KEELE
UNIVERSITY

RSC | Advancing the Chemical Sciences

SCHOOLS' ANALYST COMPETITION 2010

MIDLANDS REGIONAL HEAT

INSTRUCTION BOOKLET

ROYAL SOCIETY OF CHEMISTRY
ANALYTICAL DIVISION

LENNARD-JONES LABORATORIES
UNIVERSITY OF KEELE

Food Additives: Paranoia or Poisoning?

Welcome:

Welcome to the Schools' Analyst competition. 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 so that each of you is always busy. 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:

Your friend Katie has recently joined a local rugby club. She's been training hard and has made the girl's first team. However, after her Tuesday night training sessions she often feels unwell and has complained of nausea and skin flare-ups.

After doing some research Katie has found out that artificial food colourings can trigger allergic responses similar to the symptoms she experienced. She can think of two possible sources for the artificial food dye:

1. The rugby club is sponsored by "Powerade" who supply the club with batches of their blue sports drink free of charge. Katie suspects they might have been given a dodgy batch containing dangerous levels of an artificial blue food dye.

2. After practise night the rugby club order a takeaway from the local curry house. The curry house claims to only use natural colourings such as saffron and turmeric in their food, but Katie suspects that they might be using cheaper artificial dyes to make their food look more appealing.

Katie has been complaining loudly about her suspicions, and threatening to run tests on the leftover curry and the sports drink. When she goes back to the clubhouse to collect samples she finds there has been a fire at the clubhouse kitchen. Fortunately the fire brigade arrived in time to put out the fire with only limited damage.

Suspiciously the fire appears to have started at the door to the kitchen, rather than being caused by a faulty appliance. Katie wonders if the fire has been started deliberately to destroy evidence of misuse of food additives.

Katie is able to rescue a sample of left over curry powder and a couple of bottles of Powerade drink. She also collects a soil sample from just outside the clubhouse kitchen door to compare with a 'blank' soil sample taken 50m away from the scene of the fire.

The Tasks:

Your tasks today are:

1. To determine which food additive is present in the blue sports drink and whether it is present at safe levels.

2. To determine whether the sample of left over curry powder contains natural or artificial colourings, and to identify the type of colouring used.

3. To determine whether there are petroleum hydrocarbons present in the soil sample taken from the scene of the fire.

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:

UV/vis spectroscopy	1.5 hours
TLC Analysis	1.5 hour
GC	2 hours
Write-up / Questions	0.5 hours

Note: For the GC experiment your team will need to book three 20 minute slots on the GC sign-up sheet. We recommend you book these slots at the start of the day and plan the rest of your work around them.

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.

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.

2010 National Schools' Analyst Competition
Keele University

Important Safety Information

Lab coats and safety spectacles must be worn at all times in the laboratory (these will be provided).

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**
Acetone	Highly flammable, irritant	Fume cupboard. Wear gloves.
Hexane	Highly flammable, irritant, harmful,	Fume cupboard. Avoid skin contact, wear gloves.
Butanol	Flammable, harmful, irritant	Fume cupboard. Avoid skin contact, wear gloves.
Ethanol	Highly flammable	Fume cupboard.
Conc. ammonia	Flammable, toxic, causes burns	Fume cupboard. Avoid skin contact, wear gloves.
Tartrazine	Harmful	Do not breathe dust, avoid skin contact, wear gloves.
Sunset yellow	Irritant	Do not breathe dust, avoid skin contact, wear gloves.
Saffron	Irritant	Do not breathe dust, avoid skin contact, wear gloves.
Turmeric	Irritant	Do not breathe dust, avoid skin contact, wear gloves.
E133 Brilliant blue	Harmful, toxic	Avoid skin contact, wear gloves.
E131 Patent blue	Harmful, toxic	Avoid skin contact, wear gloves.
Sports drink	Not for human consumption	Do not ingest.

1. Determination of the dye concentration in a sports drink using UV-VIS spectrophotometry

In this experiment you will use UV-VIS spectrophotometry to identify the dye used in the sports drink. You will then construct a calibration graph by measuring the absorbance of standards of known concentrations, and use it to determine the concentration of dye in the sports drink.

Equipment:

5 x 10 ml volumetric flasks	Stock solution of E133 dye ($1x10^{-4}$ mol L^{-1})
100-1000 µl auto-pipette	Stock solution of E131 dye ($1x10^{-4}$ mol L^{-1})
Plastic pipettes	Questioned sports drink sample
UV-VIS spectrophotometer	Distilled water
Cuvettes	

Experimental procedure:

1. From the stock solutions provided, prepare a $1x10^{-5}$ mol L^{-1} solution of E131 and a $2x10^{-5}$ mol L^{-1} solution of E133 by using an auto-pipette to pipette an appropriate volume of stock solution (you will need to calculate this) into a 10 ml volumetric flask and making up to the mark with distilled water.

IF YOU ARE UNSURE OF HOW TO USE AN AUTO-PIPETTE PLEASE ASK A DEMONSTRATOR.

2. Use separate plastic pipettes to transfer a small amount of these prepared solutions, the sports drink sample, and distilled water (to use as a blank) to separate cuvettes (fill about ⅔ full).

CONSULT A DEMONSTRATOR ABOUT THE OPERATION OF THE SPECTROPHOTOMETER.

3. Using water as a blank, collect the visible absorption spectra of the 3 samples (E131, E133, sports drink) over the wavelength range 400-700 nm. Identify λ_{max} for each of the dyes, and determine which dye is present in the questioned sports drink sample. Record the absorbance of the sports drink sample at λ_{max}.
4. From the appropriate stock solution, prepare 4 calibration standards of the dye present in the sports drink (using volumetric flasks as in step 1). The concentrations of these solutions should be as follows: $2x10^{-5}$ mol L^{-1}, $1x10^{-5}$ mol L^{-1}, $0.5x10^{-5}$ mol L^{-1}, and $0.2x10^{-5}$ mol L^{-1}.
5. Transfer each solution to a separate cuvette.
6. Collect the spectrum of each solution (using water as a blank), and record the absorbance at λ_{max}.

Now plot a calibration graph of absorbance (at λ_{max}) versus concentration, and use the graph to determine the concentration of the dye in the questioned sports drink. Record your results on the answer sheet.

Results:

λ_{max} value for E133 solution:

λ_{max} value for E131 solution:

λ_{max} value for questioned sports drink sample:

The dye present in the questioned sports drink sample is:

Dye concentration (mol L^{-1})	**Absorbance**

Sports drink sample	

Attach calibration graph

Concentration of dye in sports drink (from graph): _____mol L^{-1}

Convert the molar concentration (mol L^{-1}) to mass concentration (g L^{-1}) of dye:
(Relative molar mass E131 = 582.66 g mol^{-1}, E133 = 792.85 g mol^{-1})
Show details of your calculations.

Now convert to mg L^{-1}:
(Remember that there are 1000 mg in a g)

The maximum permitted level of E133 and E131 in drinks is 50 mg L^{-1}. Is the concentration of dye in the sports drink sample you have tested within this limit?

Experiment 1 - Background Information

UV-VIS Spectroscopy

Many compounds absorb light in the near-ultra violet 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:

$$T = \frac{I}{I_0}$$

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

$$-\log_{10}T = \varepsilon cl$$

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

$$\mathbf{A = \varepsilon cl}$$

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 recorded at $\boldsymbol{\lambda_{max}}$ (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 solutions of specific concentrations. To work out the volume of stock solution required to do this, the following equation will be useful:

$$\mathbf{c_1V_1 = c_2V_2}$$

(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).

You will then use an autopipette to transfer the calculated volume (V_1) of stock solution into the volumetric flask (remember that there are **1000 µl in 1 ml**).

2. Thin layer chromatography of food colourings

In this experiment you will use thin layer chromatography to identify what food colouring(s) are present in the curry powder sample, in particular to determine whether natural (saffron/turmeric) or artificial (tartrazine/sunset yellow) colourings have been used.

Equipment:

25 ml measuring cylinder	50/50 acetone/water mixture
250 ml conical flask	Butanol
Parafilm	Ethanol
TLC plate	Distilled water
Pencil and ruler	Conc. ammonia
5 sample vials	Tartrazine (E102)
100-1000 µL autopipette	Sunset Yellow (E110)
TLC spotters	Turmeric (E100)
Heat gun	Saffron (E164)
Tall beaker and watch glass	Curry powder sample
Oven	

Experimental procedure:

1. Make up the chromatography solvent in a fume cupboard: into a 250 ml conical flask add 50 ml butanol, 25 ml ethanol, 25 ml distilled water, and 10 ml concentrated ammonia. Cover with Parafilm, swirl well, and then allow the mixture to stand with occasional swirling whilst you prepare the TLC plate.
2. Add 1 ml of the 50/50 acetone/water mixture to each of the vials containing the 4 standard dyes and the test sample.
3. Draw a line on the TLC plate about 1cm from the bottom using a pencil and ruler, and mark on positions on the line for five spots that are a similar distance apart (be careful not to disturb the silica when you do this).
4. Using separate TLC spotters, spot each dye and the test sample once onto the plate, using the heat gun to dry them gently. For saffron, spot twice more, making sure each spot is dried before you apply the next one.
5. In a fume cupboard, transfer the chromatography solvent to a tall beaker, ensuring that the solvent surface will lie below the line of sample spots on the TLC plate (a solvent depth of ~0.5cm should be adequate).
6. Carefully lower the TLC plate into the beaker, cover with a watch glass, and allow the solvent to rise up the plate (this will take at least 30 minutes).
7. When the solvent front has travelled $^3/_4$ of the way up the plate, remove the plate from the beaker and mark on the position of the solvent front. Leave the plate in the fume cupboard for ten minutes, and then dry it in the oven for 10 minutes.

Now calculate the R_f value for each spot and determine which colouring(s) are present in the test sample. Record your results on the answer sheet.

Results:

Draw a diagram of your TLC plate, labelling it clearly:

R_f value calculations:
Show details of your working out below the table.

Distance moved by solvent front = _____

Spot	Tartrazine	Sunset Yellow	Turmeric	Saffron	Test Sample
Distance moved					
R_f Value					

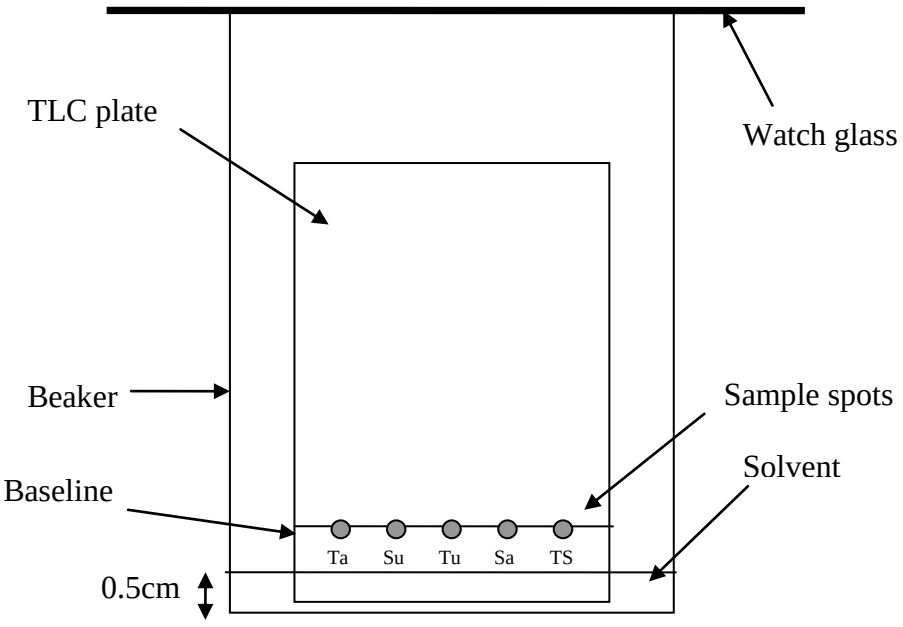
Give your deduction about the food colouring in the curry powder:

Experiment 2 - Background Information

Thin Layer Chromatography

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.

Thin layer chromatography uses a thin uniform layer of silica gel (or alumina) coated onto a sheet of glass or plastic. The silica gel (or alumina) is the stationary phase, and a suitable solvent (or mixture of solvents) is used as the mobile phase. The samples to be analysed are dissolved in a suitable medium and then applied to the TLC plate. Once dried, the plate is placed in a tank containing the mobile phase. The apparatus used for TLC is shown below:



A quantity called the $\mathbf{R_f}$ value (Retardation Factor) is used to aid identification of the components of a mixture. This is calculated using the equation below:

$$R_f = \frac{\text{distance travelled by component spot}}{\text{distance travelled by solvent front}}$$

The dyes that you will be testing in this experiment may have more than one spot, if this is the case make sure you calculate the R_f value for each spot.

3. Chromatographic analysis of petroleum hydrocarbons in soil

In this experiment you will carry out a solvent extraction using acetone in order to extract any petroleum hydrocarbons that may or may not be present in the questioned soil sample. You will then use gas chromatography to obtain GC-traces of the soil extract as well as diesel and petrol reference samples.

Equipment:

100 ml beaker Balance 25 ml measuring cylinder Foil Sonic bath Funnel and filter paper Conical flask (to collect filtrate) GC vial Plastic pipette GC instrument and computer Injection syringe	25 g questioned soil sample Acetone Petrol standard (10 µl in 1 ml of hexane) Diesel standard (10 µl in l ml of hexane) Hexane (to clean syringe)

Experimental Procedure:

1. Weigh out between 20 and 25 g (note the exact weight to 2 decimal places) of the soil sample into a 100 ml beaker.
2. Add acetone to the sample; use 1ml of solvent for each 1 g of soil.
3. Cover the beaker with foil and place it in an ultra sonic bath (ca. 30 minutes).
4. Filter the sample using a funnel and fluted filter paper. Rinse the extracted soil once with 2 ml of acetone.
5. Using a plastic pipette transfer approximately 1 ml of the extract into a labelled GC vial.

CONSULT A DEMONSTRATOR ABOUT THE OPERATION OF THE GC.

6. When the GC is ready, inject 2 µl of your prepared sample and start the data collection (this will take about 15 minutes). Rinse out the syringe using the hexane and paper towel provided.
7. You will also need to inject the petrol and diesel reference solutions that have been prepared for you and are located by the GC instrument. (This step can be done before the solvent extraction.)

The ‘blank’ soil sample that was taken 50m away from the scene of the fire has been processed for you and you will be provided with the GC trace. Now compare the GC trace of the questioned soil sample with that of the blank soil sample and the petrol and diesel reference samples, and determine if there are any petroleum hydrocarbons present in the questioned soil sample.

Results:

Chromatogram of petrol reference:

Chromatogram of diesel reference:

Chromatogram of questioned soil sample:

Are there any petroleum hydrocarbons present in the soil sample from the scene of the fire? If so, identify the type of petroleum hydrocarbon. Explain your answer.

Experiment 3- Background Information

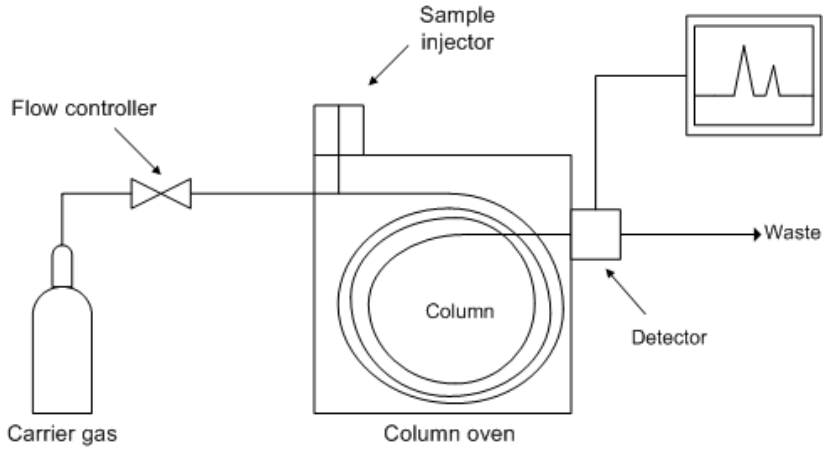
Gas Chromatography

Gas chromatography (GC), like TLC, is another separation technique. However, in GC, the mobile phase is a carrier gas (in this experiment it is helium), and the stationary phase is a liquid that is adsorbed onto the surface of an inert solid and packed into a column.

A small amount of the sample to be analysed is drawn up into a microsyringe and injected into the injector port. The injector port is set at a temperature of around 200 °C so that the components of the mixture instantly volatilise, and then they are carried onto the column by the carrier gas. The temperature of the column can be varied from about 50 °C to 250 °C, depending upon the boiling point of the sample. If a sample has a wide boiling range, then temperature programming can be useful. The column temperature is increased (either continuously or in steps) as separation proceeds. (For this experiment, a temperature programme has been set up for you.) Separation occurs in the column as a result of differential partitioning of the individual compounds between the mobile and stationary phases.

The separated components are detected by a detector, which in this case is a flame ionisation detector (FID). This involves passing the gas flow from the column through a hydrogen flame. Organic compounds passing through this flame will produce ions and electrons which create a small current across the electrodes that can then be detected. The output is recorded as a series of peaks on a time-based recorder - each one representing a compound in the mixture. The retention time of these peaks can be used for qualitative identification by comparison with known components.

The diagram below shows the components of a GC system:



Questions

1. Why was a 'blank' soil sample (collected 50 m from the scene of the fire) required for the GC analysis in experiment 3?

2. What is the name given to the phenomenon responsible for the tendency of a liquid to ascend through a piece of paper?

3. If a sports drink contained 45 mg L^{-1} of E133, how much would a person who weighed 70 kg need to drink in one day in order to exceed the acceptable daily intake of 12 mg/kg/day? Show details of your calculation.

4. The molecular formula of Sunset Yellow (E110) is $\mathbf{C_{16}H_{10}N_2Na_2O_7S_2}$. Using the periodic table provided calculate its molar mass (formula weight, g/mol). Show details of your calculation.

5. A 0.1 mol L^{-1} solution within a 1 cm path length placed within a UV-Vis spectrophotometer shows an absorbance of 0.95. Calculate the absorption coefficient ε for this compound from the relationship $\mathbf{A = \varepsilon cl}$. Show details of your calculation and work out the units of ε.