SCHOOLS' ANALYST COMPETITION 2013

MIDLANDS REGIONAL HEAT

INSTRUCTION BOOKLET

ROYAL SOCIETY OF CHEMISTRY
ANALYTICAL DIVISION

LENNARD-JONES LABORATORIES
UNIVERSITY OF KEELE

Fish kill: Who is to blame?

Welcome:

Welcome to the RSC Schools' Analyst competition at Keele University. Today's competition is based around three analytical experiments which aim to give answers to a number of questions. You will need to carry out the experiments and make decisions about the data you obtain in order to answer the questions.

To be able to complete the tasks you will need to work as a team and distribute the workload in the most effective way. Make sure you read through the safety information and the instructions before you start on any experimental work.

We hope that you enjoy your day with us at Keele University and wish you good luck in the competition!

The Scenario:

On the 22nd April 2013 a local fisherman reported a large fish kill in the River Ware. Thousands of dead fish could be seen floating on the surface of the water and environmental scientists were called in to investigate. Their preliminary investigations identified three possible causes:

- The river is located close to a water treatment facility that uses aluminium sulphate to treat water ready for drinking. In 2007 faulty storage tanks at this facility leaked 2 tonnes of aluminium sulphate into the river, polluting a 3km stretch and killing over 10,000 fish. Aluminium is very toxic to fish. Has a similar incident occurred again this time?
- The river is located close to a petroleum factory. Petroleum products contain a compound called naphthalene, which is very toxic to aquatic organisms. Since the fish kill occurred, a previous employee of this factory has contacted the Environment Agency to convey his suspicions that the company have recently started dumping the factory waste into the River Ware. If true, this could have resulted in large quantities of naphthalene being released into the river.
- There is a large farm neighbouring the river. Heavy rain was reported in the area in the weeks before the fish kill. The farmer regularly uses phosphate-containing fertiliser on his land, which could have been washed into the river as a result of the rain. Excessive phosphate levels in river water lead to a lack of oxygen, resulting in the death of fish.

Samples from the river have been obtained, and it is now up to you as analytical chemists to determine which of these possible causes, if any, is likely to be responsible for the fish kill. One important issue that will need to be addressed is the naturally occurring concentration of aluminium, naphthalene, and phosphate in the river. A water sample taken from the river before the fish deaths has already been analysed for you, the results are shown below:

Pollutant	Concentration/mg L^{-1}
Phosphate	0.1
Aluminium	
Naphthalene	0.7

The Tasks:

Your tasks today are to:

1. Determine the concentration of phosphate in the river water using UV/Vis spectroscopy.
2. Determine the concentration of aluminium in the river water using a back-titration based on complex formation.
3. Determine the concentration of naphthalene in the river water using High Performance Liquid Chromatography.

Planning:

To be successful you will need to plan how the tasks can be best distributed amongst team members to achieve the objectives in the allocated time. Our estimate of the time required for the experiments is:

UV/vis spectroscopy	2 hours
HPLC	1 hour
Titration	2 hours
Write-up / Questions	0.5 hours

Note: For the HPLC experiment your team will need to book a 20 minute slot on the HPLC sign-up sheet. We recommend that you book this slot at the start of the day.

Results:

This instruction booklet contains space for your observations as you conduct the experiments. You will have a copy of this booklet each and you should fill this in as you are conducting the experiments.

Each group will have one set of answer sheets. The answer sheets will be collected in and marked at the end of the day. They ask you to record your results and conclusions from the three experiments and also to answer a number of questions relating to the experiments. You will need to complete one set of answer sheets per team. Show all of your working out for calculations.

Units:

In this booklet you will encounter two ways of describing concentration. The first is 'molar concentration', M or mol L^{-1}, which describes the number of moles per litre of each chemical. The second is 'mass concentration', mg L^{-1}, which describes the number of mg of a particular analyte per litre of aqueous solution.

Glassware:

During the day you may need to reuse some of your glassware, if you do please ensure that you rinse the glassware thoroughly with ultra-pure water, in order to avoid contamination.

Chloe Harold & Laura Hancock 2013

Important Safety Information

Lab coats and safety spectacles must be worn at all times in the laboratory.

Do not eat or drink in the laboratory.

Long hair should be tied back.

No shorts, skirts, leggings or tights should be worn.

No open-toed shoes - only enclosed shoes may be worn in the laboratories i.e. no sandals, ballet-type shoes or flip flops.

Some of the chemicals you will be using are hazardous. The hazards and precautions associated with each chemical are listed below:

Chemical substance	Hazards	Precautions
Phenolphthalein indicator in ethanol solution	Irritant	Avoid skin contact, wear gloves.
Combined reagent	Harmful, irritant, causes burns, toxic	Avoid skin contact, wear gloves.
EDTA solution	Harmful, irritant	Avoid skin contact, wear gloves.
Zinc sulphate solution	Harmful.	Avoid contact with eyes.
Eriochrome Black T indicator	Flammable, harmful, causes burns	Avoid skin contact, wear gloves.
Dilute ammonia solution	Harmful, irritant.	Avoid skin contact, wear gloves. Do not inhale.
Naphthalene	Harmful. Toxic to aquatic organisms.	This will be in sealed vials.
Acetonitrile	Irritant	This will be in sealed vials.

Experiment 1 - Background Information

Phosphate reacts with ammonium molybdate and potassium antimonyl tartrate in an acid medium to form an antimony-phosphomolybdate complex. This complex is reduced by the addition of ascorbic acid to form an intense blue colour, the intensity of which is proportional to the concentration of phosphate in the solution.

UV-VIS Spectroscopy

Many compounds absorb light in the near-UV or visible region of the electromagnetic spectrum. UV-Vis spectrophotometry can be used to measure how much light is absorbed by a compound, and this information can be used to measure the concentration of the compound.

A spectrophotometer measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (I_0). The proportion of light that exits from the solution is called the transmittance (T) and is given by:

$$T = \frac{I}{I_o}$$

The transmittance depends on the concentration of a sample (c), and the length of the sample through which the light travels (the pathlength, l). This relationship is given by:

$$-log\ T = \varepsilon cl$$

The quantity $-log_{10}T$ is known as the absorbance (A), and the spectrophotometers you will be using today display this quantity automatically. The quantity ε is a constant called the absorption coefficient, which is specific to a particular compound at a particular wavelength. Hence, we arrive at the Beer-Lambert law which shows that the absorbance (A) of a sample is directly proportional to its concentration (c):

$$A = \varepsilon cl$$

This relationship suggests that a plot of absorbance (A) versus concentration (c) will be a straight line going through zero. The absorbance of a solution is normally recorded at λ_{max} (the wavelength corresponding to the peak absorbance of the solution).

Dilutions

For this experiment you will be required to make up dilutions from a stock solution of known concentration in order to obtain a range of solutions of differing concentration. To work out the volume of stock solution required to do this, the following equation will be useful:

$$c_1V_1 = c_2V_2$$

(Where c_1 is the concentration of the stock solution, V_1 is the volume of stock solution you need to calculate, c_2 is the concentration you want to achieve, V_2 is the final volume of the solution).

1. Determination of the phosphate concentration using UV-Vis spectrophotometry

In this experiment you will use UV-VIS spectrophotometry to construct a calibration graph by measuring the absorbance of standards of known phosphate concentration. You will then use this graph to determine the concentration of phosphate in the questioned river water sample.

Equipment:

5 x 100 mL volumetric flasks Graduated pipette & pipette filler Plastic pipettes 7 x 100 mL conical flasks 50 mL bulb pipette & pipette filler 10 mL measuring cylinder Plastic cuvettes UV-VIS spectrophotometer Graph paper	Standard phosphate solution (5 mg/L) Ultra-pure water Phenolphthalein indicator Combined reagent Questioned river water sample

Experimental procedure:

1. From the phosphate stock solution provided, prepare 5 calibration standards of the following concentrations:, 0.5 mg L^{-1}, 1.0 mg L^{-1}, 1.5 mg L^{-1}, 2 mg L^{-1} and 2.5 mg L^{-1}. Do this by accurately using a graduated pipette and pipette filler to pipette an appropriate volume of stock solution (you will need to calculate this) into a 100 mL volumetric flask and making up to the mark with ultra-pure water.

2. Pipette 50 mL of each of your standard solutions into separate 100 mL conical flasks using a bulb pipette and pipette filler. Add one drop of phenolphthalein indicator to each solution and if a pink colour develops add 2.5 M sulphuric acid drop-wise until the pink colour disappears.

   IF YOU ARE UNSURE OF HOW TO USE A GRADUATED PIPETTE AND/OR A BULB PIPETTE PLEASE ASK A DEMONSTRATOR.

3. Repeat step 2 for a blank (50 mL of ultra-pure water) and the questioned river water sample.

4. Add 8 mL of the combined reagent to each of the conical flasks. Swirl the flasks to completely mix the contents, and then allow them to stand for 15 minutes to enable a blue colour to fully develop.

5. Using a UV-VIS spectrophotometer, measure the spectrum of each solution from 1000 to 500 nm and record the absorbance of each solution at λ_{max} (~890 nm).

   CONSULT A DEMONSTRATOR ABOUT THE OPERATION OF THE SPECTROPHOTOMETER.

Results:

Phosphate concentration (mg L^{-1})	Absorbance

River water sample	

Data treatment:

Plot a Beer-Lambert calibration graph by hand of absorbance (at λ_{max}) versus concentration for the standard phosphate solutions, and use the graph to determine the concentration of phosphate in the questioned river water sample. Record your results on the answer sheet.

Attach calibration graph

Concentration of phosphate in questioned river water sample: _____mg L^{-1}

This analysis should answer the question as to whether there is a difference in the amount of phosphate in the river water sample taken after the fish deaths (the questioned river water sample) and the sample taken before the fish deaths (the comparison).

What is your conclusion?

Experiment 2 - Background Information

A method for determining aluminium concentration is to carry out a complex-formation titration with a solution of Ethylenediaminetetraacetic Acid (EDTA) of known concentration, a solution of zinc sulphate of known concentration, and Eriochrome Black T (EBT) indicator.

EDTA is a complexing agent designed to bind metal ions quantitatively. At alkaline pH (achieved using dilute ammonia solution) aluminium ions will form strong complexes with EDTA – with a 1:1 stoichiometry (i.e. 1 mole Al^{3+} ion to 1 mole EDTA), as will zinc ions.

Eriochrome Black T (EBT) is an indicator that also forms complexes with metal ions. It changes colour depending on whether or not it is complexed – it is usually blue when not complexed, and pink when complexed with a metal.

In this experiment you will add a known volume of EDTA (of known concentration) to the river water sample. The EDTA will form complexes with all of the aluminium ions in the sample. You will then add Eriochrome Black T. The solution will turn blue because the indicator will not be complexed as there will be no aluminium ions present (they are all bound to the EDTA as aluminium binds preferentially with EDTA over EBT).

$$Al^{3+} + EDTA + EBT \rightarrow Al - EDTA + uncomplexed\ EBT$$
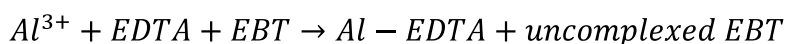
You will then titrate with a standard solution of zinc sulphate, which will form complexes with any unbound EDTA (zinc complexes preferentially with EDTA over EBT).

$$Zn^{2+} + EDTA + EBT \rightarrow Zn - EDTA + uncomplexed\ EBT$$
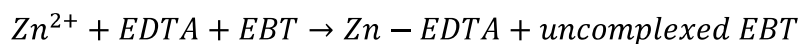
Once all the EDTA has been bound, the zinc will form a complex with the EBT, causing the solution to turn pink. This is the endpoint of the titration.

$$Zn^{2+} + EBT \rightarrow Zn - EBT$$
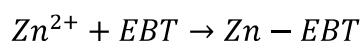
From the volume of zinc sulphate used in the titration, the volume of EDTA solution that was required to complex all of the aluminium in the sample can be worked out, which can then be used to calculate the aluminium concentration of the river water sample.

2. Determination of aluminium concentration by titrimetric analysis

In this experiment you will use a complex-formation back-titration to determine the concentration of aluminium in the questioned river water sample.

Equipment:

25 mL bulb pipette & pipette filler	Questioned river water sample
100 mL conical flask	0.001 mol L^{-1} EDTA solution
50 mL burette	0.001 mol L^{-1} zinc sulphate solution
100 mL beaker	Eriochrome Black T indicator
White tile	Dilute ammonia solution
Clamp stand & clamp	Universal indicator paper

Experimental procedure:

1. Pipette 25 mL of the questioned river water sample into a 100 mL conical flask.
2. Add 25 mL of 0.01 mol L^{-1} EDTA solution using a bulb pipette.
3. Add dilute ammonia solution dropwise until a pH of ~9 is achieved (use indicator paper).
4. Using a beaker, transfer the 0.01 mol L^{-1} zinc sulphate solution to the burette. (Do not transfer solutions above eye level).
5. Clamp the burette in place above the conical flask & place a white tile beneath the flask.
6. Add 4 drops of Eriochrome Black T indicator to the conical flask.
7. Immediately titrate the sample with 0.01 mol L^{-1} zinc sulphate solution until the colour changes from blue to pink/purple.
8. You should repeat this procedure three times to obtain consistent results.

Results:

Titration	Initial burette reading/mL	Final burette reading/mL	Volume of 0.001 mol L^{-1} Zinc Sulphate used/mL	Average volume of 0.001 mol L^{-1} Zinc Sulphate used/mL
1				
2				
3				

Data treatment:

1. Calculate the number of moles of 0.001 mol L^{-1} EDTA that has reacted with the aluminium in the water sample. (You will need to calculate the volume of EDTA that was required to complex all of the aluminium).

$$moles \ mol \ = concentration \ mol \ L^{-1} \ \times volume \ (L)$$

2. EDTA reacts with aluminium ions in a 1:1 ratio, so the number of moles of EDTA is equal to the number of moles of aluminium. From this and the volume of the river water sample that you used, calculate the concentration of aluminium in the sample.

$$concentration \ (mol \ L^{-1}) = \frac{moles \ (mol)}{volume \ (L)}$$

3. Convert the molar concentration (mol L^{-1}) to mass concentration (g L^{-1}) (molar mass Al = 26.98 g mol^{-1}), then convert to mg L^{-1} (remember that there are 1000 mg in a g).

Concentration of aluminium in questioned river water sample: _____mg L^{-1}

This analysis should answer the question as to whether there is a difference in the amount of aluminium in the river water sample taken after the fish deaths and the sample taken before the fish deaths (the comparison).

What is your conclusion?

Experiment 3- Background Information

For this experiment the river water sample has been pre-treated for you by a technique called solid phase extraction (SPE). 1 L of the river water was passed through an SPE cartridge, isolating any naphthalene present in the sample, which was then eluted from the cartridge with acetonitrile.

High Performance Liquid Chromatography (HPLC)

Chromatography involves the separation of components of a mixture. There are many different chromatographic methods of analysis, but they all work on the same principle; they all have a *stationary phase* (a solid, or a liquid supported on a solid) and a *mobile phase* (a liquid or a gas). The mobile phase, containing the components of the mixture, passes over (or through) the stationary phase. The different components travel at different rates according to how strongly each component is adsorbed onto the stationary phase, and are thus separated.

In HPLC, the mobile phase is a liquid (for this experiment it is 80% methanol:20% water), and the stationary phase is made up of very small porous particles packed into a column. A small portion (20 μL) of the sample to be analysed is injected into the system using a special sampling loop. The components of the mixture are then carried through the column by the mobile phase solvent. Separation occurs in the column as a result of differential partitioning of the individual compounds between the mobile and stationary phases. Compounds that have a high affinity for the stationary phase, spend more time on the stationary phase, and hence take longer to move through the column, compared with those that have a lower affinity. The affinity depends on the chemical structure of the compound. As the compounds leave the column they pass through a detector, which results in a peak on the chromatogram.

The time taken for a compound to travel through the column is the called the *retention time*. This can be used to identify the compound present by comparing with a standard. In this experiment you will inject a naphthalene standard and record its retention time. You will then inject the questioned sample under exactly the same conditions and determine whether naphthalene is present (i.e. is there a peak present with the same retention time as naphthalene).

The area underneath the peak is proportional to the amount of compound that has passed the detector. This can be used to calculate the concentration of the compound in the sample. One way of doing this is to inject known concentrations of the compound, calculate the area under the peaks, and then produce a calibration plot of area vs. concentration. This has been done for you for this experiment.

3. Chromatographic analysis of naphthalene

In this experiment you will use High Performance Liquid Chromatography (HPLC) to determine whether naphthalene is present in the questioned river water sample, and at what concentration.

Equipment:

HPLC instrument and computer	Naphthalene reference sample
Injection syringe	Questioned sample
	Acetonitrile (to clean syringe)

The equipment and chemicals for this experiment are located by the HPLC instruments in the analytical lab.

Experimental Procedure:

CONSULT A DEMONSTRATOR ABOUT THE OPERATION OF THE HPLC.

1. Inject the naphthalene standard.
2. Once the run has finished (this will take about 5 minutes) determine the retention time of naphthalene.
3. Inject the questioned sample.
4. Once the run has finished (this will take about 5 minutes) determine whether naphthalene is present in the sample. If naphthalene is present calculate the area under the relevant peak.
5. If necessary, use the calibration graph provided to calculate the concentration of naphthalene in the river water.

Results:

Retention time of naphthalene reference sample:

Is naphthalene present in the river water sample? Explain your answer.

Area under naphthalene peak (if present) _____

Attach HPLC trace.

Concentration of naphthalene in questioned river water sample: _____mg L^{-1}
Show full details of your working.

This analysis should answer the question as to whether there is a difference in the amount of naphthalene in the river water sample taken after the fish deaths and the sample taken before the fish deaths (the comparison).

What is your conclusion?

Questions

1. A solution with a 1 cm path length placed within a UV-Vis spectrophotometer shows an absorbance of 0.85. The absorption coefficient ε for this compound is 11900 $mol^{-1}dm^{3}cm^{-1}$. Determine the concentration of this solution from the relationship $A = \varepsilon cl$. Show details of your calculation and work out the units.

2. The zinc sulphate solution that you used in experiment 2 was prepared using zinc sulphate heptahydrate ($ZnSO_4.7H_2O$). Calculate the mass of zinc sulphate heptahydrate required to prepare 25 mL of a 0.001 mol L^{-1} zinc sulphate solution. Show units and details of your calculation.

3. Name two other types of chromatography other than High Performance Liquid Chromatography.