A Spectrophotometric Analysis of Calcium in Cereal

In this laboratory exercise, we will determine the amount of Calcium in a serving of cereal. We will do this by grinding and ashing the cereal, dissolving the remains in a strong acid and then performing a Flame Atomic Absorption (AA) analysis on the resulting solution. Because the cereal matrix may interfere with the analysis, we will use a Standard Addition methodology in performing the analysis.

Spectrophotometric analyses rely on the ability of the analyte (molecule, atom, or ion) to absorb or emit electromagnetic radiation. In Absorbance Spectroscopy an incoming photon interacts with the analyte, and if the energy of the photon matches the energy difference between two of the analyte's quantum states, then the photon is likely to be absorbed.

In general, solution phase Absorbance Spectroscopy suffers from rather low sensitivities and broad absorbance bands. Atomic Absorbance Spectroscopy provides for much better sensitivity to the analyte, an ability to resolve multiple elements simultaneously via sharp absorbance bands, and a quick method of analysis.

In Atomic Absorbance Spectroscopy, the analyte is first atomized and the resulting gaseous atoms are excited by absorption of radiation at an appropriate frequency. There are several techniques available for atomization of a sample; flames, furnaces or plasmas. In our case, we will first “ash” our cereal sample by combusting away the organic constituents of the cereal, leaving behind the calcium. The ash will next be dissolved in strong acid and then we will use an Air/Acetylene flame to atomize the calcium in the solution.
In Flame AA, a nebulizer first creates a fine mist from the analyte solution. This mist is then mixed with the flame’s fuel and is then injected into a burner. Within the flame, the sample is atomized; free but unexcited gaseous atoms of the analyte are generated. Light from a Hollow Cathode Lamp impinges upon the flame, exciting the analyte atoms. This then passes into a monochromator where the photons of different wavelengths are dispersed. The appropriate wavelength is then selected and the radiation passes to a detector. The resulting signal is then analyzed and passed to a Readout Device such as a computer monitor where the spectrum is displayed.

The Transmittance of the source radiation:

\[ T = \frac{P}{P_0} \]  
(Eq. 1)

is converted to its Absorbance (A):

\[ A = -\log T \]  
(Eq. 2)

And, the Absorbance, as usual, follows Beer’s Law:

\[ A = \varepsilon b c \]  
(Eq. 3)

where \( \varepsilon \) is the Molar Absorptivity of the analyte atoms, \( b \) is the path length and \( c \) is the concentration. In the case of Flame AA spectroscopy, it is difficult to measure the pathlength of the light source through the flame and the concentration of analyte species in the flame is not knowable directly. However, the pathlength through the flame can be held constant. And, the nebulizer uses a constant flow of analyte solution such that the concentration of analyte in the flame is proportional to the concentration of the analyte in the solution. Thus, we expect, the Absorbance will be proportional to the concentration of the analyte in solution:

\[ A = mc + b \]  
(Eq. 4)

where \( m \) is a proportionality constant (slope of the calibration curve) and \( b \) is the instrument response when the analyte concentration is zero (y-intercept of the calibration curve).

In this laboratory exercise, we will use a Perkin Elmer A Analyst 100 spectrometer to make the needed absorbance measurements.
This is a double beam instrument set-up for an Acetylene flame and background correction. A Calcium Hollow Cathode Lamp with a resonance line at 422.7 nm will be used as a light source.

We will take a pulverized sample of our cereal and Dry-Ash it in a Muffle Furnace to destroy the organic matter present. The sample will then be dissolved in a 6M HCl solution which is then injected into the Flame AA spectrometer. The resulting Ca absorbance signal will be measured to determine the concentration of the Calcium in the original cereal sample.

In many types of analytical methods, the other constituents in the sample besides the analyte (the “matrix”) can affect the response of the instrument to the analyte. Flame AA is known for being strongly affected by a sample’s matrix. For example, in a sample containing both phosphate and calcium, the phosphate can bind to the calcium and prevent it from being atomized and detected during the flame AA analysis. In some cases, it is possible to add substances to the sample to be analyzed to prevent or reduce a specific matrix effect. In the case of phosphate and calcium, lanthanum ion (La$^{3+}$) is often added to the sample. The La$^{3+}$ is strongly bound by the phosphate, freeing the calcium ion to be detected during the flame AA analysis. In this case, the lanthanum ion is referred to as a “releasing agent.”

Because the cereal “matrix” may affect the absorption signal, we will use a two-point Standard Addition technique in analyzing our sample. Standard Addition techniques are useful when the
sample composition is unknown and the sample matrix, everything in the sample except the analyte, may influence the signal from the analyte.

In the Standard Addition methodology, a known quantity of the analyte is added to the sample. From the increase in signal due to the added analyte, the amount of analyte in the unknown can be deduced.

In the two-point Std. Addition method, a volume \( V_{\text{sam}} \) of the unspiked analyte solution at concentration \( C_{\text{sam}} \) is diluted to a much larger total volume \( V_f \). Another aliquot of the sample, of the same volume, is then spiked with a volume \( V_{\text{std}} \) of a standard solution of the analyte of concentration \( C_{\text{std}} \) and also brought to the same final volume \( V_f \). Because the Absorbance measurement is proportional to the Concentration of the analyte (see Eq. 4), we have:

\[
A_{\text{sam}} \sim \frac{(C_{\text{sam}} V_{\text{sam}})}{V_f} \quad \text{(Eq. 5)}
\]

and:

\[
A_{\text{sam+std}} \sim \frac{(C_{\text{sam}} V_{\text{sam}} + C_{\text{std}} V_{\text{std}})}{V_f} \quad \text{(Eq. 6)}
\]

where \( A_{\text{sam}} \) is the absorbance of the unspiked sample and \( A_{\text{sam+std}} \) is that of the spiked sample. A simple ratio of these two equations yields:

\[
\frac{A_{\text{sam}}}{A_{\text{sam+std}}} = \frac{(C_{\text{sam}} V_{\text{sam}})}{(C_{\text{sam}} V_{\text{sam}} + C_{\text{std}} V_{\text{std}})} \quad \text{(Eq. 7)}
\]

This can then be solved for the concentration of the analyte in the unspiked sample:

\[
C_{\text{sam}} = A_{\text{sam}} C_{\text{std}} V_{\text{std}} / (A_{\text{sam+std}} - A_{\text{sam}}) V_{\text{sam}} \quad \text{(Eq. 8)}
\]

For our analysis, we will crushed our cereal sample and dry-ash it in a muffle furnace to remove the organic matter. The residue will then be taken up in an acidic solution. The Calcium absorbance of an unspiked aliquot, and that of a spiked aliquot, will both be taken. From these measurements, the concentration of the Calcium in the unspiked sample can be determined. This will then allow us to determine the amount of Calcium in the original cereal sample.
Pre-Lab Calculations

1. 1 L of our Standard Ca solution is to be prepared at 1000 µg/mL from primary grade Calcium Carbonate (CaCO₃) that has been dried. How many grams of the primary standard are required to make this solution?

2. This standard is to be diluted to 20 µg/mL to produce 500 mL of Working Standard. How many milliters of the Primary Solution are required to make the Working Standard?
Procedure

Sample Preparation

1. Crush a 5g sample of the cereal using a mortar and pestle.

2. Place ~0.5g of the mashed cereal into each of 3 weighed glazed silica crucibles and weigh the crucibles again.

3. Place each sample and an empty crucible in a muffle furnace at 600°C for two hours.

4. Remove the crucibles from the furnace and allow them to stand for 30 minutes.

5. Add 5 mL of 6M HCl to each crucible. This should dissolve the residue.

6. Transfer each solution to a separate 100 mL volumetric flask. (Be sure to rinse the crucibles appropriately.) Dilute each with distilled Water and mix thoroughly.

7. For each sample, pipet a 5 mL aliquot into a 50 mL volumetric flask, add 5 mL of La matrix modifier and dilute with distilled Water to the mark.

8. For the Standard Addition method, pipet another 5 mL aliquot into a 50 mL volumetric flask. Pipet 20 mL of a 20 µg/mL standard Ca solution into this flask as well. Add 5 mL of La matrix modifier. Dilute to the mark with distilled Water.

9. Repeat this process for each sample.

10. For the blank, simply dilute a 5 mL aliquot with 5 mL La matrix modifier to 50 mL in a volumetric flask.

Absorbance Measurements

1. Your laboratory instructor will demonstrate the use of the Flame AA spectrometer.

2. Measure the absorbance of the 422.7 nm Calcium line for each spiked and unspiked solution, as well as the blank.

3. Subtract the absorbance value of the blank from each of the other measurements.

4. Determine the Concentration of Calcium ($C_{sam}$), in units of µg/mL, for each of the three samples. Use these determinations to calculate the amount of Calcium in the three cereal samples. Report this in units of µgCa/gCereal.
5. Use the average of these results and the Serving Size of the cereal to calculate the %RDA for Calcium in the cereal. The Recommended Dietary Allowance is 1200 mg Ca for an age group of 19-24 years.