

Experiment 5

Atomic Absorption Spectroscopy

Exercises to be completed BEFORE coming to the laboratory:

1. Summarize the safety information and precautions required for this experiment in your lab book;
2. Write a short paragraph summarizing the objectives of the experiment.
3. Explain the following terms in relation to the experiment: atomized sample, absorption line, matrix effects and laboratory standard.
4. Outline how you will prepare your calibration standards (Section 3.2) using the supplied stock solution - Iron (III) standard = $1000.0 \text{ mg L}^{-1} \text{ Fe}$. Complete the table below.

Required concentration	Concentration pipetted	Pipette volume	Final volume	Dilution factor
100 mg L^{-1}	1000 mg L^{-1}	25 mL	250 mL	10
20 mg L^{-1}	100 mg L^{-1}	20 mL	100 mL	5
15 mg L^{-1}			100 mL	
10 mg L^{-1}			100 mL	
5 mg L^{-1}			100 mL	
$*2.5 \text{ mg L}^{-1}$			100 mL	

*Use 10 mg L^{-1} iron solution to prepare 2.5 mg L^{-1} iron standard.

Chemicals used

Sulfuric acid ($18 \text{ and } 1 \text{ mol L}^{-1}$) <i>Description:</i> colourless solution <i>Risk:</i> corrosive, potential for burns, harmful by ingestion <i>Precautions:</i> avoid contact with the skin and eyes <i>Disposal:</i> solutions to drain with running water	Sodium EDTA solution (4 mmol L^{-1}) <i>Description:</i> colourless solution <i>Risk:</i> harmful if swallowed, skin and eye irritant <i>Precautions:</i> avoid contact with the skin and eyes <i>Disposal:</i> solutions to drain with running water
Hydrochloric acid (5 mol L^{-1}) <i>Description:</i> colourless solution <i>Risk:</i> corrosive, potential for burns, harmful by ingestion <i>Precautions:</i> avoid contact with the skin and eyes <i>Disposal:</i> solutions to drain with running water	Iron sulphate solution (4 mmol L^{-1}, acidified) <i>Description:</i> yellow solution <i>Risk:</i> harmful if swallowed, skin and eye irritant <i>Precautions:</i> avoid contact with the skin and eyes <i>Disposal:</i> solutions to drain with running water

Nitric acid (9 and 2 molL ⁻¹) <i>Description:</i> colourless solution <i>Risk:</i> corrosive, potential for burns, harmful by ingestion <i>Precautions:</i> avoid contact with the skin and eyes <i>Disposal:</i> solutions to drain with running water	o-Phenanthroline Solution (4 mmolL ⁻¹) <i>Description:</i> colourless solution <i>Risk:</i> harmful if swallowed <i>Precautions:</i> avoid contact with skin/eyes <i>Disposal:</i> solution to drain with running water
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General Hazards:

- ◆ In this experiment, you will perform a wet digestion technique to extract the iron from the Nori sheet samples. The wet digestion reagents are concentrated acids that are strongly corrosive and some reagents. The procedure results in production of toxic fumes. **This must be performed in a fume hood.** If a spillage occurs, inform a demonstrator and dilute with water before cleaning up.
- ◆ **Only a qualified laboratory staff member may start and close-down the atomic absorption spectrophotometer.** Acetylene-air mixtures can be dangerously explosive and compressed acetylene gas pressurized above 150 kPa can be explosive. Acetylene cylinders must be kept away from the flame. The acetylene and air flows must be appropriate for the burner fitted and interlocks enabled, and these gas flows must be checked before flame ignition. Adequate gas pressure but be present for the length of the experiment. The burner head and nebulizer assembly must be fitted correctly, ensuring no gas leakage occurs. The drain from the nebulizer must be through a water trap, with no organic solvent build-up present. The exhaust from the acetylene flame may contain toxic metal atoms and other toxic compounds and so exhaust must vent into an adequate fume cupboard. **The flame must not be left unattended.**

Total time: 3 hours

You need to allocate your time carefully for this experiment. The absorption measurements should be started at least 50 minutes before the end of the session. It is advisable to start the sample dissolution before proceeding to the preparation of the standards.

Work in pairs

Each pair will prepare a set of standard solutions, and each student in the pair will be individually assigned to prepare samples from either an iron tablet or Nori sheet. Your lab report will include the analysis of both samples.

1. Introduction

Atomic absorption spectroscopy, AAS, is a widely used technique in analytical chemistry, being both selective and sensitive. In this experiment you will investigate aspects of the determination of iron by AAS, and some methods of sample treatment that are suitable as a precursor to the AAS analytical technique.

Experimental conditions must be chosen so that an AAS analysis performs adequately (e.g. through choice of the most appropriate absorption line of the analyte). The analyst must also ensure the concentration/s of analyte and standard solutions are appropriate (i.e. fall within the working range of the instrument under the prevailing conditions) and correct for any possible interferants.

2. Theory

Atomic absorption spectroscopy (AAS) uses the absorption of light to measure the concentration of gas-phase atoms. In an AAS experiment, solid and liquid samples are vaporised in a flame (or graphite furnace). The basic elements of an AAS instrument are illustrated in Figure 1.

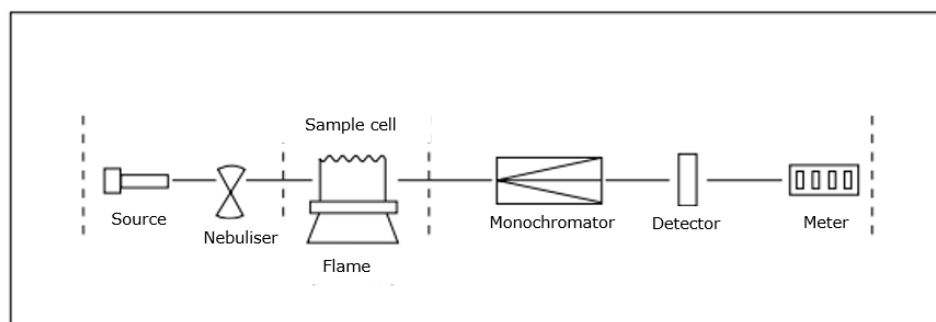


Figure 1: Schematic illustration of an AA spectrophotometer

In this experiment, the method that is used to introduce the liquid samples into the flame involves dispersal of the sample into a fine spray using a nebuliser. The spray is then de-solvated into salt particles in the flame and those particles are subsequently atomised (converted into gas-phase neutral atoms, ionic species and molecular species in the ground state). All of these conversion processes occur in geometrically definable regions of the flame and so it is important to set the instrument parameters such that the light from the source is directed through the region of the flame that contains that maximum number of neutral atoms.

The gas-phase atoms absorb light and make transitions to higher energy levels (excited electronic states). Individual elements will absorb wavelengths differently, and these absorbances are measured against laboratory standards. In effect, AAS takes advantage of the different radiation wavelengths that are absorbed by different atoms. As the electrons return to their original energy state, they emit energy in the form of light. This light has a wavelength that is characteristic of the element. A detector measures the wavelength of light transmitted and the radiant energy corresponds directly to the wavelength which is absorbable by the atomized sample. The amount of light that is absorbed by the sample is proportional to the number of atomized atoms.

It is possible that other atoms or molecules apart from those of the element being determined will absorb or scatter some radiation from the light source. These species could include un-vaporized solvent droplets, or compounds of the matrix that are not removed completely. One way that modern AA spectrophotometers accommodate this is by using two light sources. One light source would be a specific hollow cathode lamp appropriate to the element being measured (emits light at the appropriate absorption line) while the second light source (often a deuterium lamp or halogen lamp) produces broad band radiation (not a specific absorption wavelength, but a wavelength range). The total absorption (absorption due to analyte atoms plus background) is measured with the specific light from the hollow cathode lamp and the background absorption is measured with the light from the continuum source. During a measurement, the instrument alternates between using the two light sources and subtracting the background absorption from the total absorption provides the absorption arising from only analyte atoms.

In AAS experiments, a calibration is always used. Several samples of known concentration are measured under the same conditions as the samples of unknown concentration. Additionally, it is important to measure a laboratory standard for the element of interest.

3. Experiment

For trace analysis all glassware must be clean and rinsed with deionised water. Sets of glassware are labelled and must only be used for the solutions indicated by the label.

Your lab report will include the analysis of both iron samples, even though you only prepared one of these. It is advisable to start the sample dissolution before proceeding to the preparation of the standards.

3.1 Preparation of sample solutions

The treatment that a sample containing iron requires to release all the iron into solution and make it suitable for aspiration into the flame AAS instrument, depends on the sample. Usual procedures can be classified as dry ashing or wet digestion; here you will use wet digestion techniques.

Samples provided include iron supplement tablets and a dietary iron source (Nori sheets). You will be directed to the sample for which you will determine the iron content. Digestion procedures are detailed below. The solutions resulting from these procedures may need further dilution in order to bring the iron concentration within the range of the calibrating standards.

Iron Supplement Tablets – one student (Preparation can be performed in open laboratory)

- (a) Accurately weigh an iron supplement tablet and record details of make, content and batch number in your lab book. Carefully grind the tablet to a fine powder in a mortar and pestle. Weigh approximately 200 mg of the powder accurately in a weighing bottle, and then by difference determine the exact weight transferred into each of two 100 mL volumetric flasks. Record all masses in your lab book.
- (b) Add 20 mL dilute (1 mol L^{-1}) sulfuric acid and 30 mL H_2O to each flask, and then shake vigorously for 10 min. Mix thoroughly, and then place in the ultrasonic bath for 5 min before making up to volume. If necessary, filter the solutions prior to the next step. The filtration is carried out after the mixture is made to volume, obviating the quantitative filtration of large volumes. **The filtered solution obtained at this stage will be shared**

with students performing the MAS experiment – you will require at most 25 mL of the solution.

- (c) Dilute the samples down 50 times so that they are within the range of the calibrating standards (record exactly how this dilution is done).

Nori Sheets – one student (to be done in duplicate)

Digestions are to be done in fume hood

- (a) Weigh accurately one Nori sheet (tear into small pieces) into each of two 100 mL conical flasks, add few drops of antifoam and place a cold finger condenser in each flask.
- (b) In a fume hood, **cautiously** add about 15 mL of analytical reagent grade concentrated nitric acid in three 5 mL lots using the dispenser provided and when initial reaction has subsided, **carefully** add about 10 mL of concentrated sulfuric acid.
- (c) When Nori sheets have dissolved gently heat the flask and contents on a hotplate. After reaction appears complete (about 20 min), rinse down the flask walls and condenser with a little water, and gently heat without condenser until the nitrogen oxide fumes have dissipated. Leave to cool then dilute to about 30 mL with deionised water.
- (d) Quantitatively transfer the solution **and** any unreacted material (probably cellulose) to a 50 mL volumetric flask. Make up to the mark, and after shaking well, filter about 10 mL through an acid-washed (Whatman 541 etc) filter paper, or centrifuge to obtain the instrumental sample free of any solids.

3.2 Preparation of calibration standards

The following standard solution is provided: Iron (III) standard = $1000.0 \text{ mg L}^{-1} \text{ Fe}$

Prior to coming to the laboratory, you should have completed prelab question 4 outlining how you will prepare the calibration standards.

- (a) Prepare a working solution that contains 100.0 mg L^{-1} iron, using the provided iron (III) standard (1000 mg L^{-1}), pipette (25 mL) and volumetric flask (250 mL). Use this working solution to (in pairs) prepare five calibration standards that contain 20.00, 15.00, 10.00, 5.00 and 2.50 mg L^{-1} iron (III) in 100 mL volumetric flasks. **These solutions should be**

stabilised against the hydrolysis of the iron (III) cation by the addition of 5 mL of dilute nitric acid to each flask before making up to the mark with DI water.

Note: when making large dilutions it is best to dilute stepwise, e.g. to dilute a solution 500 times, dilute 10.00 mL to 250.0 mL (25 ×) and then dilute 5.00 mL of this solution to 100.0 mL (20 ×, overall 25 × 20 × = 500 ×). Do not dilute 1 mL to 500 mL.

3.3 Measurement of absorbances using the AA spectrophotometer

Allow 50 minutes for these measurements.

- (a) Prepare a table in your lab book (Table 1 below can be used as a template) to record your measurements. Alternatively, an electronic copy of your results can be emailed to yourself using the USB stick provided by a demonstrator and the computers in the anteroom.

Table 1

Solution	Reading #1	Reading #2	Reading #3
Type 1 water (zero solution)			
2.5 mg L ⁻¹ standard			
5 mg L ⁻¹ standard			
10 mg L ⁻¹ standard			
15 mg L ⁻¹ standard			
20 mg L ⁻¹ standard			
20 mg L ⁻¹ laboratory standard			
Iron tablet sample #1			
Iron tablet sample #2			
Nori sheet sample #1			
Nori sheet sample #2			

- (b) Your demonstrator will demonstrate setting up the AAS flame spectrophotometer, including the choice of lamp, wavelength, slit width / band pass, lamp current, burner alignment, gas flow settings, the zeroing of the absorbance scale, detailing, as well, all necessary safety measures.

- **Record the operating conditions for the AAS measurements (lamp, wavelength, slit width / band pass)**
- Under the supervision of a staff member set the absorbance zero whilst aspirating deionised water. [This is an experimental reading.]

- It is common practice to read three consecutive values of absorbance, once the reading has reached a steady value indicating that your solution has completely washed out the nebuliser tubing.
- (c) Continue (in the one session) to aspirate all your standard solutions and your treated samples into the AAS flame and record the absorbances at the 248.3 nm line. As a check on the accuracy of your dilutions, compare the absorbance of your 20.0 mg L⁻¹ standard with that for the 20 mg L⁻¹ laboratory standard.
- If the absorbance of a sample is beyond that for the standards, the sample "instrument" solution will need to be *quantitatively diluted*, and the absorbance remeasured.
 - If there is any delay with preparing any of the instrument samples, rerun the standards again along with the samples.
 - ***Make sure you record all readings, including those of your partner. Also make sure you both have information about the tablet recorded in your lab book.***
 - If results appear anomalous report to the laboratory supervisor immediately.

Note: a common cause of unexpectedly low or fluctuating readings is the blocking of the aspiration tube at the nebuliser.

3.4 Matrix effects and interferences in iron flame AAS

Identification and removal of interferents is important for the accurate determination of iron.

In this experiment the iron concentration will be kept constant at a nominal 10 mg L⁻¹ and any effect of possible interferents at the 200 mg L⁻¹ or 5 × 10⁻³ mol L⁻¹ concentrations will be found by looking for changes in the absorbance from that of the provided 10.0 mg L⁻¹ iron standard. The effects of oxidation level change and the effects of o-phenanthroline will be investigated.

These samples will be run for the whole group and the results shared.

Measure the absorbances of the **supplied solutions** that are all nominally 10 mg L⁻¹ in iron. The one labelled "No Interferent" is the solution to which the others can be compared. A table, such as that outlined below, can be used to record your data.

Table 2

[Fe] (mg L ⁻¹)	“Interferent”	Absorbance (248.3 nm)		
		Reading #1	Reading #2	Reading #3
10	“No Interferent”			
10				
10				

4. Report

Your lab report should start with a summary of the safety information and any precautions required for this lab, followed by the experimental procedure (third person, past tense description of how the experiment was performed). The report should be divided into two further sections – corresponding to the sections below. You must show working for each of the calculations below including the calculations of uncertainty.

4.1 Results

- (a) Report the absorbances for all of the solutions (this should already have been done as part of your record keeping during the experiment using Table 1 and Table 2).
- (b) **Prepare a calibration graph** from the absorbances of the standard iron solutions. To do this, plot the concentration vs the average absorbance. For each data point calculate the standard deviation and plot it as an error bar as shown in the graph on next page. Fit the calibration data using a polynomial least squares line fitting (2nd order polynomial). You can use any appropriate software including the EXCEL file “POLY.xls” with a 2nd available on the computers in the anteroom. **Do not use a linear fit or join points by straight lines.** Note that many AAS calibration plots are curved, concave downwards.

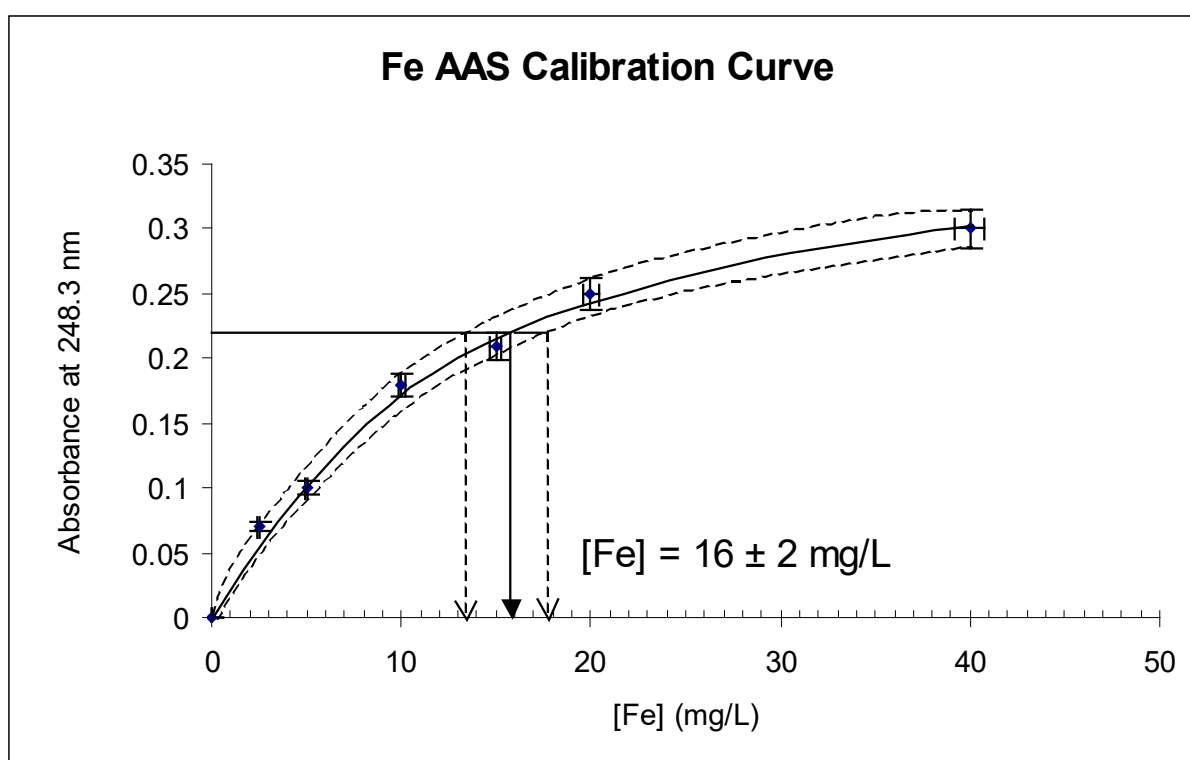
Using the average of your 3 absorbance readings for each unknown solution, determine the concentration of iron in all the unknown solutions and associated uncertainty. The EXCEL file “AAS.xls” can also be used for this purpose, using the parameters for the quadratic fit obtained using “POLY.xls”.

- (c) **From the concentration of iron in the unknown solutions obtained from (b), calculate and tabulate the iron content of the *original* solid or liquid samples** in appropriate concentration units (e.g. for solid samples such as Nori sheets and iron tablet, use % by wt., or ppm wt./wt., for solutions use mg L⁻¹ or ppm). These calculations require you to identify the dilutions carried out in preparing the sample solution from the original solid or liquid sample.

From this value for the concentration of iron in the iron tablet, calculate the mass of iron sulphate in the iron tablet, and **compare this value with the label claim.**

- (d) **Quantitatively estimate the uncertainties** in your calculated iron content results for the samples you prepared in duplicate (i.e. either Nori sheets or iron tablet samples), taking into account the uncertainties in both the measurement of concentration and absorbance, and the interpolation from the calibration plot.

Note: the uncertainty in your value for the concentration of the sample solution can be estimated by using the uncertainty in concentration and absorbance values to plot maximum and minimum lines on your graph. Interpolating from these maximum and minimum graphical lines gives an estimate of uncertainty in your iron content results.



Do your replicate determinations agree within the estimated uncertainty?

- (e) Report the percent interference (reduction or enhancement) of the interferents.

$$\% \text{ Interference} = \frac{A_{\text{in the absence of interferent}} - A_{\text{in the presence of interferent}}}{A_{\text{in the absence of interferent}}} \times 100$$

Calculate, where appropriate, the **mole or atom ratio** of interferant to iron for the matrix effect and interferant section of the experiment (Section 3.4).

4.2 Questions

1. When preparing the samples for AAS (Section 3.1), why is the filtration or centrifugation carried out after the solution has been made up to volume?
2. Report the working range of sample concentrations, both in mg L^{-1} and in mol L^{-1} , found for the AAS conditions used. [The working range for spectrophotometric measurements is often taken to be the concentrations giving $A = 0.1$ to $A = 1.0$, but in many AAS calibration curves the upper absorbance is limited by excessive curvature of the calibration curve.]
3. Would the method of standard additions likely to be of use in the determination of iron by flame AAS? (*Look at the shape of the calibration curve.*)
4. AAS is usually regarded to be a highly selective method of analysis. Do your calculated % Interference results agree with this statement?
5. If you have done the Fe-MAS experiment (Experiment 6), compare the answer you obtained for the amount of iron in the Ferrogradumet tablets with the answer obtained in by MAS. Are they similar or different? Which method do you think give better results? Justify your answer.

5. References

1. J. C. Van Loon, *Analytical Atomic Absorption Spectroscopy Selected Methods*, Academic Press, New York (1980).
2. B. Welz and M. Sperling, *Atomic Absorption Spectrometry*, 3rd Ed, Wiley-VCH, New York (1999)
3. Harris, D. C. *Quantitative Chemical Analysis*, W. H. Freeman and Co.: New York, 7th ed. 2007. Sections 4.8, 6.4, 18.1-18.5, 20.1-20.3 and 21.1-21.5.