ROYAL SOCIETY OF CHEMISTRY
ANALYTICAL DIVISION
NE Region

SCHOOLS' ANALYST COMPETITION 2010

Regional Heat

A possible case of poisoning ?

INSTRUCTION BOOKLET

Welcome to the Schools' Analyst competition! We hope that you will all enjoy participating in this practical competition and, who knows, a few of you may become analysts later on in your careers.

Analytical Science is all about problem solving, whether it be forensic work, or keeping industrial processes running when they hit snags, or in the more intellectual sense of working out exactly what experiments are necessary to perform an analysis in the laboratory. You, therefore, are going to tackle a problem today.

You should already have enough background knowledge (but feel free to ask a demonstrator if there is anything that you do not understand), but you will need to show common sense and good organisational skills.

First you will have to decide how to tackle your problem, so that you can distribute the workload among your team of three so that each of you is always busy. Then you will need to carry out the experiments, perform the calculations and make some decisions based on the data obtained. A few questions complete the exercise.

Please read and understand the instructions before commencing.

THERE IS A STRICT TIME LIMIT OF THREE HOURS FOR THE EXERCISE.

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Health and Safety

This is a practical exercise, so normal rules for safety in the laboratory apply.

Wear laboratory coats and safety spectacles at all times.

Do NOT eat or drink in the laboratory.

Always use the pipette fillers provided, and handle glassware carefully to avoid breakage and cuts.

Keep long hair under control.

IF IN DOUBT ABOUT ANYTHING THEN ASK A DEMONSTRATOR.

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THE ORGANISERS THANK YOU FOR PARTICIPATING AND WISH YOU ALL THE BEST FOR YOUR FUTURE.

A possible case of poisoning?

The scenario

A wealthy, elderly and very eccentric distant relative has recently come to stay with your family. Soon after her arrival she started complaining about feeling ill and is now complaining about being poisoned! She knows that you have some chemical knowledge and asks you for help – she trusts you as you will not inherit any of her wealth!

After careful consideration of her diet you come up with three possibilities. Most of the vegetables that you eat at home are grown by your father on his allotment, which is close to a motorway and a battery factory. Both the motorway and the factory could give rise to contamination of the vegetables by airborne lead particles. Lead is very poisonous and you decide to analyse some vegetables by digesting some lettuce leaves in dilute nitric acid, which should take the lead into solution as Pb^{2+}.

She previously lived in the state of Georgia in the U.S., where some natural sources of water are high in lithium. This water is marketed as affecting mood, which lithium does, and you suspect that she has been buying lithium carbonate tablets from an Internet vendor in a small South American country. This could be causing her to feel unwell and she agrees to provide you with a sample of urine to test.

Finally, she has been refusing many of the meals prepared in your home and has been eating take-away meals from the Curry House next door. You have read that there is a problem with some restaurants using food colourings such as tartrazine and sunset yellow rather than traditional additives such as saffron. Food additives such as tartrazine can cause pseudo-allergic responses in some people. You obtain a sample of curry powder to analyse for the additives.

The task

Your task today is to make the measurements on the three samples provided and then deduce, if possible, if there is any source of poisoning.

Planning

To be successful you will need to plan how each member of the group will use their time. Our estimate of the time required for the experiments is

Lithium analysis	1.5 - 2.0 h
Lead analysis	2.0 - 2.5 h
Chromatography	1.0 - 1.5 h
Calculations	0.5 - 1.0 h each

Experiment devised and developed by Roger Jewsbury and Margaret Scott, University of Huddersfield.

1. Determination of lithium by flame emission

In this experiment you determine the concentration of lithium in a solution by measuring the emission of light from the atoms when heated in a flame. You calibrate the atomic emission spectrometer with standards of known concentration and then run your test sample.

Equipment

5 x 100 ml volumetric flasks
10 ml graduated pipette
5 ml graduated pipette
250ml beaker
Wash bottle containing deionised water
Pipette filler
Flame emission atomic spectrometer
Li^+ stock solution (2.0 mmol L^{-1} Li^+)

Procedure

1.1 From the lithium stock solution, prepare four working calibration standards containing 0.025, 0.050, 0.100 and 0.200 mmol L^{-1} Li^+ in deionised water by pipetting an appropriate volume of the stock solution into a 100 ml volumetric flask and making up to the mark with deionised water.

1.2 Take the four concentration standards, the test solution and deionised water to the flame emission spectrometer.

ASK A DEMONSTRATOR TO SHOW YOU THE FLAME EMISSION SPECTROMETER AND THEN FOLLOW THE INSTRUCTIONS PROVIDED.

1.2 Flush the spectrometer thoroughly by aspiration of deionised water for 5 minutes before starting the analyses.

1.3 Zero the instrument with deionised water.

1.4 Run each calibration standard and record the results.

1.5 Run the test sample and record the result. Check that the result is within the calibration range. If it is not, then it will be necessary to accurately dilute your test solution.

Calculation

Draw a calibration graph with concentration as the x-axis and emission reading as y-axis. The relationship should be linear so draw the best fit straight line. Use the fitted line to convert the emission reading of the test sample to a concentration. Correct for any dilution of the test sample that you may have made.

Chemical background

The heat of the flame (around 2000 °C) will evaporate the water, then vaporise the ions in the solid firstly into molecules and then into neutral atoms. In some of the Li atoms, the outermost electrons in the 2s orbital will be excited to the vacant higher energy 2p orbital by the heat. These excited atoms will return to the more stable ground state by emitting the excess energy as light at 670.8 nm. The intensity of the light emitted will depend on the number of excited Li atoms which in turn will be proportional to the concentration of Li in the original solution, providing the other conditions remain constant.

Physiology of lithium

Lithium is an element thought to have no natural biochemical role. We know from its chemistry that it likes to bind to oxygen atoms in compounds and it may act by competing with other elements for some sites in proteins thus affecting enzymes. Its first medical use was for hyperactivity but it is not known how it works and its effect was discovered by accident, when a lithium salt was used in place of a sodium salt to change the solubility of a medicine.

It is extremely dangerous to take lithium salts except when blood levels of lithium are being monitored and several of the early patients to whom it was prescribed died. I would not drink natural water if I thought it contained any significant quantity of lithium salts.

Most lithium is excreted with a matter of hours in urine, so measurement of lithium in urine would give an indication of recent ingestion of lithium. Any significant level of lithium in urine would thus indicate lithium intake.

2. Determination of lead ions by dithizone

Pb^{2+} forms a complex with the dithizone reagent, which is coloured, and the absorption can be measured at 510 nm. The absorption measured as absorbance (see below) will be directly proportional to the concentration. In this experiment a calibration graph is constructed using standards and then the concentration of the test solution determined.

Equipment

Visible spectrophotometer
1cm path-length glass cells
6 x 100ml volumetric flasks
10ml graduated pipette
10ml bulb pipette
25ml bulb pipette
Pipette filler
7 Pasteur pipettes
7 stoppered boiling tubes in stand
Pb^{2+} standard solution (100 mg L^{-1})
Dithizone reagent solution
Dichloromethane
Deionised water in wash bottle
50 ml beaker
Marker pen or labels

Procedure

2.1 From the lead stock solution, prepare five working calibration standards containing 5, .10, 15, 20 and 25 mg L^{-1} Pb^{2+} in deionised water by pipetting an appropriate volume of the stock solution into a 100 ml volumetric flask and making up to the mark with deionised water.

2.2 For each of the five standards pipette 10.0 ± 0.1 ml of the standard solution into a boiling tube. (Ensure you know which tube is which.)

2.3 Pipette 10.0 ± 0.1 ml of deionised water into a boiling tube. This will be the blank.

2.4 Pipette 10.0 ± 0.1 ml of test sample into the remaining boiling tube.

2.5 Add, using a pipette, 25.0 ± 0.2ml of the dithizone solution to each tube, stopper the tube and extract the lead into the dichloromethane layer by repeated inversions for about 1 minute. R*egularly loosen the stopper to relieve any build up of pressure*. As the lead ions are extracted into the organic layer there will be a distinct change in colour.

2.6 Once the extractions are complete, take the seven boiling tubes to the spectrometer along with some dichloromethane.

ASK FOR A DEMONSTRATION OF THE USE OF THE SPECTROPHOTOMETER.

2.7 Set the spectrometer to 510 nm.

2.8 Fill a cell with dichloromethane, making sure that you hold the cell by the frosted faces and that the transparent optical faces are clean. Set the instrument zero.

2.9 Empty the contents of the cell into the organic waste container and after drying the cell with tissue, fill with the blank solution obtained by transferring some of the lower organic (coloured) layer in the boiling tube to the spectrometer cell using a Pasteur pipette. *(Avoid the transfer of aqueous layer to the cuvette by wiping the outside of the pipette with tissue and delivering the solution to the upper wall of the cuvette.)* Make a note of the absorbance reading.

2.10 Repeat for each of the concentration standards in order of increasing concentration, noting each absorbance reading. *(Again ensure that there are no aqueous droplets present by drying the cuvette with tissue.)*

2.11 Measure the test solution in the same way ensuring that the absorbance falls within the concentration range. (If it does not it will be necessary to accurately dilute the solution.)

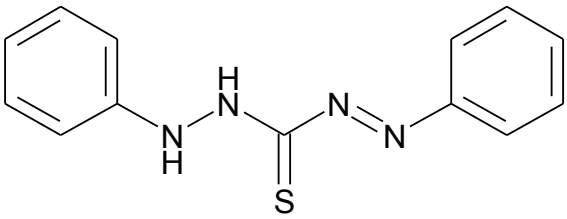
Calculation

Subtract the blank absorbance from both concentration standard and test sample absorbances.

Draw a calibration graph with concentration as the x-axis and corrected absorbance reading as y-axis. Include 0 in your concentrations. The relationship should be linear and in this case you should draw the best fit straight line. If a linear fit appears inappropriate then you should draw a smooth curve. Use the fitted line or curve to convert the corrected absorbance reading of the test sample to a concentration. (Correct for any dilution of the test sample that you may have made.)

Chemical background

Diphenylthiocarbazone, commonly called dithizone, is negatively charged in alkaline solution and forms a neutral co-ordination complex with Pb^{2+} which can be extracted in dichloromethane.



A good analytical reagent for a metal ion should form a very strong complex with the metal ion of interest but also be very specific, that is not react with any other metal ions in solution. Dithizone is such a reagent, which is reasonably specific for Pb^{2+} forming a red complex. The colour can be used to determine the concentration of the lead (II) ions. See below for explanation of spectroscopy.

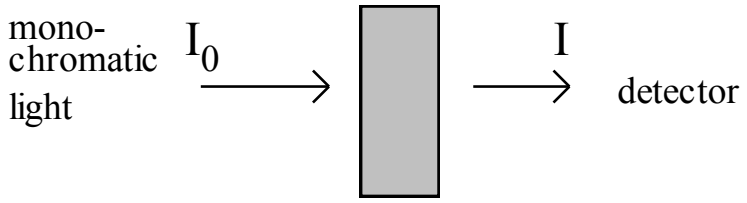
UV / visible spectrophotometry

Light can be split into the spectrum of colours that we see in a rainbow; different colours signify different wavelengths and, therefore, different energies. We call a beam of light of one colour monochromatic. Light will be absorbed by an atom, ion or molecule when the energy of one

quantum of a particular wavelength of light matches the energy required to cause an electron in an outer orbital to jump to a higher energy level.

Each absorption band is caused by the transition between a given pair of energy levels; because the energy level differences vary with different electronic structures, absorption spectra can often be used to help identify the analyte atom, ion or molecule.

The technique of spectrophotometry relies on the absorption of light by the analyte; the intensity of a beam of light is measured in the absence then presence of analyte and the decrease in transmitted intensity is used to determine the analyte concentration.



The Beer-Lambert law expresses the relationship between absorption and concentration:

$$A = \varepsilon cd$$

where A = absorbance, ε = molar absorptivity (L mol^{-1} cm^{-1}), c = concentration (mol L^{-1}) and d = optical path-length (the distance that light travels through the sample, in cm). If this relationship is valid, then a graph of absorbance against concentration for a solution will be a straight line, which passes though the origin.

This is the basic equation of spectrophotometry. The spectrophotometer can only measure the intensity of light, however, so we need an additional relationship linking absorbance to I and I_0. This is $A = \log (I_0/I)$.

Toxicology of lead

There is no physiological requirement for lead and its compounds are considered to be extremely dangerous, as they are cumulative poisons. Human exposure to lead has been through a variety of sources including plumbing and emissions from vehicles using petrol containing organo-lead compounds such as lead tetraethyl. Whilst there will be some lead in the soil, little is taken up by plants and most of the lead contamination of plants arises from airborne deposition of particulates onto the plants. The EU permitted maximum for lead in food is 1 mg of Pb for 1 kg of dried food.

The toxicological problems of lead probably arise from binding of Pb^{2+} ions to N and S centres of proteins and to DNA.

3. Chromatography of food additives

In this experiment you use thin layer chromatography to distinguish between three food colourings and by comparison identify the food colouring in the test sample.

Equipment

TLC plates (10 x 10 cm, silica gel.)
or (15x 15 cm, silica gel.)
Chromatography tank
Capillaries for spotting
Gloves
4 sample vials
Hot air drier
Microspatula

Acetone
Methyl ethyl ketone
Butan-1-ol
Ammonia solution (conc.)
250 ml glass stoppered bottle
25 ml measuring cylinder

Procedure

3.1 Make up the chromatography solvent in a fume cupboard. Into a 250 ml glass stoppered bottle, add 25 ml butanol, 15 ml butanone, 5 ml aqueous ammonia and 5 ml deionised water. Swirl well and allow to stand with occasional swirling for 10 minutes to ensure complete mixing.

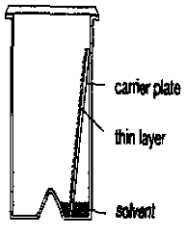
3.2 Place 20 ± 1 ml of solvent in the chromatography tank, close the lid and allow to stand for at least 20 minutes with occasional swirling whilst preparing the plate.

3.3 The solid standards and test sample are dissolved in a 50/50 acetone/water mixture in the sample vials provided. The azo dyes are more intense than the saffron and so less of these will be needed. Dissolve the amount you can get on the end of a microspatula (approximately 5 mg) of tartrazine and sunset yellow in about 1 ml of acetone/water solvent each. For saffron and the test sample use about twice as much solid in the same volume of solvent.

3.4 Mark on the TLC plate positions for four spots with pencil about 1.5 cm away from the bottom of the plate and a similar distance apart.

3.5 Using a drawn out capillary tube, spot each dye onto the plate, using the hot air drier to dry each spot. For the tartrazine and sunset yellow use one application. For the saffron and test sample, once the first spot is dry make another application up to a total of five.

3.6 Carefully lower the plate into the tank ensuring that the spots are above the level of the solvent. Cover the tank and allow the solvent to rise up the plate.

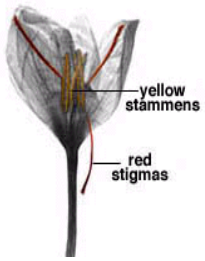


3.7 Once the solvent has risen at least ¾ of the way up the plate, carefully remove the plate from the tank and dry for about 10 minutes in the oven.

Calculation

Compare the chromatogram for the test sample with those of the known standards and hence deduce the components of the test sample. Support your deduction by reporting R_f values. (See below.)

Chemical background



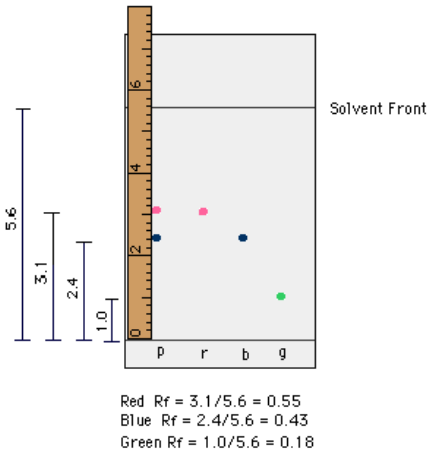
Saffron refers to the stigmas of the plant Crocus sativus. It is orange in colour and has a particularly unusual taste. There are a number of chemical constituents including picrocrocin and crocin.

Food colourings such as tartrazine and sunset yellow are azo dyes and are not chemically related to the components of saffron.

Azo compounds, which contain the –N=N- group, are usually strongly coloured. Several have been approved for food use but since the 1970s it has become apparent that some people can become sensitised to these compounds.

Thin layer chromatography (TLC)

There are many chromatographic methods of analysis, all of which involve separation of components of a mixture using a stationary and a mobile phase. In TLC, a liquid phase moves over a stationary flat surface such as silica gel. The solutes migrate at a different rate across the surface and are thus separated. Each result is expressed in terms of a Retardation Factor, R_f, which is the distance travelled by the solute divided by the distance travelled by the solvent. TLC has the advantage that it is easy to carry out and inexpensive but the precision of the results is poor being only about 10%. In this case as the compounds are coloured they can be seen directly.



Name of School or College: .....

Team members: .....

.....

.....

Flow chart for experimental design and work allocation:

Results

1. Determination of lithium by flame emission

Standard solutions

Concentration:				
Emission reading:				

(Attach calibration graph.)

Emission reading of test solution _____

Concentration of lithium in test solution (from graph): _____ mmol L^{-1}
(Keep to a realistic number of significant figures.)

Estimation of uncertainty

> Obtain a very rough estimate of the uncertainty in the answer by looking at the distance between the points and the fitted line on your calibration graph. Assume for simplicity that all the error is in the reading. Use the data point which is furthest from the line and convert an error in the reading to an error in the concentration and assume that this will be the worst case uncertainty in the concentration of the answer.

Correction factor for dilution of test solution (if appropriate) _____

Concentration of lithium in supplied solution is _____ ± _____ mmol L^{-1}

Calculations:

2. Determination of lead (II) ions

Concentration standard solutions

Concentration:					
Absorbance:					

(Attach calibration graph)

Absorbance of test solution _____

Concentration of lead (II) in test solution (from graph): _____ mg L^{-1}
(Keep to a realistic number of significant figures.)

Estimation of uncertainty

> Obtain a very rough estimate of the uncertainty in the answer by looking at the distance between the points and the fitted line on your calibration graph. Assume for simplicity that all the error is in the reading. Use the data point which is furthest from the line and convert an error in the reading to an error in the concentration and assume that this will be the worst case uncertainty in the concentration of the answer.

Concentration of lead (II) in test solution is _____ ± _____ mg L^{-1}

Calculations:

3. Chromatography of food additives

Draw the important features of your chromatogram.

Give your deduction about the food colouring in the curry powder.

Give your supporting evidence including R_f values.

Results and conclusion.

Enter your results here with your estimates of the uncertainty in the answers and units.

(Use a realistic number of significant figures.)

Lithium concentration _____ ± _____

Lead (II) concentration _____ ± _____

Food colouring identified _____

Your conclusion about these results.

(Comment on what you deduce from each of the values.)

Questions

1 What is the chemical formula for lithium carbonate?

2 To obtain a solution that is1 mmol L^{-1} in Li^+, what concentration (in mmol L^{-1}) of lithium carbonate would you use?

3 What is the concentration of Li^+ in mg L^{-1} in a solution which is 1 mmol L^{-1} in Li^+? (RAM of Li is 6.94)

4 What was the fuel gas used in your flame emission instrument?

Write a balanced equation for its combustion.

5 What sort of battery contains lead?

6 Suggest a possible reason why plants take up only a small proportion of lead from the soil?

7 Why do you think that the different compounds are retarded by the silica gel in thin layer chromatography?