Analysis of Ca, K, Na, and Mg in Liquid Milk and Egg White Samples Using Microwave Plasma-Atomic Emission Spectrometry (MP-AES)

1 Optical emission spectroscopy (OES)

In atomic emission spectrometry (AES) components of the sample are converted to atoms or elementary ions using atomizers. A fraction of these species is excited to higher electronic states and as they relax back to their ground states ultraviolet and visible line spectra arise – emission spectrum – that can be used for elemental analysis. For every atom, there exists hundreds of possibilities by which it may returns to the ground state, each of these having its own probability. Even the simplest sample contains an enormous number of emission lines. Consequently, the emission spectra can be very complex containing thousands of spectral lines accompanied by a continuum background and OES requires high performance instruments capable of locating weak intensity lines in close proximity to other much more intense.

The quantification of elements by emission spectroscopy implies that a relation exists between the concentration and the intensity of the corresponding light emission. They make use of protocols which comprise a calibration curve from standard solutions of the analyte.

For a population of n excited atoms, the emitted light intensity I_e depends upon the number of atoms dn that return to the ground state during the interval of time dt ($dn/dt = kn$). Since n is proportional to the concentration of the element in the hot zone of the instrument, the emitted light intensity I_e which varies as dn/dt, is itself proportional to the concentration:

$$I_e = k \cdot C$$

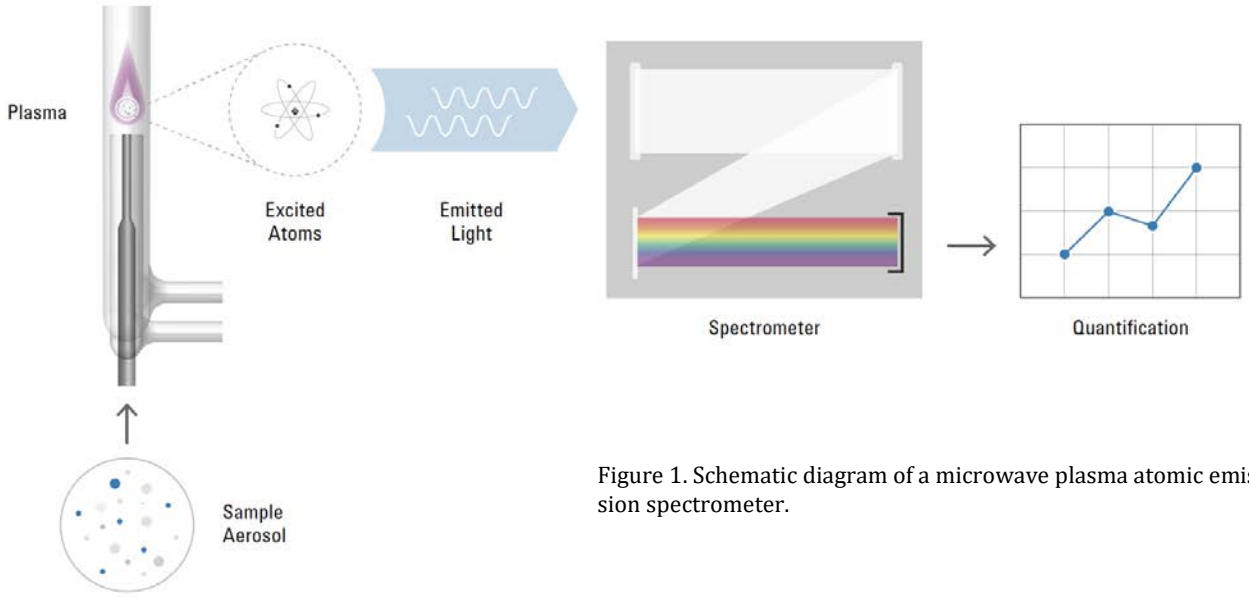

Figure 1. Schematic diagram of a microwave plasma atomic emission spectrometer.

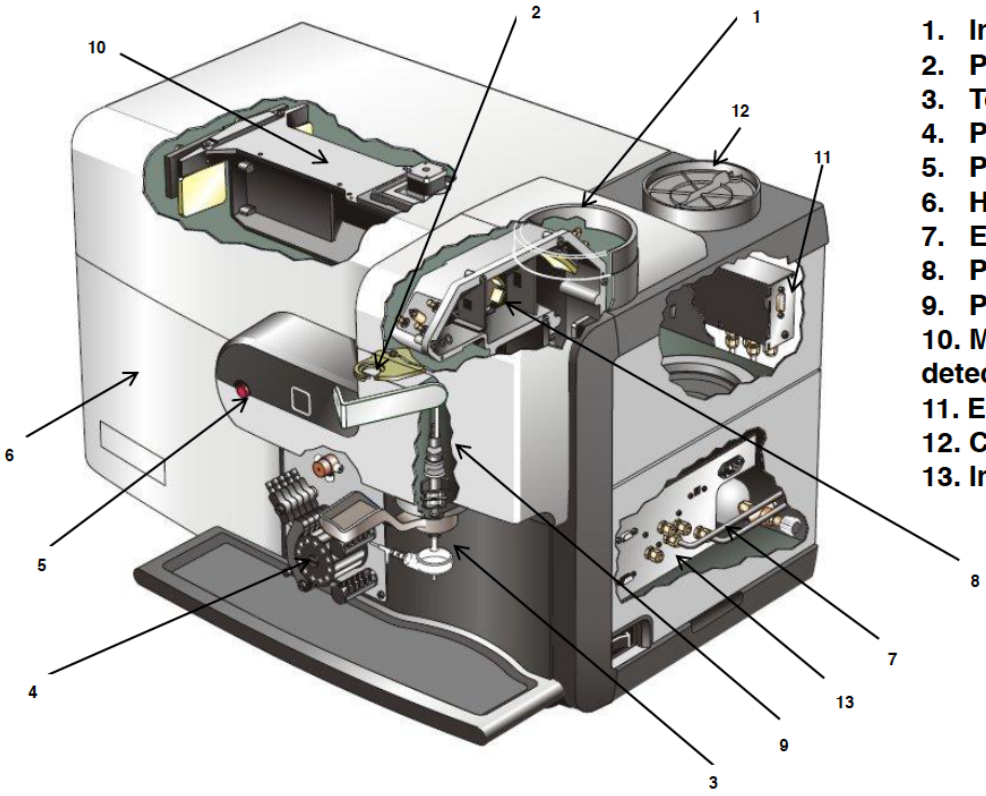

Figure 2. Schematic diagram of the Microwave Plasma-Atomic Emission Spectrometer (MP-AES).

This expression is only valid for low concentrations in the absence of self-absorption or ionization. To conduct an analysis by emission, the response of the instrument requires a calibration with a series of standards to find the calibration constant *k*.

Typical OES instruments comprise of several parts:
- *Atomizer* which is responsible for bringing the sample in the form of excited and/or ionized atoms.
- *Nebulizer* introduces the sample in the form of a fine spray of droplets into a nebulizer.
- High resolution optical equipment *monochromator* and *detector* (PMT or diode array).
- A computer essential to control the entire instrument.

1.1 Atomization methods

To convert a sample to free atoms, excited or ionized, various procedures are employed based upon gas plasmas, sparks, lasers or glow discharges. The plasma sources are the most important and most widely used type of atomization used in atomic emission spectroscopy. A plasma is electrically conducting gaseous mixture containing a significant concentration of cations and electrons. The concentration of the two is such that the net charge is zero. The plasma is considered to be the fourth state of matter. There are several options as to what type of plasma can be used in AES with the *inductively coupled plasma* (ICP) using argon gas being most commonly used in emission spectroscopy. The *microwave-induced plasma* (MIP or MP) using nitrogen gas is also used. An example is the instrument which we have in our lab – Agilent 4200 Microwave Plasma-Atomic Emission Spectrometer (MP-AES) shown in Figure 2. The plasma in this instrument is generated and sustained by coupling energy from the magnetic field of the microwave source, which provides a robust, high temperature source in conventional torches, approx. 5000 K. This allows the plasma to be sustained with nitrogen. By comparison, typical atomization temperatures in flame emission spectroscopy are in the range 2000–3000 K. As a result of higher temperatures of plasma compared with flame, formation of atoms and ions in nearly chemically inert environment is favoured in plasma resulting in fewer chemical interferences. The ionization interference effects are also small with plasmas owing to the high and relatively constant concentration of electrons in plasma. The nitrogen gas used in this instrument to sustain the plasma is obtained from

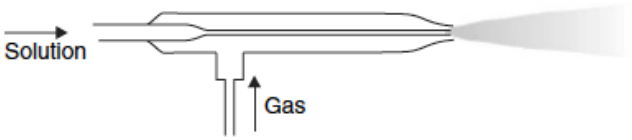

Figure 4. A Nebulizer.

air using a nitrogen generator, reducing operating costs over the argon–operated ICP.

1.2 Introduction of Solution Sample

Micro-volumes of sample solutions are introduced at a constant flow with a pneumatic *nebulizer* via a tube of small diameter directly into the plasma. The sample must arrive in the form of droplets which do not exceed a few microns in diameter. To this end a nebulizer containing two inlets is used, one for the sample solution, the other for a gas serving to generate the aerosol (Figure 4). The sample is transported to the tip by the Bernoulli effect (aspiration). The high-velocity gas breaks up the liquid into fine droplets of various sizes, which are then carried into the plasma.

1.3 Plasma Source Spectrometers

Instruments for emission spectroscopy are of two basic types: sequential and simultaneous multichannel. Sequential instruments are programmed to move from the line from one element to that of the second, pausing at each line to measure its intensity with a sufficient signal-to-noise ratio. Multichannel instruments are designed to measure simultaneously the intensities of emission lines for a number of elements. Both sequential and multichannel emission spectrometers use grating spectrometers to isolate a limited, narrow, and continuous group of wavelengths called bands. Usually, the grating is of holographic type with 2400 or 3600 groves per millimeter.

In sequential spectrometers scanning of a wavelength range is typically accomplished by rotating the grating with a digitally controlled stepper motor so that different wavelengths are sequentially and precisely focused on the exit slit. Alternatively, the grating is fixed and the exit slit and the detector are being moved. Some instruments have two sets of slits and detectors, one for the UV region and one for the visible (Figure 3). With complex spectra made up of hundreds of lines, scanning a significant wavelength region is impractical. To overcome this problem, the monochromator scans very rapidly, to a wavelength near the line of interest. The scan rate is then quickly reduced so that the instrument scans across the line in a series of small steps (0.01 to 0.001 nm). Instruments which use this technique are called slew-scan (or fast scanning) spectrometers.

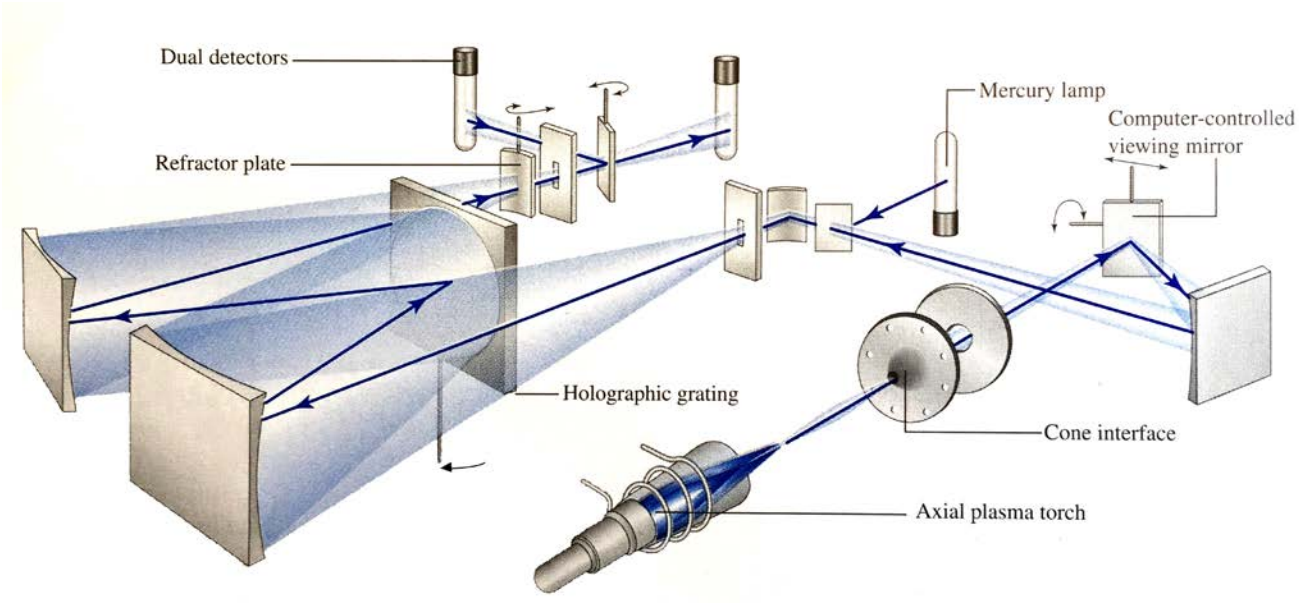

Figure 3. Optical diagram of a sequential emission spectrometer. All moving parts are under computer control and their modes of motion are indicated by the arrows. Moving parts include the holographic grating, a mirror for transducer selection, a refractor plate for optimizing signal throughput., and the viewing mirror to optimize the plasma viewing position.

A simultaneous multichannel instrument incorporates either a polychromator or a spectrograph. Polychromators contain a series of photomultiplier tubes for detection, but spectrographs use two-dimensional charge-injection devices (CIDs) or charge-coupled devices (CCDs) as transducers.

The Agilent MP-4200 instrument used for this experiment directs axial emission from the vertical oriented nitrogen plasma into the fast-scanning monochromator optics. Wavelength specific emissions are detected using a high efficiency CCD.

2 Applications of Plasma Sources

Plasma sources produce spectra rich in characteristic emission lines, which makes them useful for both qualitative and quantitative elemental analysis.

2.1 Sample Preparation

Samples for analysis are typically dissolved or suspended in aqueous or organic liquids. With plasma emission, however, it is possible to analyze solid samples directly utilizing electrothermal vaporization, laser or spark ablation, and glow-discharge vaporization. Suspension of solids in solutions can also be handled with specially designed nebulizers (Babington).

2.2 Elements Determined

Plasma emission spectrometry is generally limited to the determination of about 70 elements. While all metallic elements can be determined without difficulties a vacuum spectrometer is necessary for the determination of boron, phosphorus, nitrogen, sulfur, and carbon because the emission lines for these elements lie at wavelengths less 180 nm, where components of the atmosphere absorb radiation. The usefulness for the alkali metals is limited by, first, the operating conditions which are suitable to accommodate most other elements are unsuited for alkali metals and, second, the most prominent lines of Li, K, Rb, and Cs are located at near-infrared wavelengths, which lead to detection problems with many plasma spectrometers that are designed primarily for ultraviolet detection.

2.3 Line Selection

More than 70 elements have several lines that can be used for identification and determination purposes. Thus, a suitable line for determination of any of these elements can usually be found. Selection depends on a consideration of what elements other than the analyte may be present in the sample and whether there is any likelihood that lines of these elements will overlap analyte lines.

2.4 Calibration Curves

For quantitative analysis of an analyte a calibration curve is obtained by plotting an electrical signal proportional to line intensity versus analyte concentration for several standards. Usually, calibration plots are linear, however, departures from linearity might occur when large concentration ranges are covered. A major cause of nonlinearity is *self-absorption*, in which the output signal is reduced because of absorption by ground state atoms in the medium. Self-absorption becomes evident at high analyte concentrations and causes the calibration curve to bend toward the horizontal axis. Other possible sources of nonlinearity of calibration plots include erroneous background correction, ionization, and nonlinear response of the detection system.

To compensate for several types of both random and systematic errors an internal standard method is often used in emission spectrometry. An internal standard is a substance that is added in a constant amount to all samples, blanks, and calibration standards in an analysis. Calibration then involves plotting the ration of an analyte signal to the internal-standard signal as a function of the analyte concentration of the standards. This ration for the samples is then used to obtain their analyte concentrations from a calibration curve. The improvement in the calibration curve is observed when the internal standard is used if the analyte and internal-standard signals respond proportionally to random instrumental and method fluctuations, the ratio of these signals is independent of such fluctuations. If the two signals are influenced in the same way by matrix effects, compensation of these effects also occurs resulting in smaller scatter of the calibration plots.

2.5 Interferences

At low analyte concdentrations the argon or nitrogen ions in plasma recombine with electrons giving rise to the background emission. To correct for this type of interference background readings are taken on either side of the line of interest. Modern instruments usually include software designed to make background correction automatically or under operator control.

Another type of interference commonly encountered in Microwave Plasma (MP) or Inductively Coupled Plasma (ICP) spectra for many elements are – spectral interferences – which are always possible due to the multitude of the lines present. To avoid these type of errors requires knowledge of all the components of the analyte solution, which is not always possible. The software for modern instruments is usually equipped with a database of spectral lines which can assist in selecting lines which do not cause spectral interferences. However, it will not prevent interferences from elements in the sample solution the user is not aware of.

2.6 Detection Limits

Detection limits with plasma sources are generally comparable or better than other atomic spectral procedures. Howere, detections limits are not the same for all elements. Some elements might have detection limits below 1 ppb while for others the detection limit might be around 500 ppb.

3 Experimental

Milk is a complex colloidal system with distinct components such as fat emulsion, casein micelle suspension, and an aqueous phase [1]. It has been demonstrated that some analytes can be distributed differently in each of these phases [2] and problems related to sample nebulization and atomization interferences may compromise accuracy and precision in the direct analysis of milk solutions. An interesting strategy to overcome some of these problems is to dilute samples in a mixture of tertiary amines. This reagent can dissociate casein micelles and stabilize cations present in the aqueous phase.

In this experiment a microwave plasma atomic emission spectrometer, the Agilent 4200 MP is used for elemental determination of Ca, K, Mg, and Na in fresh milk and powdered egg whites after acid digestion. Quality assurance is performed by analyzing a Certified Reference Material (CRM).

3.1 Standards and reagents

Analytical grade concentrated nitric acid (HNO_3 67–69%) was used for sample digestion. The deionized water used was obtained from a Milli-Q™ Water System. Calibration and accuracy verification standards were prepared using Agilent Calibration Standards.

3.2 Sample preparation

Milk samples were purchased from a supermarket in Houston and need to be digested before analysis by MP-AES.

To prepare egg and milk samples, accurately weigh and transfer 0.25 g of powdered egg whites, and (separately) 1 g of fresh milk into clean vials. Add 6 mL of nitric acid to each vial. Place the samples on a hot plate in a fume hood and gently heat, avoiding boiling, for about 30 minutes. Prepare the egg and milk solutions in triplicate for the experiment.

Prepare at least 600 mL of 5% nitric acid to be used as a blank and and in the next step.

Dilute each sample to a total volume of 10 mL with deionized water. Further dilute each solution to 100 mL with the 5% nitric acid.

If it is not already made, a reference solution of the Certified Reference Material with a concentration of 500 mg/L in 5% HNO_3 should be diluted to a concentration of 50 mg/L (1 mg/L = 1 ppm) to make a stock solution.

Use the 50 ppm Certified Reference Material stock solution to prepare four calibration standards with concentrations of 10, 5, 1, and 0.1 ppm in 5% nitric acid in order to be within the linear concentration range of the MP-AES instrument.

Prepare seven samples of Certified Reference Material (5 ppm in 5% nitric acid) in order to evaluate the accuracy of the analytical procedure.

3.3 Elemental determination

3.3.1 Starting the system

The plasma is extremely hot (about 6000 °C) and operates using high-levels of microwave energy. The plasma emits high intensity light. Always wear appropriate eye protection if viewing the plasma. Close contact with the operating plasma can result in severe heat burns to the skin, and exposure to the microwave radiation can cause sub-surface skin burns.

Refer to the Figure 5 for an overview of the MP-AES instrument

1. Check that the exhaust and intake lanes are secured to the MP-AES instrument.
2. Ensure that gas lines are connected to the MP-AES instrument and that the gas supplies are turned on and

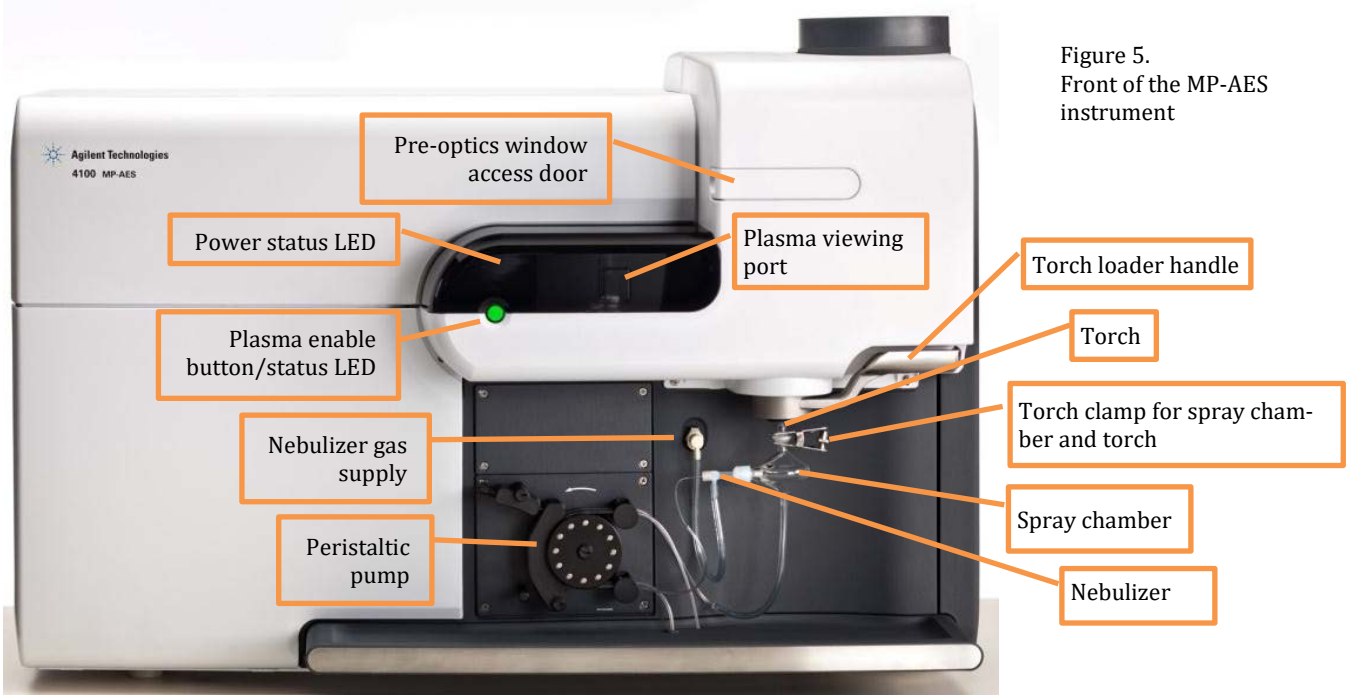

Figure 5.
Front of the MP-AES instrument

set to the correct values. **Turn on the air compressor and the nitrogen generator**.

3. Check that the preoptic window is installed and the interlock is engaged.
4. Check that the torch is clean and in a good condition.
5. Insert the torch and completely close the torch handle. Fit the spray chamber socket to the ball joint on the base of the torch and secure using the torch clamp.
6. Check that all tubing on the spray chamber, nebulizer and peristaltic pump are correctly connected.
7. Switch on the computer.
8. Turn on the MP-AES instrument.
9. Ensure that the Plasma Enable Switch is in the Enable state (pushed in).
10. Start the MP Expert software.
    a. Click Instrument (top-left) and in the dialogue box, click "start purge"
    b. Wait 30 minutes for the instrument to warm up and the nitrogen to reach maximum purity within the system.

3.3.2 Preparing for analysis

The plasma cannot be run without the spray chamber and nebulizer gas supply connected. Doing so will damage the torch.

1. Click the plasma button in the MP Expert software. Alternatively, choose **Plasma On** from the arrow under the **Plasma** button.
2. Ensure the peristaltic pump is correctly set up (refer to the Peristaltic Pump section of the Help). Adjust the pressure bars on the peristaltic pump for even sample flow.
3. Place the sample tubing from the peristaltic pump into the rinse solution and the drain tubing into the drainage vessel
4. Click the **Pump** button in the MP Expert software and choose **Normal (15 rpm)** from the arrow under the **Pump** button. The pump will be initialized and the solution will begin aspirating.

3.3.3 MP-AES Instrument Wavelength Calibration

Instrument wavelength calibration is achieved using an Agilent calibration standard with multiple elements dissolved in 5% nitric acid. This is completed before any experiment is run.

3.3.4 Running Samples

1. Create a new worksheet. Click New from the Start page or the file menu.
2. On the 'Elements' page, select the element from the 'Element' drop-down box or type the element name

or symbol. In this experiment, you will be looking at Ca, K, Mg, and Na. To select a wavelength you wish to use do one of the following.

a. Click  to select the primary wavelength for the selected element.
b. Highlight the wavelength you wish to use from the list of available wavelength displayed and click **Add.**

The element will appear in the table with the selected wavelength and default settings selected.

3. Check that there are no interferences or other analytical lines close to the selected analytical line. Their relative frequencies will govern how close the lines can be to each other. For example, if your matrix contains an element that is not of analytical interest, but is a potential interferent and has a line close to one of the selected analytical lines, the concentration of that element in the matrix will determine whether you need to choose another analytical line.
4. Make any required adjustments to each element including selecting a different wavelength, entering additional information into the "Label" column, selecting the type of sample (choose from analyte, internal standard, interferent) and the type of background correction is to be used.
5. In the 'Conditions' page, allow for auto background correction, and 3 second read times. Use 3 replicates for each solution at a normal pump speed (15 rpm) with manual sample introduction. Allow for a 15 second uptake time and a stabilization time of 15 seconds.
6. In the 'Standards' page add the number of standards to be used for making a calibration curve of solutions with known concentrations as stated in the sample preparation section. Type in the corresponding concentrations of each element for each of the calibration standards (i.e. for the 10 ppm calibration standard, each element will have a concentration of 10 ppm.) Allow for linear calibration fits for each of the elements studied.
7. In the 'Sequence' page, add the appropriate amount of samples as described in the sample preparation section. Do not forget to include the Certified Reference Material solutions. Make sure to label each of your samples appropriately.
8. Before running the method you have just set up, click on the 'Analysis' page and ensure that all the samples are selected. Save your worksheet before continuing on to the next step.
9. Click 'Run' at the top of the page. A dialogue box will appear for you to "Present blank". Insert the solution tubing into your blank solution and push the pressure bar tensioners against the pressure bars until you hear a click. This will put pressure on the peristaltic pump tubing and cause the blank to be pumped into the spray chamber. Press "Continue" to begin data acquisition. Note that the program will collect three points for each element for each of the samples run.
10. Following the same procedure as for the blank, run the four calibration standards in the order in which they were written into the program in step 6. The program will automatically generate a calibration curve of concentration versus intensity for each of the elements. This curve will be used to determine the concentration of each element in the egg and milk samples.
11. Run the digested egg whites and milk samples, then the Certified Reference Material solutions.
12. Print the report of the gathered data, including the generated calibration curves.

3.3.5 Turning off the MP-AES Instrument

1. Rinse the spray chamber by aspirating DI water for a few minutes.
2. Remove the solution tubing from the water and using the peristaltic pump, pump all solution from the sample line, nebulizer, and spray chamber. Continue to run for another 30 seconds to ensure that the sample introduction system is solution free.
3. Extinguish the plasma by choosing **Plasma Off** from the **Plasma** button drop-down arrow. The peristaltic pump stops automatically when the plasma is extinguished.
4. Loosen the pressure on the peristaltic pump tubing by releasing the pressure bars.
5. Turn off the main power switch on the instrument and the nitrogen generator.

4 Discussion Questions

4.1 Calibrations and Concentrations

1. In your lab report, include the calibration curves for each of the elements analyzed during the experiment. Comment on the R^2 values of the plots. How could they be improved?
2. Using the data for the three egg white samples, calculate the average, standard deviation, and percent relative standard deviation (%RSD) for the concentration of each element. Do the same for the three milk samples. Do the samples show good reproducibility between each other?

3. Calculate the amount of each element (in mg/kg, mg of element per kg of egg or milk) originally in the egg whites and milk. Include and comment on the relative standard deviations (%RSD).

4.2 Quality Assurance and Applications

1. Calculate the average concentration (in ppm), and its standard deviation, for each element in the Certified Reference Material solutions. Does the calculated concentration match the reference value of 5 ppm per element? Comment on the precision and accuracy of the MP-AES instrument.
2. Describe a real-world application of the MP-AES technique. (Think of the type of information you can gather from this technique. Answer the question: in what sort of industry experiment would knowing such information be useful and how?)