# Royal Society of Chemistry
# Analytical Division
# East Anglia Region

# 2011 National Schools' Analyst Competition

## East Anglia Region Heat
## April 2011

## School of Chemistry
## University of East Anglia
## Norwich

# Important Safety Information

Eye protection is compulsory in the laboratory. Safety spectacles or goggles must be worn at all times.

You are advised to wear disposable gloves at all times.

Pipetting by mouth is FORBIDDEN. Please use the fillers provided, If you do not know how to use them, please ask.

Some of the chemicals you will be using are hazardous. The hazards and precautions associated with each chemical are listed below. Please read this information and ensure that you understand it, then sign the page to confirm that you have read and understood the information.

| **Chemical substance** | **Hazards** | **Precautions** |
|---|---|---|
| sucrose | None in this context | |
| caffeine | Harmful if swallowed | Do not ingest, avoid skin contact, wear gloves |
| Potassium hydrogen phthalate | irritant | Do not breathe dust, avoid skin contact, wear gloves |
| Sodium hydroxide | Corrosive, causes severe burns | Avoid skin contact, wear gloves |
| Phenolphthalein indicator solution | Irritant, may be carcinogenic | Avoid skin contact, wear gloves |

I have read and understood the safety information given above, and agree to abide by the safety rules set out.

Signature: _____

Print Name: _____

Signature: _____

Print Name: _____

Signature: _____

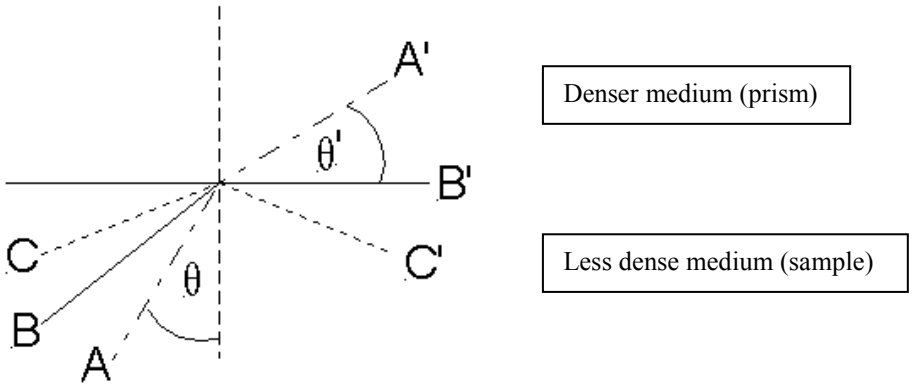
Print Name: _____

**In the laboratory exercises, you will be analysing the composition of a very well known product –Coca Cola. This introduces some important analytical techniques and also provides a good example of some of the practical problems encountered when working with real samples, rather than convenient test materials. Below, we provide some theory behind the methods you will be using to help you to understand the analytical principles involved. Please don't worry if you don't fully understand it all. You will be given detailed instructions on the day, and it is still possible to do very high quality practical analysis without understanding all the underlying theory (many analysts don't!).**

**Brief Theoretical Background to the Techniques used in the Analytical Exercises**

**Sugar Estimation by Refractometry**

The Index of Refraction, or Refractive Index, of a substance is the ratio of the speed of light in a vacuum to the speed of light in the substance. The speed of light in a vacuum is a maximum (there is nothing to impede its propagation). The speed of light in any other substance is lower than the speed in a vacuum, hence the refractive index of any susbstance is always greater than 1. For example, water has a value of about 1.33, and window glass about 1.52. The refractive index of a substance can be measured using an instrument called a refractometer. There are many different types, with different optical configurations. The one you will use is an Abbé refractometer. This makes use of the fact that the critical angle for refraction of light passing from one medium to another depends on the refractive indices of the two media. The Abbé refractometer measures the critical angle for light passing from a glass prism of known refractive index to a substance of unknown refractive index. The critical angle depends on the




refractive indices of the two media.

At angles of θ below the critical angle, refraction occurs (e.g. A-A'). At the critical angle, the light travels along the interface between the two media (e.g. B-B'). Beyond the critical angle, total internal reflection occurs (e.g. C-C')

When a substance is dissolved in water, the refractive index of the solution almost always increases (since the solute almost always has a higher refractive index than water). The refractive index is approximately proportional to the concentration of the solute in the solution (strictly, it depends on the volume fraction of each component). A good way of estimating concentration is thus to measure the refractive indices of a series of calibration solutions of

known concentration and plot a calibration graph. The concentrations of unknown solutions can then be determined by measuring the refractive index and interpolating from the graph to convert refractive index into concentration. The technique is not very sensitive, but works well when relatively high concentrations are being measured, since making the measurements is quick and easy (once you have got used to using the refractometer!).

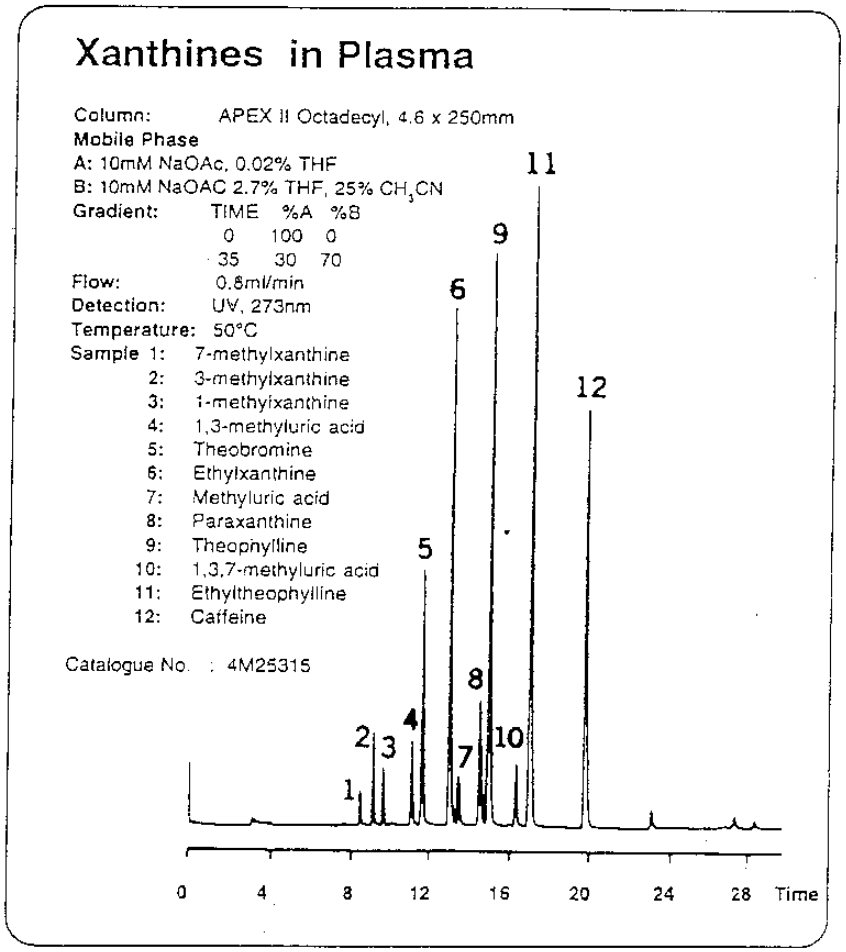
Coca Cola contains a relatively high concentration of sugar. The type of sugar used depends where the Coca Cola is made. In the USA, high fructose corn syrup (a mixture of glucose and fructose, produced from maize starch) is used. In the UK, sucrose is generally used since it is much more readily available. In this exercise you will be measuring the sucrose content of UK produced Coca Cola, by making up and measuring known standard solutions of sucrose and plotting a calibration graph, from which the concentration in the Coca Cola can be determined by interpolation.

**Caffeine Quantitation by HPLC Analysis**

Chromatography is a generic term for a family of separation techniques including paper chromatography (which you might have done), thin-layer chromatography and the two main instrumental methods, gas chromatography (GC) and high performance liquid chromatography (HPLC). GC and HPLC are extremely powerful instrumental methods of analysis and are widely used in many analytical situations. This is because they can **separate** the various components present in complex mixtures (most analytical samples are complex mixtures of one sort or another), and also **quantitate** the different components present.

In all types of chromatography, there are two distinct phases, a mobile phase and a stationary phase. In HPLC, the mobile phase is a liquid, and the stationary phase is made up of very small porous particles packed into a column. A small sample (usually 10-20 µL - 1 µL = 1 $mm^3$) of the analytical mixture is injected into the system using a special sampling loop through which the mobile phase solvent (under high pressure - required to drive the solvent through the small particles in the column) is flowing at a constant rate. The components of the mixture partition between the mobile and stationary phase in a continuous on/off process as they are carried down the column by the mobile phase solvent. Compounds that have a high affinity for the stationary phase, spend more time on the stationary phase, and hence move more slowly along the column, compared with those that have a lower affinity. The affinity depends on the chemical structure of the analyte molecule. This results in separation of the components of the mixture as they move along the column. As each analyte emerges from the end of the column it passes through a detector, which signals its presence by recording a peak on the chromatogram. The whole chromatogram will contain several (maybe many) peaks, each of which signals the presence of at least one analyte.

A typical chromatogram is shown below:




The time taken for a peak to emerge from the column after injection, its **retention time**, is a measure of **analyte identity**, when compared with standard analytes run under the same chromatographic conditions

The area underneath the peak can be integrated to give a measure of the **amount** of .analyte present. Again this needs to be **calibrated** using some kind of standardisation method. In this exercise you will be using the method of **standard addition**.

**The method of standard addition**

This is an alternative method to the more familiar technique of running a series of external standards of known concentration and plotting a calibration graph (as you will do for the refractometry experiment). Because it needs only two measurements, it is a very economical technique when only one or a few samples need to be analysed.

Equal quantities (weight or volume) of sample are added to two flasks of the same volume. To each flask is then added any necessary reagents or buffers. One flask is then made up to volume with the appropriate solvent (water in the Coca-Cola case). To the second flask is added a known amount of the pure standard analyte. This flask is then also made up to volume with solvent.

Both flasks are then analysed. Assuming that the analytical signal (area under chromatographic peak in this case) is proportional to analyte concentration, we can express the analyte concentrations in the two flasks as follows

Sample flask $Y_0 = KC$

Standard addition flask $Y_1 = K(C + C_s)$

Where:
K is the sensitivity of the method (a constant - which you don't actually need to know…)
$Y_0$ and $Y_1$ are the analytical measurements
C is the concentration of analyte in the sample flask
$C_s$ is the concentration of the added standard in the standard addition flask

By solving these two equations simultaneaously, it can be shown that:

$$C = \frac{Y_0 \times C_s}{(Y_1 - Y_0)}$$

From which C, the concentration of analyte in the sample flask, can be calculated from the known value of $C_s$ and the measured values $Y_0$ and $Y_1$.

**Phosphoric Acid Estimation by Titration**

Titration is a simple, inexpensive, precise, accurate and robust method of analysis, which is still very widely used in analytical chemistry, despite the development of a vast array of complex instrumental methods. Very many different methods of titration have been developed to measure a wide range of different analytes using e.g. acid/base, redox and complexiметric methods. A weakness of titration is that it is not always easy to apply to complex mixtures, as one component present sometimes interferes with the titration of another component.

Acid base titration is probably a method that is familiar to you. It is a very good way of measuring the **concentration** of an acid or base, by titrating it against a base or acid of accurately known concentration (either because you made it up accurately or because you **standardised** it beforehand).

In the analysis of Coca-Cola acid base titration is used to quantitate phosphoric acid. Unfortunately this is complicated by two factors. Firstly, Coca-Cola is dark brown, which masks the colours of the indicators that would usually be used to indicate the end point of the titration. Secondly, the concentration is rather low, which makes the end point of the titration less distinct and hence harder to measure accurately.

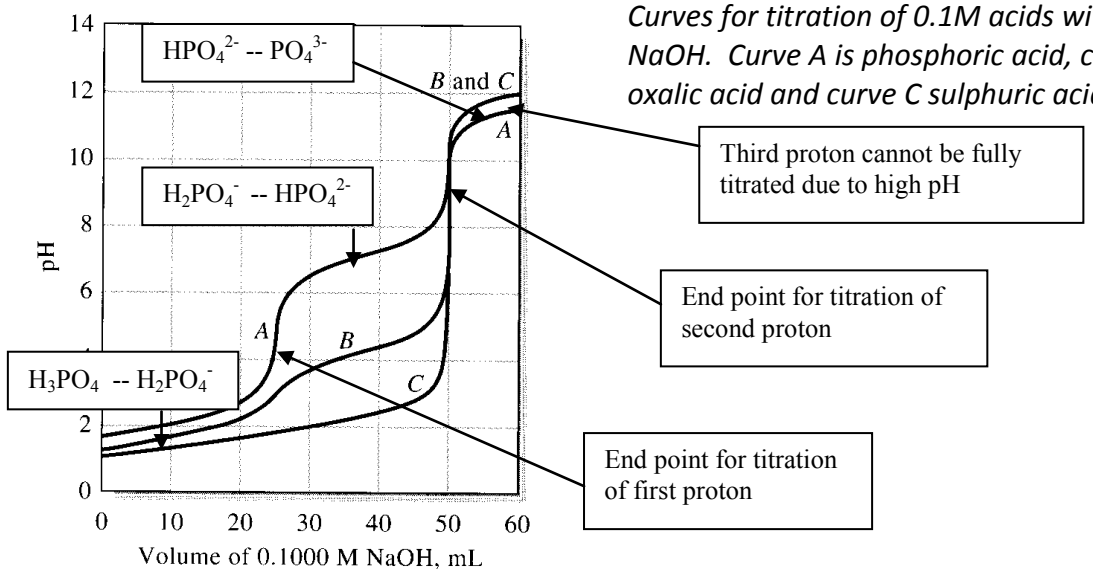
To overcome these problems, the course of the titration will be followed **potentiometrically,** using a **pH meter** to measure the concentration of $H^+$ ions present in solution. A pH electrode has a special sensitive surface, which responds to the concentration of $H^+$ ions by producing an electrical signal, which can be measured and converted to pH by a meter. This allows you to dip the electrode in a solution and measure the $H^+$ ion concentration quickly and easily. Using

this method, you can follow the progress of a titration by measuring the pH after each small addition of acid or base and plotting a titration curve, which can then be analysed to extract analytical information.

The titration of phosphoric acid is quite complex because it is a triprotic acid. This means that it can liberate 3 protons by dissociation as follows:

$H_3PO_4 \Leftrightarrow H_2PO_4^- + H^+ \Leftrightarrow HPO_4^{2-} + H^+ \Leftrightarrow PO_4^{3-} + H^+$

Usually, with acids that are polyprotic, it is difficult to distinguish between the individual ionisations and only the overall process can be easily measured by titration. In the case of phosphoric acid, however, the equilibrium constants for the three separate ionisations are very far apart. This means that the first ionisation is essentially complete before the second ionisation begins i.e. all the phosphoric acid molecules lose one proton before any of them lose their second proton and so forth. Because of this, the three separate processes can be easily identified in the titration curve for phosphoric acid.




*Curves for titration of 0.1M acids with 0.1M NaOH. Curve A is phosphoric acid, curve B is oxalic acid and curve C sulphuric acid.*

In Coca-Cola, the phosphoric acid is present as a mixture of $H_3PO_4$ and $H_2PO_4^-$, so some of the first ionisation has already occurred. Using the pH meter to monitor the pH however, the point at which the first ionisation ends and the second ionisation begins can be easily identified (it is the point where the pH increases rapidly). The second ionisation ($H_2PO_4^-$ -- $HPO_4^{2-}$) can then be fully titrated to its end point (the next point where the pH undergoes a rapid rise). This is what will be done in the Coca-Cola experiment. Due to the rather low concentration of phosphoric acid in Coca-Cola, the rise in pH at the end point is quite small, but using a pH meter to follow the changes in pH, it is relatively easy to identify on the titration graph. You will receive instructions on the day to explain exactly how to analyse your data.

# Introduction

In today's laboratory exercises you will be analysing a very familiar product, Coca-Cola, using analytical methods typical of those that would be used in a production plant for quality control purposes. You will be quantitating the sugar, caffeine and phosphoric acid content of standard Coca-Cola.

There are three different tasks to perform. Two of them, Refractometry and High Performance Liquid Chromatography (HPLC), require quite complex instrumentation that you probably would not have at school. The third task is based on an acid base titration. Most of you will probably be familiar with simple acid base titrations. In this case though, the titration is complicated by several factors, necessitating a rather more sophisticated approach. Some theoretical background to the techniques and how they work is presented on the separate theory sheet. Hopefully, you will have had time to read this before you came to the competition today.

Although today's analytical challenge is competitive, please do not be disheartened if your team does not win. The main objective of the competition is to give students the opportunity to gain experience of chemical analysis that they probably could not do at school. We hope you will all work hard and learn something new in the process, but at the end of the day it is also supposed to be a positive and fun experience, and a taster of what further study in chemistry might be like. Do not panic if you make a mistake, or one of your experiments goes wrong - it is all part of the learning experience…

## First things First…

Coca Cola presents at least two problems to the analyst

- It is highly coloured
- It is very bubbly

It is difficult to do much about the colour, but this interferes with some typical analytical processes, such as the colour change of indicators, so we have to design experiments to get around this problem.

The bubbles make it difficult to measure volumes accurately, since bubbles keep forming and sticking to the walls of glass vessels. Since the bubbles are formed from carbon dioxide, some of this gas also dissolves, producing carbonic acid, which complicates titrations. The best way to remove this problem is to boil the cola. This drives off the dissolved gas and breaks down the carbonic acid.

**The cola samples supplied have been boiled for you to save time, so you do not need to do this.**

**Experiment 1**

Estimation of Sugar Content by Refractometry

*Making up calibration solutions of sucrose*

In a plastic weighing boat, weigh out approximately 4.00g of sucrose (it doesn't need to be exactly 4.00g, but you need to record the weight you used to two decimal places on the results sheet). Use the top pan balances, not the accurate analytical balances as it will be quicker and they have sufficient accuracy for this experiment.

Transfer the sucrose to a 100ml volumetric flask using the small funnel (make sure it is dry!). Tap the funnel to ensure that all the sucrose has gone into the flask. Rinse the weighing boat with a little distilled water and add to the flask. Add distilled water to just below the final volume and shake the flask until all the sucrose has dissolved. Make up to exactly 100ml using a Pasteur (dropping) pipette and thoroughly mix again.

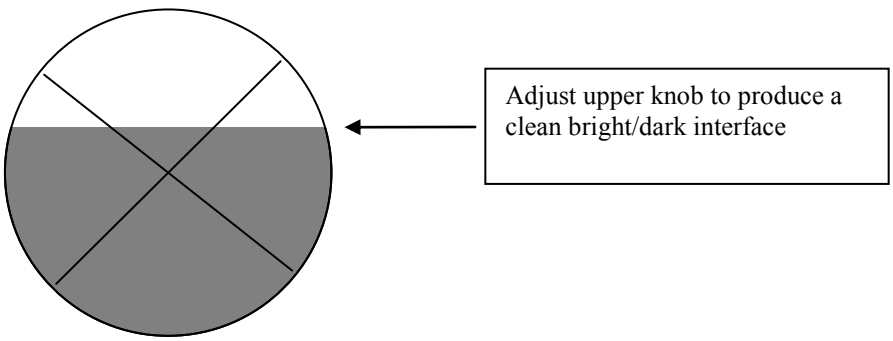
Repeat the above using 8.00, 12.00 and 16.00g of sugar, so that you end up with four calibration solutions of known concentration. Ensure you label your solutions and mix them thoroughly.

*Measuring Refractive Index*

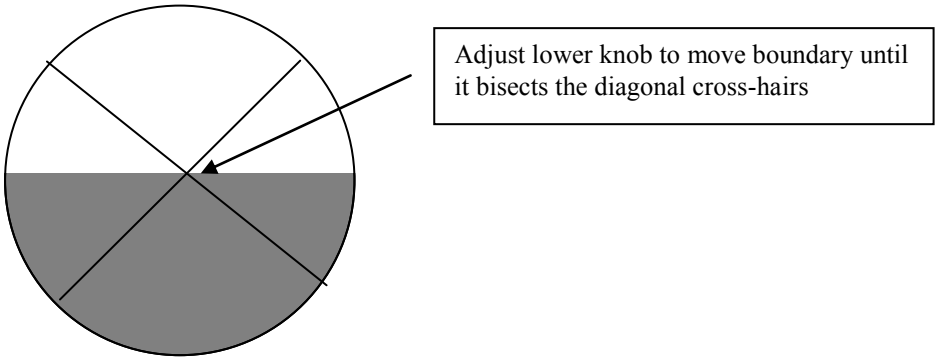
You now need to take your solutions, along with a sample of your degassed Cola (in a small beaker) to the refractometers where a demonstrator will show you how to measure the refractive index of each. The method is described below, but will make much more sense to you when you actually use the instrument.

Place a few drops of solution on the bottom prism, and close the cover. Look through the upper eyepiece and turn the upper knob on the right hand side until a clean light/dark boundary is seen with no colour.

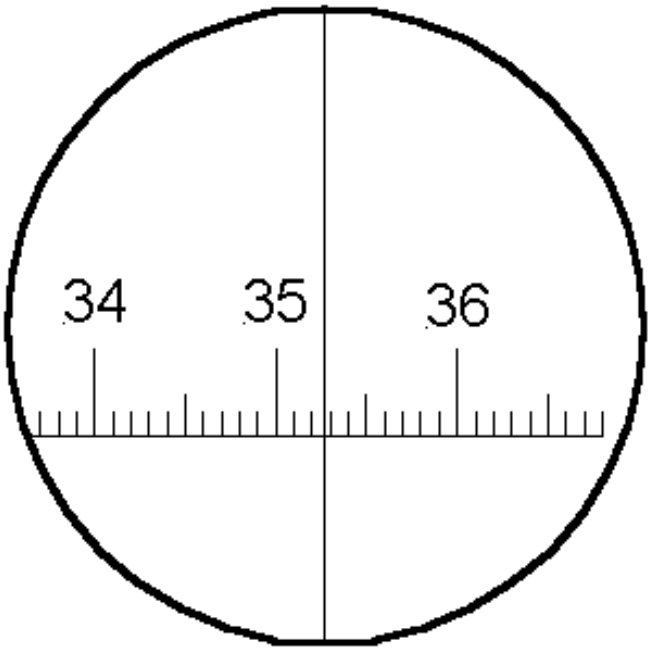
If it is slightly mis-adjusted you will see red/orange or blue/purple colours near the boundary.



Now adjust the lower knob on the right hand side to move the position of the light/dark boundary until it exactly bisects the diagonal cross-hairs.




The instrument is now correctly adjusted and you can read off the refractive index by looking at the scale in the lower eyepiece. The reading is determined at the point where the vertical line bisects the scale.




The refractive index will be 1.???? (in this case it is between 1.35?? and 1.36??)

The third decimal place is determined by counting the number of small scale increments between 1.35 and 1.36 before the vertical line is reached (in this case it is 2, so the reading is 1.352?)

The last decimal place is estimated from the position of the vertical line between the two small scale increments. (Imagine you had another 10 very small

increments in this space. Where would the line bisect the scale? In this case it is about 0.7 or 0.8)

The final reading is thus 1.3527 (or maybe 1.3528)

Once you have measured a sample and recorded the result, open the cover and wipe both surfaces with damp tissue to remove your sample. Dry with another tissue, and then add a few drops of your next sample. Continue adjustment and measurement as above.

Once you have measured and recorded the refractive index for distilled water and all your samples, you will need to analyse the data. This is described on the results sheet.

(I suggest that one team member should do this, while the others start the next exercise).

## Experiment 2

Estimation of Caffeine by High Performance Liquid Chromatography (HPLC)

*Making up the Caffeine Standard Solution*

In a weighing boat, accurately weigh about 50mg of caffeine (you will need to use the four figure analytical balances for this). Record the exact weight of the caffeine.

Transfer the caffeine into a 250 mL volumetric flask, using distilled water to wash any residues in the weighing boat into the flask. Make up to just below the line and shake the flask until all the caffeine has dissolved. Make up to exact volume using distilled water and a Pasteur pipette. Mix thoroughly again.

Using a 25 mL pipette, transfer 25 mL of the caffeine solution into a 250 mL volumetric flask, make up to volume with distilled water and mix thoroughly. This solution is your CAFFEINE STANDARD SOLUTION.

*Making up the solutions for HPLC analysis*

Using a 5 mL pipette, transfer 5 mL of Cola into a 50 mL volumetric flask. Make up to exact volume with distilled water and mix thoroughly. This is your cola sample for analysis. Label the flask

Using a 5 mL pipette, transfer 5 mL of Cola into a second 50 mL volumetric flask Using a 25 mL pipette, transfer 25 mL of caffeine standard solution (see above) into the flask. Make up to exact volume with distilled water using a Pasteur pipette and mix thoroughly. This is your cola sample with added standard. Label the flask.

*HPLC Analysis of the Samples*

Take your samples to the instrument lab, where a demonstrator will show you how to inject your samples into the HPLC instrument. Please be patient if you have to wait a few minutes while other samples are run. Once you have the chromatograms and data for your two samples, you can calculate the amount of caffeine in your cola sample (see results sheet for instructions on how to do this).

(I suggest that one team member should do this, while the others start the next exercise).

## Experiment 3

Estimation of Phosphoric Acid by Acid/Base Titration

*Preparation of standard Potassium Hydrogen Phthalate solution*
In a weighing boat, accurately weigh (to 4 decimal places - use an analytical balance) about 1g of potassium hydrogen phthalate. Record the exact weight used.

Transfer the potassium hydrogen phthalate into a 250 mL volumetric flask, using distilled water to wash any residues in the weighing boat into the flask. Make up to just below the line and shake the flask until all the potassium hydrogen phthalate has dissolved. Make up to exact volume using distilled water and a Pasteur (dropping) pipette. Mix thoroughly again.

*Preparation of Sodium Hydroxide solution*
In a weighing boat, weigh about 2.00 g of sodium hydroxide pellets. The exact amount is not important (anything between about 1.80 g and 2.20 g will be fine).

Transfer the sodium hydroxide pellets into a 250 mL volumetric flask. Make up to just below the line and shake the flask until all the sodium hydroxide pellets have dissolved. Make up to volume using distilled water. Mix thoroughly again.

Using a 50 mL pipette, transfer 50 mL of the sodium hydroxide solution to a 500 mL volumetric flask. Make up to volume with distilled water and mix thoroughly. THIS IS THE SODIUM HYDROXIDE SOLUTION YOU WILL USE IN THE TASKS BELOW.

*Standardisation of the sodium hydroxide solution*
( I suggest that one team member does this, while the other two work on the titration of the Coca-Cola - see below)

Using a glass funnel, fill the 50 mL burette with sodium hydroxide solution, and clamp it in the burette stand (take care - burettes are fragile!). Record the starting volume reading (it should not be deliberately set to exactly zero, as this may introduce bias in your volume readings). Using a 25 mL pipette, transfer 25 mL of potassium hydrogen phthalate solution into a suitable conical flask (e.g. 100 mL or 250 mL). Add about 3 drops of phenolphthalein indicator solution, and titrate with sodium hydroxide from the burette until a pale pink colour is seen, which persists for some time (at least 30 seconds). Record the final volume reading from the burette.

Refil the burette and repeat the above titration. If the volume of sodium hydroxide solution used differs by more than 0.20 mL, repeat the titration a third time (if you have time).

Using the calculation sheet to help you, calculate the exact concentration of the sodium hydroxide solution you have made up.

*Potentiometric Titration of Coca-Cola*

Fill the burette with sodium hydroxide solution, read and record the initial volume and carefully clamp it in the burette clamp of the titration apparatus.

Using a 25 mL pipette, transfer 25 mL degassed cola into the 100 mL beaker containing the magnetic stirrer bar. Switch on the magnetic stirrer and adjust the speed by turning the knob until the stirrer bar is rotating steadily but without splashing (a setting of 3 or 4 should be about right). Remove the pH electrode from its beaker of distilled water and carefully clamp it in place so that the end is dipping into the cola, but it is not being hit by the rotating stirrer bar (please take special care, pH electrodes are expensive!). Allow the pH meter to settle until a reasonably stable reading is obtained. Record the pH (to 2 decimal places). It should be somewhere between pH2 and pH3. If it is not, please ask for advice as there may be a problem with the pH electrode…

Add about 1 mL of sodium hydroxide solution from the burette. Wait until the pH meter stabilises again, and record the new pH value, along with the exact volume reading. Continue making 1 mL additions of sodium hydroxide and recording the pH until 30 mL has been added or you have reached a pH value above 9. It does not matter if you add slightly more or less than 1 mL, just record the volume added and the pH, so you can plot it in the right place on the graph. When you have finished, switch off the magnetic stirrer.

Plot a graph of pH against volume of NaOH solution added. Use a pH range from 2.00 to 12.00 and a volume range from 0.00 to 30.00 mL. If you are organised you can plot this graph as the experiment progresses.

Using your potentiometric titration graph, calculate the volume of NaOH solution required to neutralise the phosphoric acid, and hence the concentration of phosphoric acid in Coca-Cola. The method is explained on the results sheet.