtain readings. Average the readings for each of the standards and for the vitamin extract. Prepare a standard curve by plotting concentration on the *x* axis and instrument reading on the *y* axis. Determine the concentration of the vitamin extract from the graph. Alternatively, if available, a plotting program can be used to prepare the graph and a linear least squares analysis to determine the concentration of the vitamin extract. In order to determine the total amount of zinc in the vitamin, multiply the concentration obtained from the graph by the dilution factor (100) and also multiply by the total volume of the original extract (100 mL). Compare the value obtained to the amount listed on the vitamin bottle.

Analysis of copper

It is not necessary to dilute this extract.

The entire procedure must now be repeated for the analysis of copper. Place a copper hollow cathode lamp in the instrument. Set the monochromator at 3,247.5 Å (the first resonance line for copper). Aspirate the highest concentration of copper standard and make all of the previous adjustments to

optimize the conditions for copper. (Adjust the nebulization rate, the fuel-oxidant ratio, and flame position.) Follow the same procedure used for the analysis of zinc. Obtain measurements for each of the copper standards, beginning with the highest concentration first. Obtain three readings of the nitric acid extracted vitamin. Notice that the flame becomes brightly colored when aspirating the vitamin extract. This is due to the sodium and other metals extracted with the copper. (Sodium produces a bright yellow flame, potassium produces an orangish-yellow flame, and calcium produces a red flame.) Prepare a standard curve for copper and determine the concentration of the vitamin extract. In order to calculate the total amount of copper, it is necessary to multiply the concentration of the extract by the total initial volume (100 mL). Alternatively, the data can be analyzed using graphing software and a linear least squares analysis to determine the concentration of the vitamin extract. Compare the total amount of copper extracted to the amount stated on the vitamin bottle.

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