# Iron in Breakfast Cereal by Atomic Absorption Spectroscopy (AAS)

**Background:** Iron is one of the most important of the essential minerals in our diet, because it is the element that is responsible for the oxygen-carrying ability of hemoglobin in red blood cells. Iron is also present in a number of other biologically significant molecules. In this lab you will analyze breakfast cereals, to find out the actual iron content of different brands of cereals, and to compare the experimental results with the values listed on the manufacturers' labels. The analytical technique to be used in this determination is atomic absorption spectrophotometry.

The procedure was developed from P. H. Laswick, *Journal* of *Chemical Education,* 50,132 (1973). Background information on atomic absorption spectroscopy (AA or AAS) can be found in analytical chemistry textbooks, such as *Fundamentals* of *Analytical Chemistry,* by Skoog, West, Holler, and Crouch. Seek out and read the appropriate chapters and /or sections according to your background.

**Procedure:** Choose a cereal that has sufficient iron to be analyzed - your choice must contain at least 25% United States Recommended Daily Allowance (USRDA). Weigh accurately (analytical balance) three samples, each of ~ 5 g of a cereal[1]. Put each sample into a 250 mL beaker and add 50 mL of 3 M hydrochloric acid. Cover the beakers with watch glasses and place them on a hot plate. Simmer gently for about 45 minutes, to break down the cereal and bring the iron into soluble form. Let the samples cool somewhat, and then wash down the watch glasses and sides of the beakers with small quantities of distilled water. Stir thoroughly, filter (by gravity) into 100 mL volumetric flasks, dilute to the mark, stopper and mix. Larger volumetric flasks are not advised, since the sample may be too dilute to obtain an accurate measurement of iron concentration.

If you are analyzing a food other than cereal you need to take a sample that will give approximately 35-50 mg/L of iron. Consult the manufacturer's label for information; information may be given as percent of the RDA (Recommended Daily Allowance). You will need to consult a literature source to ascertain the current RDA of iron.

Prepare standards as described below. Set up the atomic absorption instrument for iron and measure the absorbance of the standards and the sample, at the same time. (Note: The instructor will set up the instrument and demonstrate its operation; written operating instructions will also be provided).

Note: all digested cereal solutions must be centrifuged and decanted into a clean container to be made suitable for aspiration into the AAS. Any particulate matter that is accidentally sucked into the inlet tube can clog the instrument causing great problems. A centrifuge will be available near the instrument; you need only centrifuge enough for the measurements, thus a test tube full is likely to be enough. This can be done right before the sample is aspirated.

**Preparation of Standard Solutions:** A calibration curve must be prepared **each time** the atomic absorption spectrophotometer is used, because the conditions of the flame change each time the instrument is turned on. Work with a partner to prepare the standards; one partner will use one of the two methods described, the other partner will do the other of the two methods. Decide in advance, which method each partner is to use. Be certain to have both sets of standards and all samples from both partners ready, before you start you absorption measurements.

A 1000 mg/L stock solution **may** be supplied. If not, you must make a stock solution that has a concentration of ~1000 mg/L of iron. Elemental iron is a primary standard. Iron is readily oxidized by more concentrated HCl solutions to form aqueous iron chloride. Check you calculations with the instructor (weight of iron and conc/amount of HCl required) before you proceed.

Using pipets and volumetric flasks prepare a minimum of seven quantitative standard iron solutions in the *approximate* range of 5 mg/L, 10 mg/L, 15 mg/L, 25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L. Note: Depending on the sizes of pipets available, you may wish to prepare standards having concentrations slightly different from those listed above. Just make sure that you have at least seven standards that span the range between ~5 mg/L and 100 mg/L. You should need a total of no more than 100 mL or so of the 1000 ppm stock solution.

Preparation of standards is *crucial* to making accurate quantitative spectrophotometric measurements. Your data will only be as good as your standards. So, when planning the preparation of these solutions, consider how to keep the relative errors in concentration as low as possible.

**Method A:** Prepare a stock solution of 100mg/L. Use this stock solution to prepare the other standards by **serial** dilution. Keep careful records of *exactly* how you prepare each solution, *i.e.* volume of concentrated solution pipetted (size of pipet), size of receiving volumetric flask.

**Method B:** Prepare a stock solution of 100mg/L. Use this stock solution to prepare the other standards by taking the appropriate quantity of it for *each* whenever possible. (This method is often referred to as "parallel" dilution, or "direct" dilution"). As in Method A, keep careful records of *exactly* how you prepare each solution, *i.e.* volume of stock solution pipetted, size of pipet, size of volumetric flask.

**Waste Disposal**

All waste from this experiment will be collected in the **large blue non-hazardous waste container**.

In order to avoid carrying glassware around, and to avoid undo foot traffic in the laboratory, used chemicals will first be collected in the wide-mouthed polyethylene containers that are stationed at each laboratory bench. These used-chemical containers must remain in their secondary containment tray while in use, and must remain covered as much as possible. When the used-chemical container becomes approximately two thirds full it is to be dumped in the large blue (55 gallon) nonhazardous waste collection drum. Be sure to screw on the white lid before transporting the used-chemical container.

**Data Collection**

Measure the absorbance of your standards and unknowns in the following order:

- One partner's set of standard solutions (the complete set), starting from the least concentrated solution.
- Both sets of samples (3 for each partner).
- The other partner's standard solutions.

On the day that you take your measurements, both partners **must** be present; all absorption measurements on the standards and samples must be completed in a single run. Remember: *all digested cereal solutions must be centrifuged and decanted into a clean*

*container (test tube) to be made suitable for aspiration into the AAS.* Use the centrifuge placed by the AAS to spin down your samples as you work with your first set of standards.

Use distilled water containing a little hydrochloric acid (~ 1 M) as the blank; you will "zero" the absorption meter using this blank. Take the absorption readings for each of the prepared standard solutions, and for the sample. Always aspirate the blank for 1-2 minutes between the absorption readings of two solutions in order to clean up the aspirator and make sure that the blank readings are consistent. After measuring the last solution, aspirate distilled water **for a full 5 minutes** to ensure that the aspirator and flame head are clean.

**Data Analysis Methods**

Since each set of partners will have prepared standards according to Method A and Method B, you will be able to compare the accuracy and precision of standards prepared by both methods. When you plot both data sets (computer!), you may elect to use the set of standards that appears to have the least error, and then analyze the data from your sample using that set of standards. Decide carefully, for you must justify your choice of standardization curves.

Everyone's standardization data and sample data will differ, so each of you must decide how to handle your particular data. There are several reasonable methods (also known as models) to try and find the best method for your data. Note: the sample data shows the results of applying these models on the sample data given. Results for your data will vary.

The simplest method is a linear fit (models A and B), but first you must see if Beer's Law will apply. When the instrument is well aligned (and the lamp is good), the lower concentration standardization data will conform to Beer's Law. Generate plots of absorption versus concentration for the two sets of standard solutions. If the absorption of the blank is zero, then the plot should give a straight line passing through the origin at low to moderate concentrations (Why?). You should expect some deviation from your linear fit at higher concentrations. You can use this method *if your sample data falls in the linear region.* Perform a linear regression on a partial set of your standardization data (model B): include only the data points that fall within the linear region. Read the iron concentration of the sample (cereal) solution for the corresponding absorption from the plot that appears to have fewer errors.

If the standardization is curved in the region where your samples fall, you will need to employ a more sophisticated method of analysis. You may be able to **fit** your standardization data to a *higher order polynomial function* (model C polynomial expansion). For poorer data, a quadratic equation must be used (why?), but for reasonable data, usually a fourth-order polynomial fit will work. That is to say that the absorbance function (standard curve), which is a function of concentration, is approximate by a function such as:

$$A_{Fe}(c) = a_0 + a_1 c_{Fe} + a_2 C_{Fe}^2 + a_3 C_{Fe}^3 \ldots$$

where the coefficients $a_0$, $a_1$, $a_2$, $a_3$, etc. come from the polynomial fit. If the instrument was correctly calibrated, $a_0$ should be zero (why?). Beer's Law is just the first order term of the expansion, assuming a correct blank.

Note: If you are using a higher level mathematics package (more than a spreadsheet), you should try fitting using a better choice of expansion functions, such as an nth root truncated set. If you plan to try this, ask for help.

Yet another (and more approximate) method is to estimate the curve by *local linear segments* (model C). Using this method, the absorbance function (standard curve) is approximated by connect-the-dots lines between the series of data points. The concentration for each sample is calculated from the equation of a straight line for the line segment that encompasses the sample absorbance. This method is much easier to calculate, particularly without the aid of a computer, but not without a cost... The calculated concentrations will **always** be greater than the actual concentrations in solution. This error is lowest for samples that have a measured absorbance that fall near to standardization data points, assuming the standardization data is good. Clearly, this method benefits from additional standardization data points. The major weak point of this method is that each region of the standardization plot *depends only on two data points*. Thus is one data point is *off,* two line segments are affected. Still, the method has merit and may be used if a polynomial fit (or other expansion) is not possible.

**References**


1. P. H. Laswick, *Journal* of *Chemical Education,* 50, 132 (1973).

*2. Contemporary Chemical Analysis,* J. F. Rubinson, K. A. Rubinson, Prentice Hall, 1st ed., 1998.

*3. Fundamentals* of *Analytical Chemistry,* D.A. Skoog, D.M. West, and F.J. Holler, Saunders, 7th ed., 1995.

*4. Analytical Chemistry,* Gary Christian, 4th ed., John Wiley & Sons, 1986.