CHM 317H1S
Winter 2021

Section A - Flame Atomic Spectrophotometry

E: Atomic Absorption Spectroscopy

1. List of Experiments

1. Calcium–Magnesium Water Hardness

2. Sodium in Drinks by Atomic Emission

2. Locker Inventory

Glassware for each experiment may be found in designated drawers in room LM6. These should include the following items; please report any missing or broken items to your lab TA or the lab coordinator.

✓	Quantity	Item (4 drawers for each experiment)
	6	100 mL glass beakers
	10	100 mL plastic beakers
	1	10 mL graduated cylinder
	1	25 mL graduated cylinder
	2	50 mL volumetric flasks with stoppers
	1	250 mL volumetric flasks with stoppers
	13	100 mL volumetric flask with stopper
	2 ea.	1, 2, 4, and 5 mL transfer pipettes
	4	10 mL transfer pipettes
	1	1.000 mL graduate (Mohr) pipette, in $1/100^{th}$ units
	20	Pasteur pipettes with bulbs
	2	3-Way bulb pipette fillers
	14	100 mL polyethylene storage bottles
	1	250 mL polyethylene storage bottle
	2	Wash bottles
	2 ea.	Glass rods and large spatulas (Scoopulas™)
	1	Filter funnel

In addition, you should have two 20.00 mL transfer pipettes for experiment A1 and a mortar and pestle for experiment A2.

3. Technique Overview

Students should already be broadly familiar with the principles of atomic spectroscopy and the practical aspects of flame atomic absorption spectrophotometry. In brief, sample solutions are aspirated through a concentric nebulized into a spray chamber to generate an aerosol (dispersion of liquid in a gas) with a small drop size distribution. While most of the sample drains from the spray chamber, a small proportion is swept up by a fuel–oxidant mixture through a burner into a flame. The flame strips away any remaining solvent and provides sufficient thermal energy to break down the remaining chemical substances into a population of atoms which are then able to undergo atomic absorption and/or emission as a result of electronic transitions of valence shell electrons. The ability to detect those atoms depends on the hollow cathode lamp (for absorption) or the flame temperature and element (for emission) combined with a suitably sensitive photon detector.

The flame chemistry, combined with sample composition, can play a key role in either contributing to or avoiding both spectral and chemical interferences when analysing real world samples by both emission and absorption spectrophotometry. There are various methods of addressing these concerns: adjustment of fuel and oxidant type and flow rate, addition of suppressing and releasing agents, the use of background correction (BGC) systems, and the method of standard additions. This set of experiments explores all these techniques to supplement content from the prerequisite course and provide context for discussion of advanced techniques such as graphite furnace and inductively-coupled plasma atomic spectroscopy.

4. Instrumentation

During this set of experiments, you will be using one of two flame atomic absorption spectrometers:

- Perkin-Elmer AAnalyst 100 spectrophotometer (AA Winlab Analyst software)
- Perkin-Elmer AAnalyst 200 spectrophotometer (Syngistix for AA software)

Both spectrometers use double beam optics, laminar flow premix nebulizers, automatic wavelength and slit width selection, and automatic deuterium arc background correction. One instrument can optionally be used with a model HGA-800 graphite furnace accessory and AS-72 auto sampler, or a flow-injection hydride generation manifold for mercury determination.

The following pages provide general information on using the flame AA spectrophotometers in the Analest laboratory. Please take time to read through these instructions carefully **before coming to the laboratory.** You may also want to take a quick virtual tour of the instrument –see the course website for details. **Update: the software instructions refer to the AAnalyst 100 only.**

4.1 Starting the Instrument:

(a) Turning the fuel gas on: Make sure that the main valve on the acetylene cylinder in the prep room is on (open it by turning it counter-clockwise). Both gauges on the regulator assembly should register pressure; the outlet (low-pressure) gauge should read at least 15

psi. You should **not** need to adjust the valve on the regulator (the large knob between the two gauges.)

(b) There are additional single-stage regulator valves located in the gangway behind the instruments that runs between the benches. Open the air and acetylene valves by turning them counter-clockwise about two turns. You do not need the argon for these experiments, so leave the valve closed.

(c) If the instrument is not already on, turn it on using the main switch:

- AAnalyst 100: located on the right-side panel at the bottom
- AAnalyst 200: located behind the front door, on the bottom left

Also turn on and log in to the computer adjacent to the spectrometer:

- AAnalyst 100: username: chm317aa2; password: aa2 (experiment E2)
- AAnalyst 200: username: chm317aa3; password: aa3 (experiment E1)

(d) Wait until the instrument has finished its start-up and initialization sequence, then launch the appropriate software using the desktop shortcut ***without*** responding to the prompts on the instrument display.

On the AAnalst100 AA WinLab software:

(1) A window titled **Checking Connections** should open, and should contain a box with a green check mark in it indicating that the computer and instrument are communicating with one another: If the box does **not** show a green check mark, make sure that the AA is turned on. Once all is correct (i.e. the green check-mark is displayed) click on the **Exit** button.

(2) On the splash-screen that appears immediately after dismissing the **Checking Connections** dialog, make sure that the **Technique** pull-down menu is set on **Flame** and then click on the **Workspace** button. Select one of the following file in the resulting file dialog box: **CHM317CM**

4.2 Configuring the Instrument via Software:

The two software packages are laid out a little differently, but function in the same way. You will need to:

- Make sure the appropriate method has been loaded in to the software. The current method and element should be displayed in the application toolbar.
- For atomic **absorption** experiments only:
  - Check that the correct element lamp is present in the instrument.
  - Check the lamp settings: click on the Lamps icon in the toolbar and check the settings in the resulting dialogue, then turn on the lamp.

- Make sure that the sample tube on the nebulizer/spray chamber is inserted into a container (flask or bottle) or ultrapure deionised water – make sure you keep this topped up during the experiment.
- Set a **Results Data Set Name** that is unique for your demo group, and make sure any associated check box is set to save data – this will ensure your results are accessible and can be printed out at the end of the experiment.
- Make sure that the list of standards loaded in the method corresponds to those in the experiment protocol.
- Light the flame ~5 minutes prior to starting your experiments.

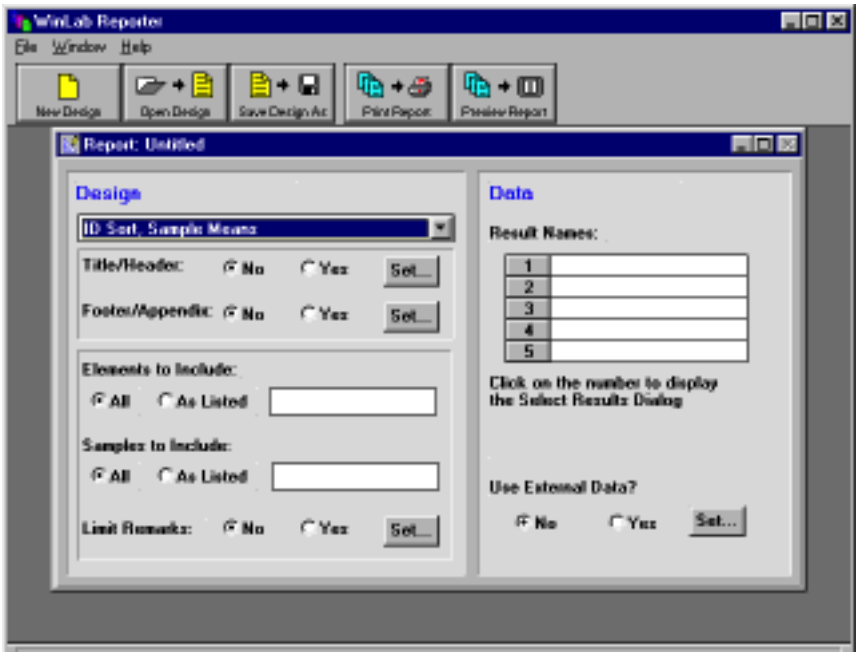
Full instructions for each instrument are included with the relevant experimental procedure.

4.3 Printing Data

Once your experiment is complete, you will need to create and print a report.

In the AA Winlab software:

- Go to the **File** menu and select **Utilities→Reporter**; this will open the **WinLab Reporter** window
- Select **Chronological, Complete** from the pull-down menu just under the **Design** heading, then click on the first number button under the **Result Names:** heading. This will open a dialog within which you can locate and select the name for your data set within the results database. Note that you can choose to sort the entries by either name or date; doing the latter makes it very easy to find your data set, as it will now be first in the list!
- Click on the **PreviewReport** button. In the preview window, click on the printer icon at the bottom to call up the print dialog and send your results to the printer. Note that, if you want to print only selected pages, you *must* use the printer icon button at the bottom of the window - the **Print** option in the **File** menu will *only* print the entire report.



AAWinLab Report Generator dialog box

In the Syngistix software:

- Click on the **Data Viewer** icon, then load your results data set.
- You can print your report in much the same way as the AA Winlab software. You also have the option to export your data as an Excel file. This will be saved in the user data folder for the Syngistix software; locate the file within the Windows desktop, and move it to the shared data drive (shortcut on desktop). You will then be able to copy this file to a USB key using one of the data terminals at the back of LM9.

4.4 Shutting Down the Instrument

(a) Aspirate deionised water for a few minutes while the flame is still on in order to clean the nebulizer/spray chamber from the last sample. In the **Flame Control** window, click the flame switch **OFF** to turn the flame off. Notify your TA that you are done with the instrument, and make sure that you remove ***all*** solutions and samples back to LM6 for cleaning.

(b) Laboratory staff will shut off the gases and bleed the lines so that there is no residual acetylene in them. They need access to the software to do this, so leave the software running and the computer logged in.

5. Experiment A1: Calcium–Magnesium Water Hardness

This experiment is based on the standard procedures for the flame atomic absorption determination of calcium and magnesium described by American Public Health Association in "Standard Methods for the Examination of Water and Wastewater", 20th edition. The same basic procedures are used by water testing laboratories in Canada, Europe, and the US. In addition to tap and fish tank water samples, you should bring in your own sample of bottled water for testing.

Although inductively coupled plasma atomic (optical) emission spectrophotometry is generally faster when analysing multiple elements, both the instrumentation cost and operating expenses are significantly higher than for flame AAS. With only two elements of interest and a large number of samples, flame AAS is therefore preferred for water hardness measurements. The method also compares favourably in terms of ease-of-use with the more traditional complexometric titrations.

After performing this experiment, you should:

- Understand how to use dual element lamps for multi-element AAS analysis
- Develop an appreciation of background correction in flame AAS

Essential Notes:

- When performing this experiment, be sure to note any practical observations, such as flame colour, in your notebook.
- Make sure you label all containers *before* use, and keep careful track in your lab notebook when dispensing solutions to make the required multi-element standards; omitting one of your solutions or using the wrong volume will result in this experiment taking far longer than it should!

Chemicals:

- 100.0 mg/L calcium and magnesium standards for atomic spectroscopy
  (As the metal nitrate in 4% nitric acid)
- Solid lanthanum trinitrate hexahydrate, $La(NO_3)_3{\cdot}6H_2O$ (M_m = 433.03 g/mol)

Sample(s):

- You will be provided with an "unknown" sample, which should be analysed along with the tap water in the lab. You should also bring your own water sample for analysis; check with your demonstrator *the week before* this experiment to find out is appropriate.

AAWinlab Software Toolbar, showing the method for experiment E2

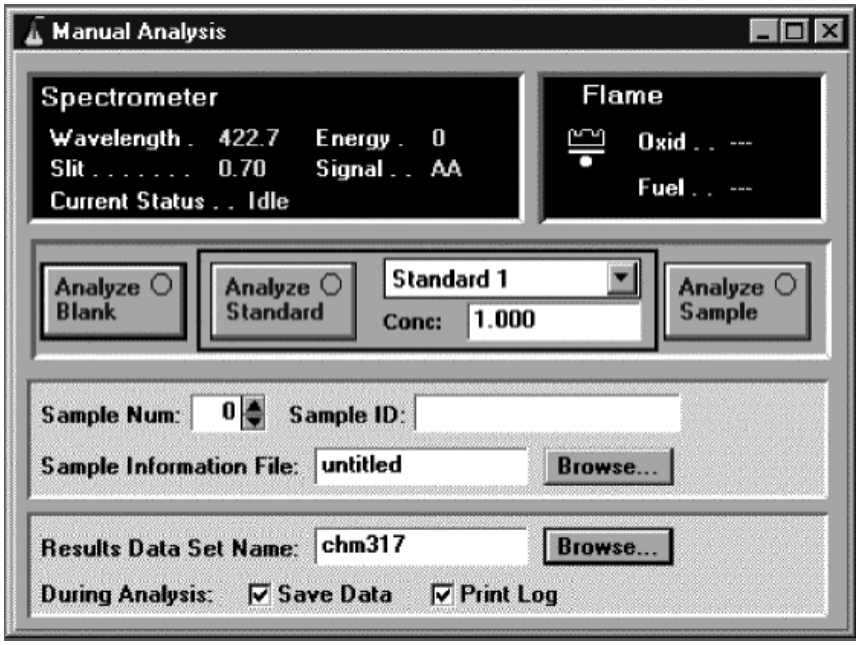
1. Using either the **File** menu or the **WkSpace** button, open the workspace "CHM317CM"; this will open and position a number of windows for you. Next, click on the **MethodEd** button and check that the method "Calcium Water" is loaded; if not, click on the **Method Editor** button and select this method, then click **OK**.

Open the **Lamps** window and select the Ca lamp, making sure that the lamp is correctly identified as Ca rather than Mg. The appropriate wavelength and slit width have already been set in the method. Check the actual position of the lamp in the carousel, and double-check the lamp name to make sure it is the right element. Allow 15 minutes for the lamp to warm up.

Due to software limitations, it is more reliable to save all your data into the software's results database and print a report at the end of the experiment. To do this, you need to create a unique entry in the results database for your experiment. In the **Manual Analysis** window (see below), click on the **Browse...** button beside the **Results Data Set Name**: entry.

In the resulting dialog box, enter a unique data set name and description for your experiment. The data set name should consist of letters and numbers *only*; do *not* include spaces or punctuation characters. When you close the dialog, the data set name should appear in the appropriate text box in the **Manual Analysis** window, and the **Save Data** box should be checked. If the **Print Log** box is checked, **uncheck** it.

➔ If you use an existing name, your data will be appended to that group's data! You only need one entry for your experiment on any given day.



AAWinlab Manual Analysis Window, showing a poor choice for the results data set name – choose something unique!

Set a unique **Results Data Set Name** for this experiment; do ***not*** change this once you have created it – use the same data set name for *all* parts of this experiment!

2. Prepare 250.0 mL of a stock solution containing about 5.0%(w/v) La^{3+} by dissolving $La(NO_3)_3 \cdot 6H_2O$ in ultrapure deionised water, transferring this to a 250.0 mL volumetric flask, and making up to the mark with ultrapure deionised water. (You may weigh out the lanthanum salt on a top-pan balance; record the weight used to 2 decimal places in your lab notebook.)

➔ You could just make this solution in the beaker. However, should you run out and have to re-make it, it will be easier to get the *same* concentration of potassium using a volumetric flask.

3. Prepare a set of calibration solutions, each of which should contain **both** Ca^{2+}, Mg^{2+}, and La^{3+} (a releasing agent) as follows: use a transfer pipette to dispense 20.00 mL of the stock La^{3+} solution into each of five 100.0 mL volumetric flasks. Using the appropriate transfer pipettes, dispense the appropriate volumes of the stock 100.0 mg/L Ca^{2+} and Mg^{2+} solutions to result in the following **concentrations** when made up to volume with ultrapure deionised water:

Flask:	**A**	**B**	**C**	**D**	**E**
$[Ca^{2+}]$ / mg L^{-1}	0.100	0.500	1.000	5.000	10.000
$[Mg^{2+}]$ / mg L^{-1}	0.050	0.100	0.500	1.000	5.000
La^{3+} / %w/v	1.0	1.0	1.0	1.0	1.0

Transfer the solutions to clean plastic bottles, being sure to rinse the bottles and caps three times with small volumes of the solution before filling them.

➔ For the lower concentrations, you will find it easier to prepare a 10.00 mg/L standard *without* the lanthanum solution, and then dispense this to make your final standards *with* the added lanthanum.

➔ Label each bottle and flask clearly *as you prepare each solution*

4. Additionally, prepare a blank by pipetting 20.00 mL of the stock La^{3+} solution into a sixth 100.0 mL volumetric flask, and diluting to the mark with ultrapure deionised water. Label the flask.

➔ At this point, one of you can start running the standards on the AA while the other prepares the samples for analysis – see step 6.

5. To prepare a sample, use a transfer pipette to dispense 10.00 mL of the sample and 20.00 mL of the stock La^{3+} solution into a clean 100.0 mL flask, then dilute to the mark with ultrapure water. In this manner, prepare samples of water from the hot and cold taps, fish tank water, and bottled water for analysis. Also prepare 100.0 mL of a solution containing

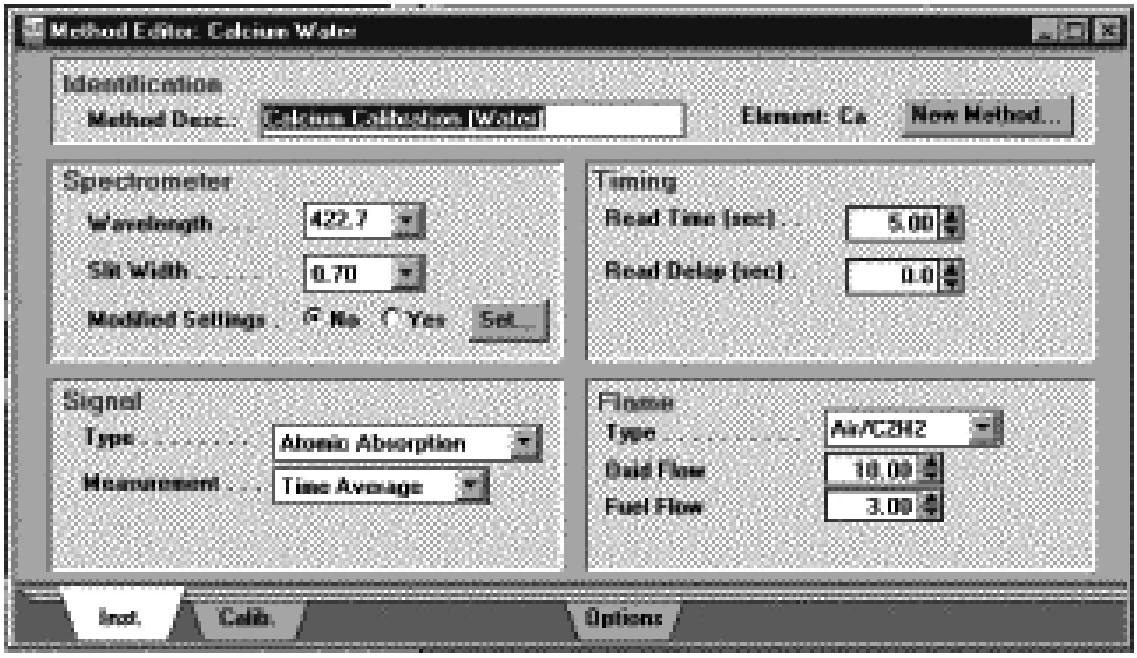
1.00 ppm Ca and 1.00 ppm Mg with **no** added La, to be run as a sample. Label these clearly!

6. To light the flame, first make sure that the plastic sample tube is immersed in the flask of ultrapure deionised water provided. Click on the **Flame** button to open the **Flame Control** window: window you will see an **On/Off** button and a **Safety interlocks** icon; this icon should show a green check mark. If the box shows a red '**X**' instead, there is either insufficient fuel pressure to light the flame, the drain trap is empty, or the waste container on the drain is full; please ask the lab instructor or manager for assistance.

ⓘ Normally, the fuel and oxidant flow rates and burner height relative to the optical path through the instrument would need to be adjusted for optimum performance. To save time, laboratory staff have performed this procedure for you.

Light the flame by clicking on the **On/Off** switch in the **Flame Control** window. The flame should be a clear blue colour. While operating the spectrometer, make sure that the instrument is always aspirating ultrapure deionised water whenever the flame is on and you are not taking readings.

7. Select **Method Editor** from the **Tools** menu or click on the **MethodEd** button, and confirm the following settings in the resulting tabbed dialog (see below). In particular, check the values of the standard concentrations listed in the **Calib** tab under **Standard Concs:** these should match the concentrations set out in the table above. Once satisfied, close the method window.



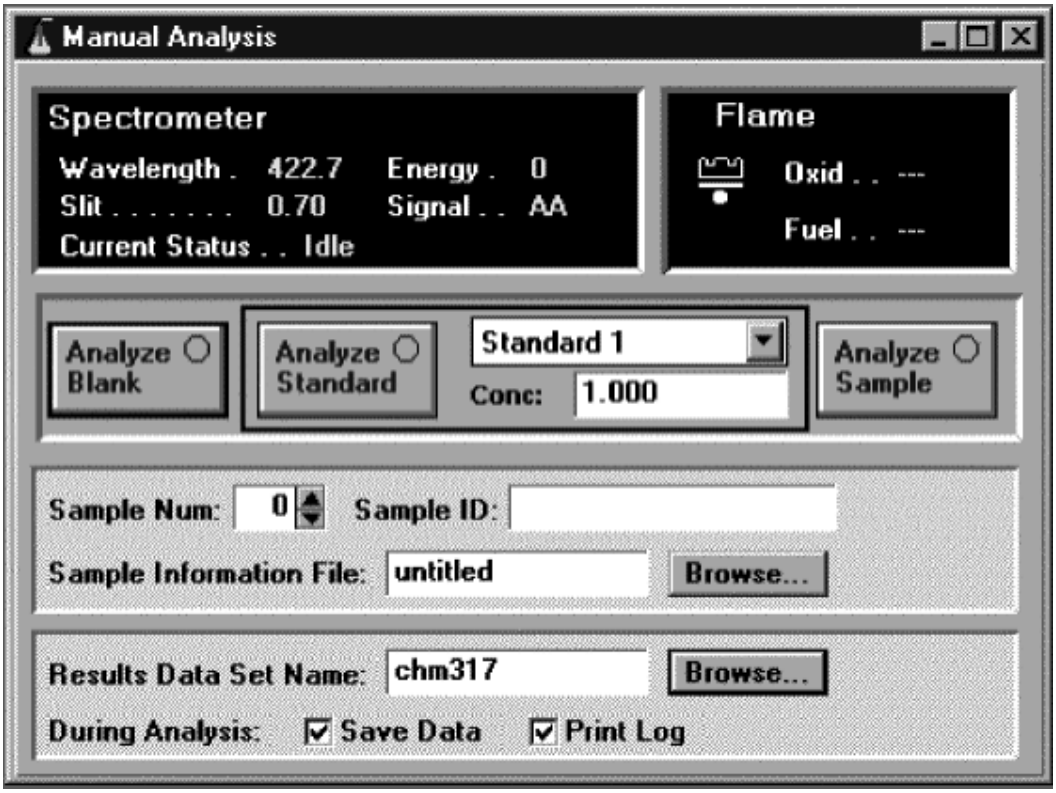
The method editor window

8. Aspirate the reagent blank solution (*i.e.* the one containing only the dilute lanthanum), and click on the **Analyse Blank** button in the **Manual Analysis** window (below). Once the

software has finished collecting the data (the current status will read 'Idle'), switch to the lowest concentration standard. Check that the window displays "Standard 1" and click on the **Analyse Standard** button. Once the software has finished collecting the data, the display should update to "Standard 2", and the calibration curve in the graphics window should also update. Continue in this manner until you have analysed all the standards in sequence from lowest to highest.

9. Now analyse each sample in turn: you may click in the **Sample ID:** field to give each sample a name, which will appear on the resulting print–out. Enter the sample ID information, aspirate the sample, and click on the **Analyse Sample** button.

➔ Do **not** change the results data set name – **only** change the **Sample ID:** field!



The manual analysis window

10. Once the analysis for calcium is completed, click on the **Method...** button and load the method **chm317MgWater** ("Magnesium in Water"). Click on the **Lamps** button and change the entry for the calcium lamp from **Ca** to **Mg**. Since the lamp is a dual-element lamp, it does not need to warm up before you continue with your measurements.

11. Repeat the analysis procedure (including the reagent blank), but this time analysing for magnesium instead of calcium. Once you have finished running all the standards and samples, check how much time you have left. If there is sufficient time available, click on the **Method...** button again, but this time load the method **chm317MgWaterBG**

("Magnesium in Water BGC"). This will enable the deuterium lamp background correction system for the method.

Click on the **Lamps** button, turn on the deuterium lamp using the button in **Lamps** window, and allow it to warm up for ~ 5 minutes before continuing. Repeat your analysis (autozero on deionised water, reagent blank, standards, and samples) for magnesium but this time with background correction on.

D. Finishing Up

12. Once you are satisfied with your data, print out the results from your analysis and shutdown both the instrument and the software as indicated in sections 4.4 and 4.5. Make sure you collect *all* your solutions, beakers, *etc*., and return them to LM6.

13. Dispose of all your solutions in the proper waste container. Used Pasteur pipettes should be rinsed thoroughly with distilled water before being disposed in the teal-coloured decontaminated glass bin. Clean all your glassware, using some alcohol on a KimWipe™ to remove any labels, and return it to the correct bench drawers.

➔ Please keep all the transfer and graduated pipettes in a different drawer to the rest of the glassware, in order to minimize accidental breakage

Check all areas where you have been working – balance, bench, and instrument – to make sure that they are clean and tidy, and that all chemicals have been returned to the correct shelves. When done, have your TA validate your lab notebooks before leaving.

E. Analysis of the data

14. Reconstruct the calibration curves for Ca, Mg, and Mg with background correction (if completed) using the blank corrected absorbance values reported by the software. Determine the linear portion of each graph, and find the best-fit straight line through the linear region by linear regression analysis. Calculate r or r^2, $s_{y/x}$, and the limits of detection and quantitation for the linear portion of each calibration curve.

➔ The on-line statistics tutorial has a section on how to find the linear region using Excel™. See http://www.chem.utoronto.ca/coursenotes/analsci/stats/

15. Use your calibration curves to determine the individual concentrations of Ca and Mg in your samples, as well as for the solution of calcium and magnesium prepared *without* the added lanthanum. Also use the linear regions of each calibration curve to determine the flame AAS sensitivity for calcium, magnesium, and magnesium with background correction enabled.

16. If you were able to obtain data for magnesium both with and without background correction, compare both the absorbance *and* concentration values you obtained by both

methods. Determine what difference, if any, there were in both the blank-subtracted absorbance values and the corresponding sample concentrations obtained for your samples. Also compare the limits-of-detection and sensitivity that were obtained with and without background correction.

6. Experiment E1: Sodium in Beverages by Atomic Emission

This experiment is an exercise in method development: although standard methods exist for many common analyses, it is often necessary to adjust these for specific samples. During the lab, you will first determine the working linear range for sodium of the flame atomic emission spectrophotometer, and estimate the sodium content of a wine sample. Using this information, you will then develop a protocol to accurately determine the sodium content of this sample using the method of standard additions. As a result, this experiment will involve more steps than would be required for the routine analysis of similar samples.

Flame AES exploits the characteristic yellow atomic emission lines of sodium to determine the concentration of this important element. Since most drinks (other than tap or mineral water) represent complex sample matrices that cause a variety of interference effects in atomic emission spectroscopy, the method of standard additions is preferred for the analysis. In particular, the alcohol content of wine affects the viscosity of the sample, which has important implications for the efficiency of aspiration and nebulization.

After performing this experiment, you should:

- Understand how to establish a protocol for standards addition
- Know how to calculate sample concentrations by standards addition
- Develop an understanding of atomic emission *versus* atomic absorption for determination of metals

Essential Notes:

- When performing this experiment, be sure to note any practical observations, such as flame colour, in your notebook.
- Sodium is one of the more sensitive elements with respect to atomic spectroscopy, and it is also prone to ionization effects. Sodium contamination is a serious source of error. To minimize this, you should:
    1. Avoid the use of detergent for cleaning glassware – these often employ sodium salts
    2. Rinse *all* glassware *thoroughly* with ultrapure water before use
    3. Transfer *all* solutions to plastic Nalgene® bottles as soon as they are made – glass contains sodium!

Chemicals:

- 1.000 g/L sodium standard for atomic spectroscopy (as $NaNO_3$ in 4% HNO_3)
- Solid potassium chloride (sodium-free)

Sample(s):

- A wine sample and a sodium "check sample" are provided. Bring your own water sample for analysis, or use the ones provided. Check with your demonstrator *the week before* this experiment to find out is appropriate.

A. Instrument setup and solution preparation

1. Make sure that the AANALYST 200 is on, and that the Syngistix software is running and connected to the instrument. Access the **Analysis** tab in the main window. From here, you can access the **Method** and **Analysis** buttons, which will allow you to see the current settings, change the method, and run the experiment.

   First, click on the **Method** button: the current method displayed beside the button should be "chm317Na1"; if it is not, use the drop down Open menu to select the appropriate method. Next, examine the settings in the **Method** window, and record the wavelength, slit width, and other parameters being used in the method. When you are done, simply close the window.

   Next, click on the **Analysis** button (the one with the test tube icon). The resulting **Analysis** window should have a display on the left with a series of buttons and menus corresponding to each step of the procedure (blank, standards, and samples) and, on the right, a series of entries for each calibration solution. The concentrations displayed should match those in the experiment. Make sure that the **Save Data** check box is enabled, and that you have entered a name for the data set – record this in your lab notebook for later reference!

2. *Ionization suppressor solution:* Weigh out sufficient potassium chloride into a 250 mL beaker to make 250 mL of a solution containing 1.0 g K^+ for each 100 mL of solution (1.0 %(w/v)). Dissolve the KCl in ultrapure water, transfer the solution to a 250 mL volumetric flask, and dilute to volume. Once the solution is prepared, transfer it *immediately* to a clean, rinsed, **labelled** plastic bottle: the solution *will* leach sodium from the glass if left in the flask, and this will adversely affect your results.

➔ You could just make this solution in the beaker. However, should you run out and have to re-make it, it will be easier to get the *same* concentration of potassium using a volumetric flask.

3. *Sodium Stock Solution:* Obtain the 1.000 g/L Na stock solution from the reagent shelf, and transfer a ***small*** volume (**~5 mL**) to a small, clean, dry beaker. Following standard operating procedures, ***accurately*** prepare 100.0 mL of a 10.00 mg Na/L solution of sodium by dilution of the stock with ultrapure deionised water. *Immediately* transfer this to a clean, rinsed, **labelled** plastic bottle.

4. *Sodium Calibration Solutions:* Prepare a set of sodium calibration solutions, each of which contains (when diluted to final volume) 0.10 %(w/v) of K^+ as ionization suppressor. Use the 10.00 mg Na/L stock solution prepared in step 3 to make standards containing 0.100, 0.200, 0.500, and 1.000 mg Na/L by accurately transferring the appropriate volume into separate clean, rinsed 100 mL volumetric flasks. Add 10.00 mL of the 1.0 %(w/v) K^+ solution to *each* flask *before* diluting to volume. Make sure the solutions are well mixed and *immediately* transfer the solutions to clean, rinsed, **labelled** plastic bottles.

5. *Reagent Blank:* Finally, prepare 100.0 mL of a solution that *only* contains 0.10 %(w/v) of K^+ as ionization suppressor (*i.e.* has *no* sodium in it). Again, *immediately* transfer this to a clean, rinsed, **labelled** plastic bottle once it has been diluted to volume and mixed.

B. Instrument Performance and Estimate of Sodium Content

6. Take your solutions through to LM9 to the instrument assigned to you. Make sure that the plastic sample tube connected to the nebulizer is immersed in ultrapure deionised water - there should be a flask with the instrument. Click on the **Instrument** tab and then the **Flame Control** button. If the **On/Off** switch in the **Flame Control** window is greyed out and inactive, there is either insufficient fuel pressure to light the flame, the drain trap is empty, or the waste container on the drain is full; please ask the lab instructor or manager for assistance.

ⓘ Normally, the fuel and oxidant flow rates and burner height relative to the optical path through the instrument would need to be adjusted for optimum performance. To save time, laboratory staff have performed this procedure for you.

Light the flame by clicking on the **On/Off** switch in the **Flame Control** window. The flame should be a clear blue colour. While operating the spectrometer, make sure that the instrument is always aspirating ultrapure deionised water whenever the flame is on and you are not taking readings.

➔ While one of you is calibrating the flame atomic spectrophotometer, another should be preparing the samples for analysis. For each sample, dispense 10.00 mL of the sample and 10.00 mL of the 1.0 %(w/v) K^+ solution into a 100.0 mL volumetric flask and dilute to volume with ultrapure deionised water. Transfer to a clean, labelled Nalgene bottle as soon as possible.

7. When operating in flame emission mode, the instrument needs to set the correct gain on the detector (a photomultiplier tube) for the range of emission intensities represented by your standards and samples. Once the flame has been on for ~ 5 minutes, select **Analyze Blank** from the main screen.

You will be asked to aspirate the **most concentrated** sample or standard; switch the sample tube from the ultrapure deionised water to your 1.000 mg Na/L standard (with 0.1%w/v K^+) and click the **OK** button.

Once the instrument has taken a reading, you will be asked to aspirate your **blank or lowest standard**. Remove the plastic sample tube from your standard, wiping it down with a KimWipe™ as you do so, and place it back in the **reagent blank** (*i.e.* the solution that contains *only* 0.1%w/v K), then click the **OK** button. Once the instrument has taken this reading, it will be ready for calibration.

8. You can now start analysing your standards, working from the lowest to highest concentration (0.100–1.000 mg Na/L). Check that the display shows the standard you expect. Place the nebuliser tube in your first standard and then click on the "Analyze

Standard" button. Once completed, switch back to the deionized water. The display should update to the next standard; repeat until all the standards have been measured. You can check the calibration by pressing the "Display Calibration" button.

➔ Should you need to repeat any standard measurement, click on the displayed concentration; a dialogue should appear allowing you to select the standard you want to measure.

9. Once you have finished calibrating the instrument, you can measure the sodium content of your samples. You can type a short text description into the **Sample ID** field of the main window to identify the sample in the database; you should, however, *also* be recording all the blanks, standards, and samples in your lab notebook as you perform the experiment! To take a sample reading, click on the **Analyze Sample** button; once the instrument has finished taking a reading, it will report the calculated concentration and you can return the sample tube to the flask of ultrapure deionised.

➔ If your sample contains more sodium than your highest standard, you will simply get an error message; if this occurs, roughly dilute your sample using a measuring cylinder until it comes within your calibration range. For example, wine samples will typically require a dilution factor of roughly 1:100 – 1:500.

Once you have finished all your measurements, turn the flame off and obtain a hard copy of your data from the database (see section 4.4, page 6).

C. Determination of Sodium Content by Standard Additions

10. Now you have first measurement of the sodium content of your samples, as well as the linear range for sodium emission, you can determine how to make up your solutions for the determination of sodium in the wine sample by standards addition. Each solution you make should:
    - Contain a final concentration of 0.1%w/v K
    - Contain a total sodium concentration that is within your linear range
    - Have total sodium concentrations covering an order of magnitude (factor of 10)

    For example, if the linear range for sodium extended to 5 mg Na/L, and your sample had an estimated concentration of 10 mg Na/L, you would want to have a set of solutions with total sodium concentrations covering the range 0.2 – 2.0 mg Na/L. To achieve this, you would pipette 2.00 mL of sample into each 100.0 mL flask, along with 10.00 mL of your 1.0 %w/v K solution. You would then add 1.00 mL, *etc.* of your 10.00 mg Na/L standard to each successive flask, so that you have one diluted sample and at least four additions. The following table shows the resulting added and total concentrations for this particular example; create your *own* table in your lab notebook, using the *actual* estimate of your *own* sample concentration as the starting point.

Estimated sample concentration: 10 mg Na/L
Concentration of standard for additions: 10.00 mg/L

Flask:	A (Sample)	B (Add. 1)	C (Add. 2)	D (Add. 3)	E (Add. 4)
Vol. 1% K (mL):	10.00	10.00	10.00	10.00	10.00
Vol. sample (mL):	2.00				
C sample (mg/L)	0.200				
Vol. std. added (mL)	0				
C added (mg/L):	0				
C total (mg/L):	0.200				

11. Once you have checked your scheme with your TA and made your sample solutions, you are ready to return to the instrument and perform the analysis. Click on the **Analysis** tab and load the method **chm317NaAdd1** then light the flame again.
12. You will need to tell the software what concentrations you are using for your additions. Click on the **Parameters** button in the main toolbar, then go to the **Calibration** tab, and. This will display a small spreadsheet-like area displaying the expected values of the concentration added to the sample. Edit this area with your own concentrations and volumes, deleting any rows that you don't need.

You should **NOT** add a row for an added concentration of zero; this is included (albeit with an odd label) in the table already. If you add a row with a zero concentration into the table, your experiment is **DOOMED!**

13. Once you are done, you are ready to analyse your wine sample. Click on the Analyse Blank button as before; you should be prompted to add the highest concentration solution (the one with the greatest amount of added standard). After this, return to your reagent blank, (the solution containing *only* 0.1 %w/v K). Continue analysing the additions. When you have measured all the solutions that you listed in the method editor, the software will extrapolate the graph and calculate the concentration of sodium in your sample.

D. Finishing Up

14. Once you are satisfied with your data, print out the results from your analysis and shutdown both the instrument and the software as indicated in sections 4.4 and 4.5. Make sure you collect *all* your solutions, beakers, *etc*., and return them to LM6.
15. Dispose of all your solutions in the proper waste container. Used Pasteur pipettes should be rinsed thoroughly with distilled water before being disposed in the teal-coloured decontaminated glass bin. Clean all your glassware, using some alcohol on a KimWipe™ to remove any labels, and return it to the correct bench drawers.

➔ Please keep all the transfer and graduated pipettes in a different drawer to the rest of the glassware, in order to minimize accidental breakage
Check all areas where you have been working – balance, bench, and instrument – to make sure that they are clean and tidy, and that all chemicals have been returned to the correct shelves. When done, have your TA validate your lab notebooks before leaving.

F. Data Analysis

16. Use Excel™ to plot the regular calibration data for sodium emission, using the mean blank corrected values. Note that, although the software refers to all readings as 'absorbance', in emission mode these are intensity values having arbitrary units. Determine the linear region of the calibration data (see the on-line statistics tutorial for details on how to do this).
17. Use the calibration curve to determine the concentration of sodium in both your water sample and the "unknown" provided; also calculate and report the uncertainties associated with these concentrations. Remember to take into account any sample dilution required in order to place the sample within the linear region of the calibration curve.
18. Use Excel™ to produce a standards addition calibration plot for your sample data, again using the mean blank corrected values provided by the software. Perform your own linear regression analysis on the data, calculating r or r^2, $s_{y/x}$, and the intercept on the concentration axis together with its uncertainty (again, the on-line statistics tutorial provides a formula for the value of s_{xE} required.) Use this to determine the actual sodium concentration of your original sample, taking into account the dilution factor inherent in the method of standards addition, and completing the corresponding error propagation. Compare this value to your original estimate.
19. Comment in your report on whether your choice of additions was appropriate for determining the sodium content of the wine sample. What steps might you take to decrease the uncertainty in the extrapolated value for the sodium concentration?

7. Elements for Report Discussion

Your formal report for this technique should describe the experiments performed and present the results obtained, commenting on any special features of the methodology and instrumentation used. From the results, you should go on to discuss what the results suggest about atomic absorption and emission, and how any limitations of the instrumentation can be mitigated. You should address the following questions in addition to any specific questions or findings from each experiment:

1. Calcium and magnesium are known to form refractory compounds with phosphate, a common interferent in water samples. Lanthanum is commonly added as a releasing agent, however it can also have another effect. Using your results from experiment A1 for the Ca + Mg sample with no added La^{3+} or phosphate, explain what this role is.

2. Atomic absorption lines are very narrow, given the relative simplicity of the electronic energy states, yet we still require a monochromator in flame atomic spectroscopy. Why is this, and where is it located in the instrument?

3. In the lab, you used air-acetylene flames as the atom source. What are the advantages and disadvantages associated with using an inductively coupled plasma source instead, and which is most appropriate for (a) atomic emission and (b) atomic absorption?

4. How does sensitivity compare between flame AAS, flame AES, and ICP-AES? What factors, or questions, would you need to take into account in choosing one technique over another for any given analysis?