Georgia Institute of Technology
School of Earth and Atmospheric Sciences

EAS 4641
Spring 2008

Lab 2
Introduction to Quantitative Analysis: Chemistry

Purpose of Lab 2:

1) Understand the general function of analytical instruments and how to calibrate. We will calibrate an IC, this calibration will be used for a later experiment to measure the chemical components of particles in the ambient air.

An IC (Ion Chromatograph) is an analytical device for quantitatively measuring the concentration of ions in aqueous samples. The instrument separates the various ions in a liquid flow and measures the conductivity of the water. To determine ion liquid concentrations a calibration curve must be constructed that will relate conductivity to liquid concentration. This is done be making liquid standards of known concentration and running them through the IC to determine the associated conductivity for each sample. To make the liquid standards Volumetric Glassware and Serial Dilutions are discussed.

Volumetric Glassware

Volumetric laboratory glassware includes volumetric flasks, pipettes, and burettes. Beakers, graduated cylinders, Erlenmeyer flasks, and test tubes are not volumetric glassware. Volumetric glassware is calibrated by the manufacturer to deliver (TD) or contain (TC) a known exact volume of liquid at standard conditions: 20 degrees Celsius and 1 atmosphere pressure. Volumetric glassware will have identification of the volume and the volume tolerance etched onto the glass. Often, ambient laboratory conditions are not identical to standard conditions, therefore the volumes dispensed or contained in volumetric glassware are often not the same as the manufacturer's specifications, but within some acceptable limits (± % tolerance) determined by the manufacturer. This slight variation in volume will cause a systematic error when making solutions that are based on volume-volume or mass-volume concentrations.

Serial Dilutions

A serial dilution is a set of solutions with exact concentrations created from a standardized primary solution of a known concentration.

For example, say a student will need to create 5 solutions (5E-5, 1E-5, 5E-6, 1E-6, and 5E-7 M $NaNO_3$) from a primary standard of $5x10^{-3}$ M $NaNO_3$ in order to complete an assignment. To do so, serial dilutions will be required from the primary standard, $5x10^{-3}$

M $NaNO_3$ (Note: the 5x10-3 M $NaNO_3$ standard will be made from a 0.5 M $NaNO_3$ solution). One viable method could be:

5×10^{-5} M = 1 ml of 5×10^{-3} M $NaNO_3$ into 100-ml volumetric flask, fill to the mark, shake well.

1×10^{-5} M = 200 µl of 5×10^{-3} M $NaNO_3$ into 100-ml volumetric flask, fill to the mark, shake well.

(Note, always start with the stock solution).

…and so on.

The mathematics involved when deciding how to construct your serial dilution follows the volumetric law:

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

Where C_1 is the concentration of the stock solution, V_1 the volume of the stock solution to be diluted into the volume V_2, to give the concentration C_2. In this case,

$$C_2 = \frac{C_1 \times V_1}{V_2}$$

For the $NaNO_3$ dilution from 1 M to 0.1 M solution, the relationship will be

$$0.1M = \frac{1M \times 10ml}{100ml}$$

Calibration Curves: How to measure a quantity of interest.

Analytical equipment is usually optimized to detect and report chemical information of one specific factor (chemical reaction, concentration, analyte, or component of the system under investigation) within a specific range of parameters and factor levels. However, not all levels of the factor can be detected. In order to accurately describe the instrument response to factor level intensity, it is necessary to test the response against a series of known standards. This is called calibrating the instrument. The result will be a plot of response vs. factor intensity, known as the **calibration curve**.

Calibration curves are functions of an instrument's responses to a range of **factor levels**. A factor level could be concentration, or chemical potential, or some other measurable quantity. In some cases, the factor level may be too low to be detected. In some cases, the factor level may be too high and overload the detector. The optimal range of a factor level for detection by an instrument is usually narrow. Ideally, this narrow range represents a linear response or output proportional to the factor level intensity. If the

factor level increases, the response increases. If the factor level decreases, the response decreases.

The calibration curve has three distinct regions. (See figure 1). The segment **Below the Limit of Detection** is where the factor level is not great enough or intense enough to produce a signal greater than the background noise of the instrument. There is a high **noise to signal ratio**. The **Linear Region** is the region where the response is proportional to the factor level. As factor level increases, response increases. The linear region extends to the **Limit of Linearity**, or the maximum factor level that will produce a linear response. Any measurements of factor levels greater than the limit of linearity will fall in the region **Beyond the Limit of Detection**, or where the detection device becomes overloaded with incoming data.

The integrity of a calibration curve depends on how much of the entire linear range is defined. In figure 2, three points denote a linear portion of the calibration curve, but there is great uncertainty. Are these points merely coincidence or are they in the linear region? In order to accurately describe such a curve, a series of know standards must be constructed to add detail to the wide interval between points (figure 3)

Figure 1: Calibration Curve

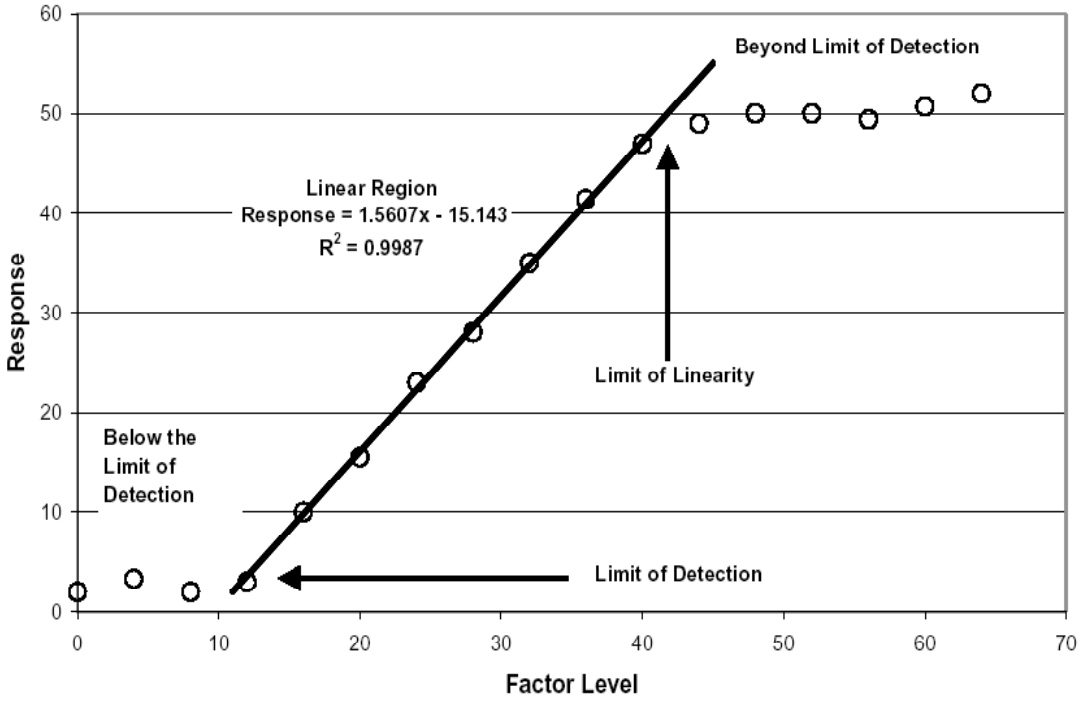


Figure 2: Calibration Curve

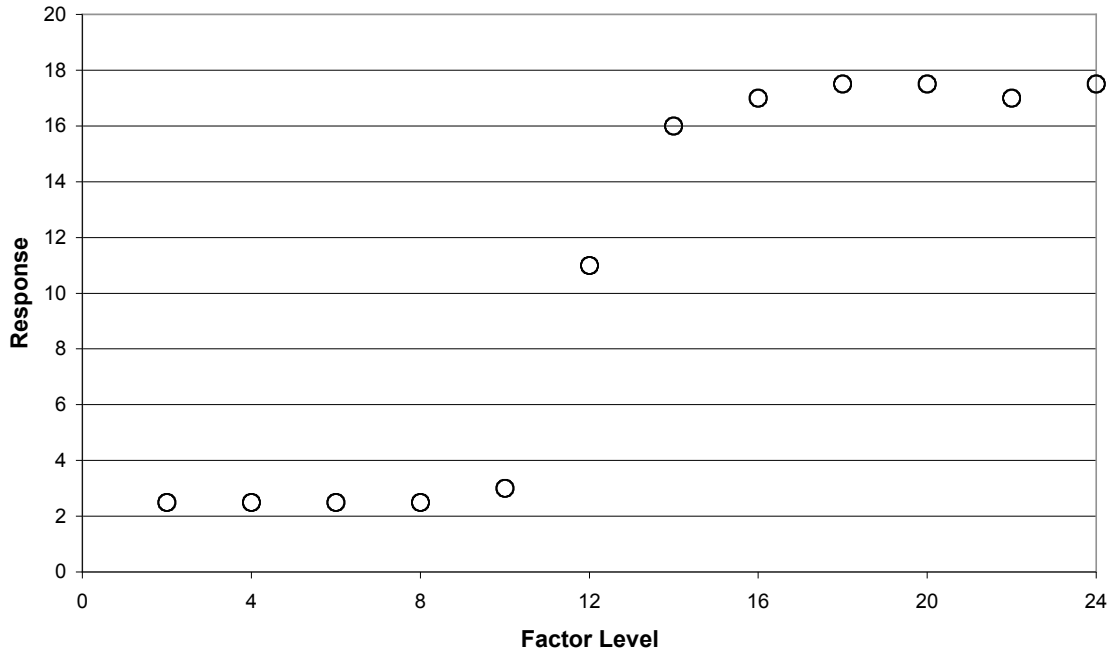
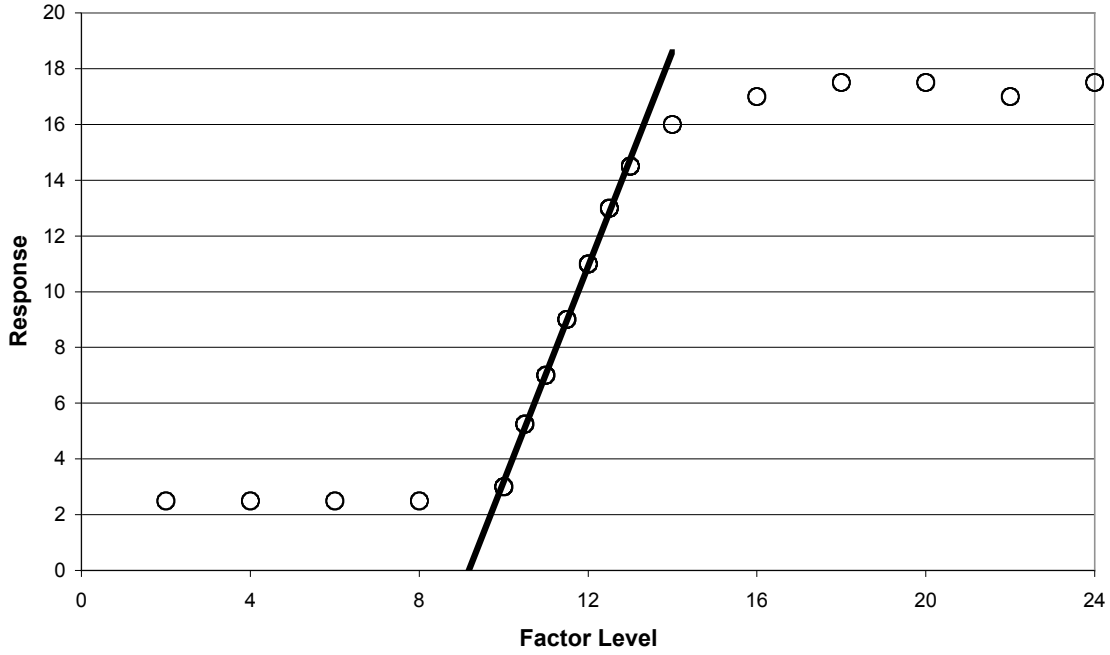


Figure 3: Calibration Curve



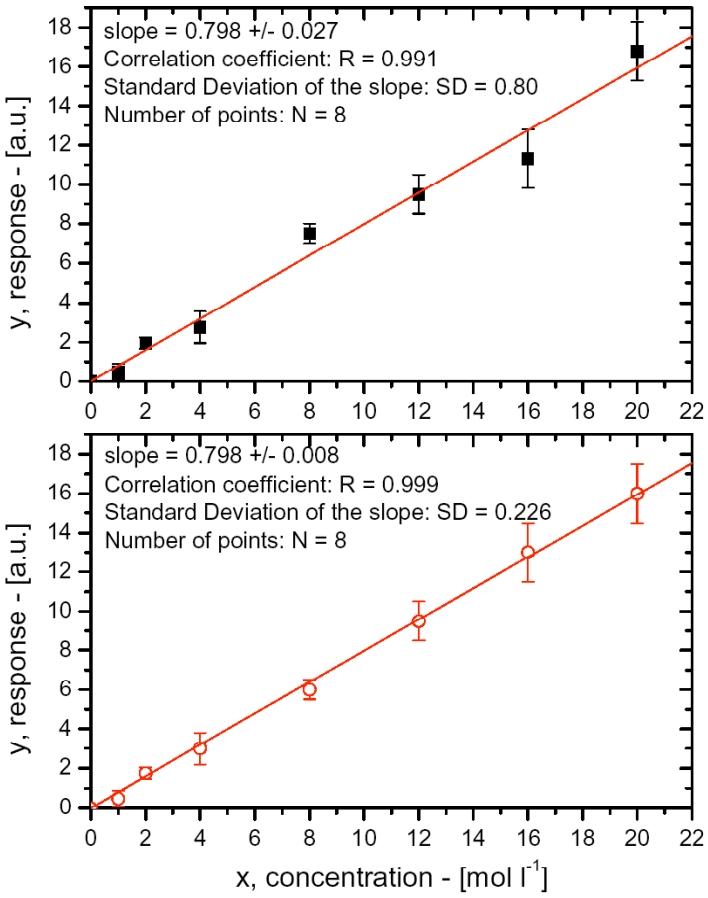
Once a reliable calibration curve has been determined, factor levels for unknown samples can be determined. The general formula for the linear portion of a calibration curve is

$$response = (slope \times FL) + b$$

where, FL is the factor level (intensity, concentration, i.e., unknown to be determined, or the input) and response is the instrument's output. The slope is the analytical slope for the detection of that factor. b is the Y-intercept (note, in many cases b = 0). Once the calibration curve is constructed from a series of standards, the unknown concentrations of a factor in a solution can be determined by rearranging the above equation to the following.

$$FL_{unk} = x_j = \frac{response - b}{slope}$$

Of course, for the calibration curve to be effective the factor must be detected and a response provided. If the response of an unknown falls outside the established linear range of the calibration curve, then additional work is required. If the response falls below the Limit of Detection, then one must concentrate the sample. If the response lies above the Limit of Linearity, then one must dilute the sample. In this lab we will always be in the linear range of the IC.



The figure to the right shows an example of a good calibration curve. The linear regression data is provided and an estimate of the measurement error is included as an error bar associated with each data point.

Experiment 2
Preparing Standard Solutions and Running an Ion Chromatograph

Calibrate an IC and determine concentration of an unknown sample.

We will use commercially available liquid standard stock solutions. The concentrations of Choride, Nitrate, and Sulfate in the stock solution will be provided by the TA. (Stock solution: 50mg/L of Cl^-, NO_3^-, SO_4^{2-})

The goal is to prepare 6 standards that will roughly cover the range of expected ambient concentrations of chloride, nitrate, and sulfate in fine particles. Each of the 6 standard solutions you prepare will contain known concentrations of chloride, nitrate, and sulfate that will be used to create separate calibration curves for each compound (ie Conductivity vs Liquid Concentration). To make the lab easier, the lab groups will be divided into two groups. Group 1 will make standards of 5, 2.5 and 1 mg/L. Group 2 will make standards of 0.5, 0.25 and 0.1 mg/L.

Each lab will make 3 solutions that will contain 3 of the following 6 concentrations: 5, 2.5, 1, 0.5, 0.25 and 0.1 mg/L of chloride, nitrate and sulfate. These will be used to calibrate the IC for the 3 compounds (Cl^-, NO_3^-, SO_4^{2-}). To do this, do the following:

1. Calculate the volume of stock solution you will need to make a solution of 100ml volume for each of your 3 concentrations above.

2. Make sure you have 3 clean 100 ml volumetric flasks. Clean by rinsing 3 times with high purity water. Pour a small (roughly 5 ml) of standard stock into a disposable cup. For each solution, pipette the calculated volume of stock from this dish into a pre-cleaned (rinse 3 times with ultrapure water) 100ml volumetric flask. Fill the volumetric flask to the 100ml mark with ultrapure water. Record the uncertainty in each step of this process (pipette volume uncertainty, volumetric flask uncertainty, and record). Assume the uncertainty of an IC measurement is ±10% (peak area is directly proportional to conductivity in this IC). Using this method make your 3 standard solutions.

3. Make the IC measurement: Fill the IC sample loop with a solution by drawing it in with a syringe. The volume of sample drawn into the sample loop should be sufficient to flush the loop a number of times. Run the IC. With the IC column you are using the peaks will come out in the following order, chloride (lowest retention time = comes out first), nitrate, and sulfate (greatest retention time). Run all you're your groups calibration standards. Record the IC data (area under curve) for each anion (chloride, nitrate, sulfate) for each standard.

4. Clean the volumetric flask by dumping out the contents and rinsing with ultra pure water.

5. The lab instructor will give you a sample of unknown concentration of chloride, nitrate, and sulfate. Run this sample through the IC and record peak areas.

Calculations

All lab groups please send me their calibration results (rweber@eas.gatech.edu). I will post them on the web so that all calibration data can be used to construct the calibration curves.

Include each of the following in your lab report

1. Create a calibration curve (graph) for chloride, nitrate, and sulfate using the standard concentrations your made and the measured IC peak area. Note that **Factor Level** is the concentration of the three ions in solution. **Detection** is the conductivity recorded as a peak area. This is a very important graph. Do a good job; include axis labels with units.

2. Using excel or other software (Matlab etc) calculate the slope (m) and y-intercept (b) for your calibration curve and the uncertainty associated with each (ie, 1 standard deviation). Note that if the data suggests, force the fit through zero so that b =0. On the graph, show the individual data points and the fit line.

3. From the calibration curve determine the chloride, nitrate, and sulfate concentration of the unknown solution. Remember to report the label of your unknown solution along with what you think is the concentration.

4. Estimate the absolute uncertainty and the relative (%) uncertainty for the concentration of the three ions in your unknown solution. You may want to put both x and y error bars on your calibration curves.