ROYAL SOCIETY OF CHEMISTRY
ANALYTICAL DIVISION
NE Region

SCHOOLS' ANALYST COMPETITION 2011

Regional Heat

The case of the Dying Marrows

INSTRUCTION BOOKLET

Introduction

In the sleepy village of Early Winter, little has changed for decades. Located twenty miles from the nearest town, the majority of the population have lived in the village all their lives and many are now retired. There is a small shop, post office, public house, school and church in the village centre. Surrounding the village are several small farms, each with a small herd of dairy cattle. Life in the village tends to focus on rural activities, with the annual village show being the highlight of the year. The prizes awarded for local produce are fiercely contested and behind the tranquil scenes emotions can run high, after all there is considerable pride at stake.

Retired Colonel Smith has run the public house for the last twelve years. He is highly respected and popular in the village; his hobby is growing prize vegetable marrows. His marrows have won the village show for three years running, much to the annoyance of Mrs Dale (who runs the small shop); she also grows marrows, and has been second place at the show for the last three years. The one thing that really annoys Mrs Dale is that she sells the plant fertiliser to Colonel Smith, the very thing that makes his marrows the best. She has often told him that one day she will "replace the fertiliser with water before you buy it, then look what will happen to your marrows".

Recently, a new family (the Deckers) moved into the village to run the post office. Mr and Mrs Decker have moved from the city to take over the post office. Their son (Jason, aged 9) and daughter (Rachael, aged 12) have joined the school but they do not like village life. The arrival of the Deckers caused some animosity in the village, since it was expected that Colonel Smith's daughter, Britney, would take over the post office. Colonel Smith was disappointed because the post office is next to the pub, and he hoped his daughter would move in next door. The relationship between the Colonel and the Deckers has not improved because Jason kept kicking his football into the Colonel's garden. Matters really deteriorated last month when the colonel finally confiscated Jason's football after it landed on his marrows. When he refused to return Jason's football, Jason swore revenge on the colonel, telling him that "something would happen to his marrows".

The village school has only one teacher, a small spinster called Miss Prim. Her pupils often call her a witch, perhaps because she has the facial features of such a character or because she has a small, evil looking black cat. She believes passionately in keeping the village exactly as it was when she was a girl, so much so that she stopped a fast food shop being opened and she tried very hard to stop Colonel Smith obtaining a music licence for his pub. Much to the relief of the village population, that plan failed and now he has regular music evenings on the last Thursday in every month. Miss Prim did not take failure lightly, and she warned Colonel Smith that she would get her own back; she told him that "something that he holds so dear may suffer".

Recently, there was scandal in the village. Colonel Smith's prize marrows started to die. He was very annoyed about this, and believed that someone has sabotaged his marrows. He is right to be concerned since no other plants or vegetables are dying in his garden. The marrow patch is quite isolated from the other parts of his garden, yet close to the outside fence. Anyone in the village could have got at his marrows, so it requires scientific investigation to identify the culprit.

The colonel's preliminary investigations have identified three possible suspects. The first is Mrs Dale. He buys his plant fertiliser from her shop and she has often threatened to empty the bottles and fill them with water. The next suspect is Jason. When he confiscated Jason's football, Jason swore revenge and threatened to do something to the Colonel's marrows. The colonel saw Jason with a large bag of kitchen salt, did he add it the marrow patch in sufficient quantity to kill the marrows? The final suspect is Miss Prim. Since he obtained his music licence, she has been very nasty to him and she knows how much he thinks of his marrows. Miss Prim bought a large bottle of silver nitrate for use at 'school' but the school does no chemistry. Did she obtain it to kill his marrows?

Colonel Smith has collected a sample of fertiliser from the bottle he bought from Mrs Dale (and a bottle of the same fertiliser bought out of town). He also collected a sample of soil from the marrow patch and another part of his garden. For simplicity, the soil samples have already been treated with dilute nitric acid and centrifuged. This has extracted the analytes from the soil, and they are now ready for use. Your mission today is to undertake the required analyses carefully, to identify the culprit, and exonerate the wrongly accused. Remember, proving who is innocent is as important as finding out who is guilty.

What is required?

There are three possible substances whose presence (or absence) may have caused the demise of Colonel Smith's marrows: fertiliser, sodium chloride, and silver nitrate. Since phosphate is a vital component of Colonel Smith's marrow fertiliser, a convenient way to detect its presence or absence is to measure the concentration of phosphate in the fertiliser by a spectrophotometric method. The presence of excess sodium (from the salt) can easily be determined by a form of atomic emission spectrometry, called flame photometry. A titration method can be used to determine the amount of silver present in the sample. One important issue that will need to be addressed is the naturally occurring concentration of sodium and silver in the garden soil – it is vital that you take this into account. This is why you have been supplied with two extracts. The first has been obtained from soil removed from the marrow patch, this is the sample. The second has been obtained from soil removed from another area in the garden where the plants are thriving. This is the comparison and will allow you to evaluate the naturally occurring background level.

In your team of three, first decide who is going to do each analysis; then read the experiment you are going to do. Each experiment has a section explaining the hazards of the chemicals you will be using; you should read this section carefully. Once you are happy with what you are going to do, you should begin with the practical work. Good luck, Colonel Smith is depending on you. The background section includes some brief information on each analytical technique.

Thanks to Dr Tom McCreedy and colleagues at the University of Hull for devising and developing the competition.

Background

In this section, the theory of each analytical method is explained. Enough detail is provided to allow you to understand what you are doing and why you are doing it.

Units and ppm

In this booklet you will encounter two ways of describing concentration. The first is "molarity". This should be familiar to you and describes the number of moles per litre of each chemical. The second is "parts per million" or "ppm". This unit may not be familiar to you, but it simply describes the number of mg of a particular analyte per litre of aqueous solution, for example 1 mg of copper in 1 litre of water would be 1 ppm copper. It is frequently encountered for elements rather than molecules, i.e. copper rather than copper sulphate.

Spectrophotometric determination of orthophosphate

Many molecular species absorb radiation in the UV or visible regions of the electromagnetic spectrum. Solutions containing such molecules may be colourless or coloured, and the amount of light absorbed depends on the concentration of the molecules. It is slightly more complex than that, indeed the absorbance (A) of any solution is related to three parameters; the molar absorptivity coefficient (ε) of the molecules at the wavelength used, the concentration of the absorbing species (c) and the path length (l) through the solution. This is expressed as Beer's law (equation 1) and describes the relationship between the measured absorbance and the concentration of the absorbing species.

$$A = \varepsilon c l \qquad \text{(equation 1)}$$

In experiments like the one here, the pathlength is usually fixed at 1 cm. This is simply achieved by using a glass, plastic, or quartz curvette that has holds a column of liquid exactly 1 cm in thickness. The molar absorptivity coefficient is usually different for each absorbing species present, and has the units of $mol^{-1}dm^3cm^{-1}$. The molar absorptivity coefficient can readily be determined by producing a calibration graph of absorbance versus concentration (measure the absorbance of a series of solutions of known concentration). Assuming a 1 cm curvette was used, then the gradient of the line is ε. Once the calibration graph has been produced, it is relatively simple to determine the concentration of the analyte in a solution where the concentration of the analyte is not known. The absorbance of the solution can be read from the graph and by rearranging equation 1, the concentration can easily be calculated.

The only problem with this method is that many species do not absorb light at a convenient wavelength or there are interferences. An alternative approach is to measure the analyte indirectly, by derivatising it to a species that absorbs at a suitable wavelength. Ideally this procedure should eliminate any interference since the derivatising reagent should only react with the analyte of interest. Orthophosphate is a good example where direct analysis by spectrophotometry would be difficult. Instead, we react the orthophosphate with ascorbic acid and ammonium molybdate to form the phosphomolybdneum blue complex. This gives a blue solution whose absorbance is conveniently measured at 660 nm. It is sufficiently specific for phosphate to give an accurate result. One potential problem that can be encountered is the presence of traces of phosphate on glassware from residual detergent. This causes all solutions to develop a blue colouration.

Atomic emission spectrometric determination of sodium

When ions are heated in a flame, some of them become electronically excited. In order to return to the electronic ground state, they have to emit light at a characteristic wavelength. The amount of light is proportional to the temperature of the excitation source and the concentration of ions present. Thus if a flame in used to excite the ions, and the flame temperature is constant, then the amount of light emitted will be proportional to the concentration.

While all elements can be made to emit light, the temperatures required can be extreme. For some elements, e.g. lithium, sodium, and potassium, emission can be obtained at much lower temperatures. Flame photometry uses a cool flame to excite the ions, and thus only a few elements emit. This means that the emission spectrum is very simple, and unwanted emission wavelengths can be readily removed using a filter. Here, we use a flame photometer to measure the sodium emission from a suspect sample. Aqueous samples of sodium ions are aspirated into the methane/air flame and a percentage become electronically excited. As they return to the ground state, they emit light; this gives rise to the characteristic yellow colour in the flame. A filter is used to block out the other wavelengths so that only that due to the sodium emission reaches the detector. The amount of sodium in the sample can easily be determined from a calibration graph. This is produced by first analysing a set of calibration standards and plotting a graph of sodium concentration vs. instrument reading. The unknown concentrations can then be determined from the graph.

Titrimetric analysis of silver

Silver can be determined using many analytical techniques, but a straightforward method is to titrate the silver solution with ammonium thiocyanate solution using iron(III) as the indicator. The method we are using here is adapted from Volhard's original method. The silver solution is pipetted into a conical flask, acidified with nitric acid and then iron(III) is added as the indicator. At this stage, the solution is colourless. The burette is filled with thiocyanate solution, and then it is slowly delivered into the conical flask. The thiocyanate reacts with any available silver to form silver thiocyanate; this is the preferred reaction, so while any silver remains uncomplexed no other reaction will occur.

$$Ag^{+} + SCN^{-} \leftrightarrow AgSCN$$

As soon as all the silver is used, the excess thiocyanate forms a complex with the iron producing a reddish-brown colouration. The end point has been reached when the solution turns pale brown.

$$Fe^{3+} + SCN^{-} \leftrightarrow [FeSCN]^{2+}$$

Experiment 1: Determination of phosphate by the molybdenum blue reaction

Risk assessment

The chemicals in this experiment are of low hazard; however, some are mildly corrosive and/or toxic. Just remember to use good laboratory practice at all times and avoid getting the chemicals onto your skin, or in your eyes or mouth.

Equipment

- 100 ppm orthophosphate stock solution (as 100 ppm phosphorus)
- Suspect fertiliser solution (sample)
- Fertiliser solution prepared from known source (comparison)
- 0.6% ammonium molybdate solution (in 0.4 M nitric acid)
- 1.5% *L*-ascorbic acid solution
- 8 x 100 mL volumetric flasks
- 1, 2, 5 mL pipettes
- 2 mL, 5mL pipettes for reagents
- Pipette filler
- Deionised water
- Labels

Experimental procedure

1. Take 8 100 mL volumetric flasks, and pipette 2 mL of ascorbic acid solution and 5 mL of ammonium molybdate solution into each.

2. Fill the first flask to the mark with deionised water and label as 0 ppm phosphorus.

3. Into the second flask, pipette 1 mL of phosphate stock solution, make up to the mark then label this flask 1 ppm phosphorus.

4. Repeat this procedure with four more flasks using 2, 3, 4, and 5 mL of the stock solution and label these flasks 2, 3, 4, and 5 ppm phosphorus respectively.

5. In the seventh flask, pipette 10 mL of the fertiliser sample and dilute to the mark. Label this as “sample”.

6. Into the final flask, pipette 10 mL of the comparison fertiliser, make up to the mark and label this as “comparison”.

7. Shake all the flasks, and measure as soon as an instrument is available, shake well just before measurement.

8. Consult a demonstrator about the operation of the spectrophotometer.

9. Check the wavelength on the spectrophotometer is set to 660 nm then measure the absorbance of your calibration standards, the sample and the comparison. Note each reading down below. You should obtain two readings for each solution.

1. Results for phosphate determination by the molybdenum blue reaction

Calculate the average absorbance for each solution and record the values in the table below.

Phosphorus/ppm	0.0	1.0	2.0	3.0	4.0	5.0	Sample	Comparison
Average reading								

Calculation

1. Plot a graph of concentration vs. average absorbance for the phosphate absorbance.
2. Use your graph to determine the concentration of phosphate in the comparison and sample.
3. The concentration of phosphorus in the sample was _____ ppm
4. The concentration of phosphorus in the comparison was _____ ppm

Outcome of the analysis

This analysis should answer the question as to whether there is a difference in the amount of phosphate in the sample and the comparison.

My conclusion is that:

Experiment 2: Determination of sodium by flame photometry

Risk assessment

All the chemicals used in this experiment are of low hazard. Just remember to use good laboratory practice at all times: avoid getting the chemicals onto your skin, or in your eyes or mouth.

Apparatus

Liquid extract from marrow patch (sample)
Liquid extract from another part of the garden (comparison)
Sodium chloride (Analar, or equivalent)
1 x 1 L volumetric flasks
5 x 100 mL volumetric flasks
10 and 20 mL pipettes
Pipette filler
Deionised water
Labels

Experimental procedure

1. Prepare a 100 ppm stock solution of sodium (from sodium chloride) by weighing 0.190 g of sodium chloride, and dissolving it in deionised water. Make up to 1 L.

2. By dilution of the 100 ppm stock solution, prepare five calibration solutions in the range 0 - 40 ppm sodium.

3. Consult a demonstrator about the operation of the flame photometer.

4. Check the filter on the flame photometer is set to sodium, and then aspirate your 40 ppm calibration solution into the flame photometer to check that the response is on the scale.

5. Once you are happy that the top calibration standard is on scale, aspirate each of your standards in increasing concentration order and then the comparison and sample. Note down each reading below, it is best to take two readings for each solution.

6. If either your comparison or sample gives a reading above your top calibration reading, you will need to dilute them.

2. Results for sodium determination by flame photometry

Calculate the average reading for each solution and record the values in the table below.

Solution/ppm	0	10	20	30	40	Comparison	Sample
Average reading							

Calculation

1. Plot a graph of concentration vs. average reading for the sodium emission.
2. Use your graph to determine the concentration of sodium in comparison and sample
3. The concentration of sodium in the comparison was _____ ppm
4. The concentration of sodium in the sample was _____ ppm

Outcome of the analysis

This analysis should answer the question whether there is a difference in the amount of sodium in the comparison and sample.

My conclusion is that:

Experiment 3: Determination of silver by thiocyanate titration

Risk assessment

All the chemicals used in this experiment are of relatively low hazard, but some are mildly corrosive and/or irritant. Extra care should be taken when using the 6 M nitric acid - this is quite corrosive. Remember to use good laboratory practice at all times: avoid getting the chemicals onto your skin, or in your eyes or mouth.

Apparatus

0.1 M silver nitrate standardised solution
0.1 M potassium thiocyanate solution (approximate)
Ferric alum indicator
6 M nitric acid
Liquid extract from marrow patch (sample)
Liquid extract from another part of the garden (comparison)
250 mL conical flask
5 mL and 20 mL pipettes
2x 10 mL measuring cylinders
or other means of dispensing indicator and nitric acid
Pipette filler
Microburette
Deionised water

Experimental procedure

1. Fill the microburette with 0.1 M potassium thiocyanate solution.

2. Pipette 5 mL of the silver nitrate standardised solution into a conical flask. Add 1 ml of ferric alum indicator solution, then 10 mL of 6 M nitric acid.

3. Titrate this solution, remembering to carefully shake the solution on a regular basis. The end point is when the solution turns **pale** brown.

4. Note the titration volume and then repeat the procedure with the 20 mL sample and then 20 mL comparison instead of the silver nitrate.

5. Repeat steps 2-4 using the average values of each of the titrations in the subsequent calculations.

3. **Results for the determination of silver by thiocyanate titration.**

:	Inititial Burette Reading	Final Burette Reading	Titre (mL)	Average titre (mL)
Standard Silver Nitrate				
Marrow Patch Sample				
Comparison Sample				

Calculation

1. The standard silver nitrate solution will allow you to calculate the exact molarity of the potassium thiocyanate solution.

2. Once you have this information, use it to calculate the amount of silver present in the sample and comparison.

3. The concentration of the potassium thiocyanate is _____ M

4. The concentration of silver in the comparison is _____M

5. The concentration of silver in the sample is _____M

Outcome of the analysis
This analysis should answer the question whether there is a difference in the amount of silver in the comparison and sample.
My conclusion is:

Overall conclusions from the analyses of ‘The Case of the Dying Marrows‘

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Questions

1. Why is orthophosphate an important constituent of fertiliser?

2. Name another common household product that might contain phosphate?

3. If 100 mL of water contains 10 mg of potassium, what is the concentration in ppm?

4. If 10 mg of potassium chloride were dissolved in 1 L of water, what would the concentration of potassium be in ppm?

5. Atomic emission spectrometry using a methane/air flame can be used for other ions in addition to sodium, name 2 of them?

6. What would happen if you prepared a silver nitrate solution using tap water rather than deionised water?