Royal Society of Chemistry
Analytical Division
East Anglia Region

2013 National Schools' Analyst Competition

East Anglia Region Heat
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School of Chemistry
University of East Anglia
Norwich

Analytical Challenge for the Day

Your analytical challenge is to analyse a pharmaceutical formulation to verify whether or not its composition is as claimed.

The formulation contains ascorbic acid (vitamin C), paracetamol and sucrose.

Background to the Techniques you will use in the Laboratory

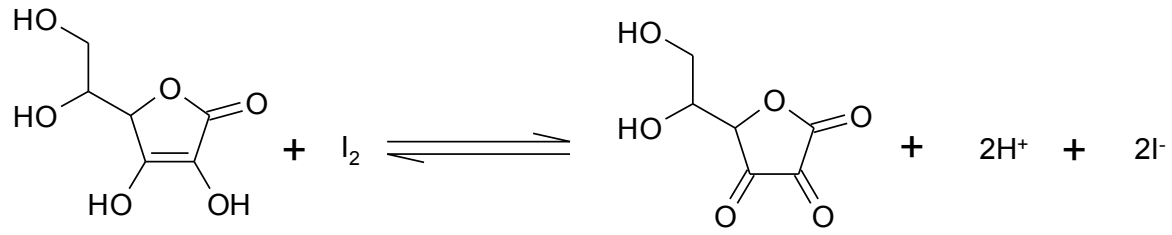
Quantitating Ascorbic Acid by a Direct Redox Titration

Redox titrations are based on the transfer of electrons between the analyte and the titrant, hence in the process one species becomes oxidised while the other is reduced. Many different species can be used as the titrant, depending how strong an agent is required to oxidise or reduce the analyte. Typical reagents you might have come across include dichromate, permanganate and iodine.

Iodine is a moderately strong oxidising agent. As the analyte is oxidised, iodine becomes reduced to iodide according to the following equation:

$$I_2 + 2e \rightleftharpoons 2\,I^-$$

Iodine can oxidise ascorbic acid as follows:

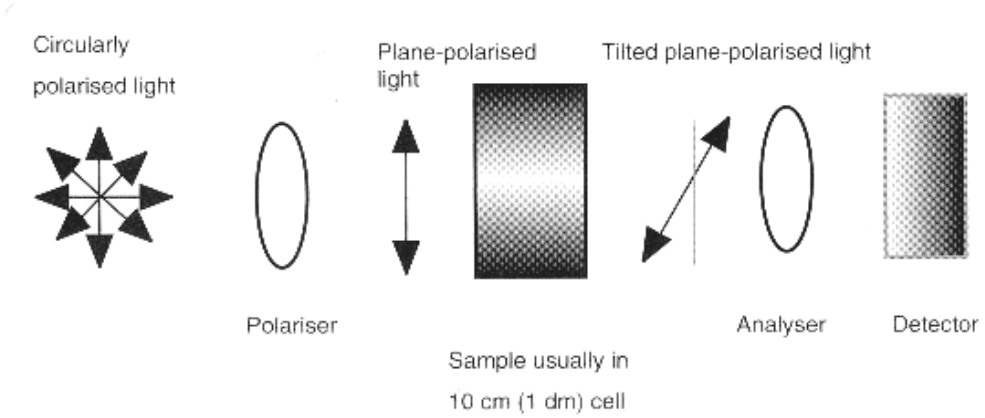


Iodine is relatively insoluble in water, but its solubility can be greatly increased by dissolving it in potassium iodide. This forms the I_3^- ion, which is much more soluble than I_2, but it releases I_2 freely to react, so the solution can be considered to be I_2 for practical purposes. In this form it is a useful reagent for a wide range of redox titrations. The indicator used for iodine titrations is soluble starch. Iodine complexes strongly with amylose chains (linear polymers of D-glucose) in the starch, producing an intense blue-black colouration. The appearance or disappearance of this colouration indicates the presence or absence of I_2.

Ideally, iodine solutions would be standardised using standard sodium thiosulphate to determine their precise concentration before use. This step is omitted for the competition due to lack of time, but solutions made up gravimetrically from sublimed iodine (high purity) are usually very close to the expected concentration, so any error due to this should be minimal.

Quantitating Sucrose in a Mixture using Polarimetry

An important property of many molecules is chirality. Chiral molecules are mirror images of one another and their 3D structures cannot be superimposed. The two mirror images are called enantiomers, and their absolute configurations are defined as "D" or "L" according to a convention. Such molecules have identical physico-chemical properties, but they can be distinguished from one another based on particular properties related to their shape or, as in this case, based on their interaction with light. Chiral molecules have the ability to rotate the plane of polarised light, dependent on their absolute molecular configuration. For any given molecule, the D enantiomer will rotate the light in one direction and the L enantiomer will rotate it exactly the same amount in the opposite direction (for solutions of equal concentration). The optical rotation is measured by preparing a solution of the substance and determining, by the use of a polarimeter, the direction and degree to which the plane of polarisation of a beam of plane-polarised light is rotated upon passage through the solution. This is also a means of measuring the presence of a chiral molecule in solution, since non-chiral molecules cannot rotate the plane of the polarised light. It cannot identify the species present, however, it can only tell you that <u>something</u> chiral is present.



The magnitude of the rotation (α_D^T) is dependent on concentration (c), length of sample tube (l), solvent, temperature (T) and wavelength (the D line of sodium is usually used).

The specific rotation is expressed as:

$[\alpha]_D^T = 100\alpha_D^T / lc$

Where l is expressed in dm and c in % w/v

In the pharmaceutical formulation, the main component is (claimed to be) sucrose. Sucrose is chiral, hence it should be possible to quantitate it using polarimetry.

Ascorbic acid is also chiral, so this presents a potential source of interference and hence error. The likely concentration of ascorbic acid is much lower than sucrose, however, and its specific optical rotation value is smaller, hence this error is likely to be relatively small (you could theoretically correct for it once you have determined the ascorbic acid concentration by titration – see above, but you are not required to do this).

Quantitating Paracetamol in a mixture 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 on the next page.

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. Because it needs only two measurements, it is a very rapid and 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 this 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 $Y_0 = KC$

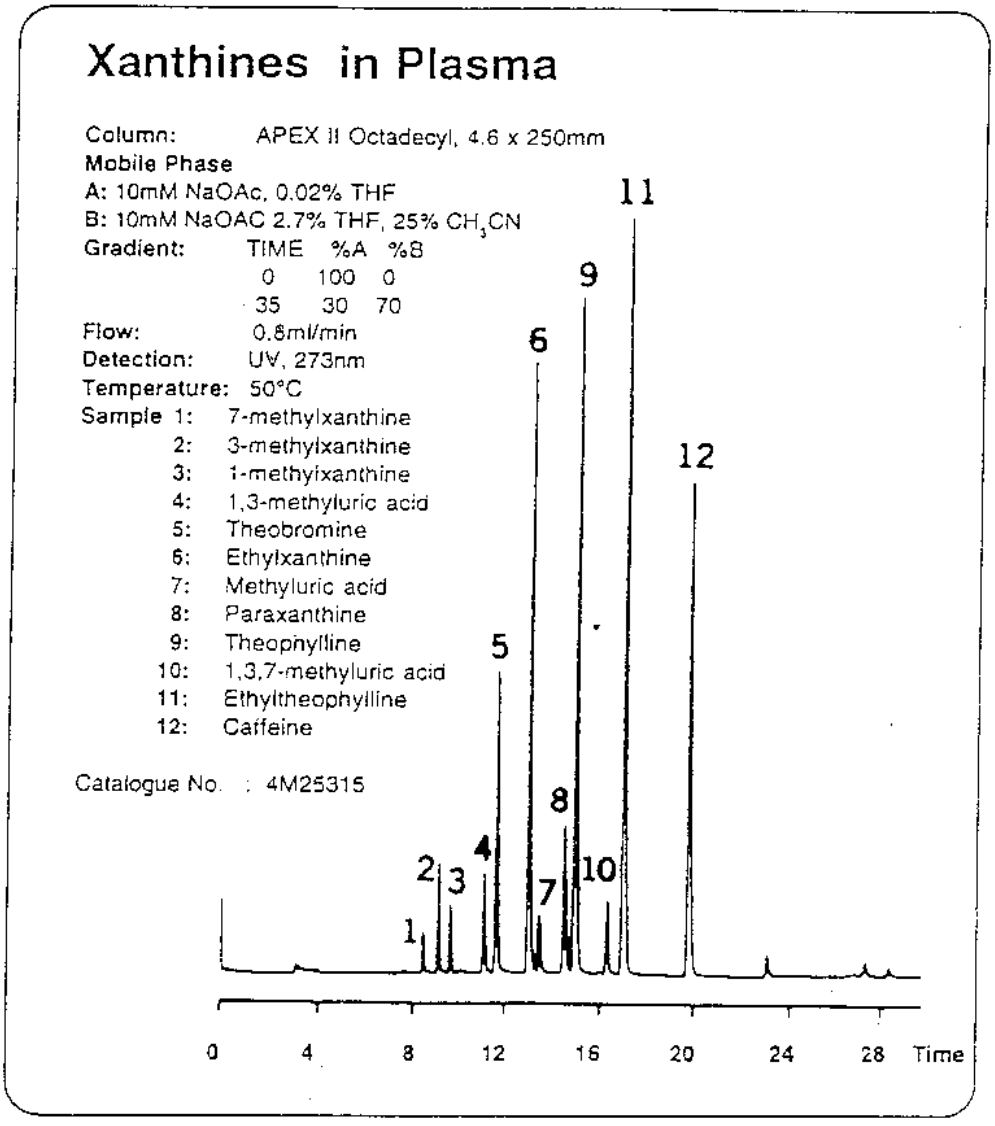
Standard addition flask $Y_1 = K(C + C_s)$

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 (values that come from the instrument when the samples are analysed)
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.



Experimental Protocols

1 Estimation of Sucrose by Polarimetry

Using a weighing boat (white diamond-shaped plastic type), weigh about 5.00 g of the pharmaceutical formulation. It does not need to be exactly 5.00 g, but you should record the weight to two decimal places.

Transfer the solid to a 50 mL volumetric flask (use a powder funnel – a funnel with a very short stem) to help you. Add about 40 mL distilled water and shake thoroughly until all of the solid is dissolved. Make up to exact volume with distilled water and mix thoroughly again.

Dissolve 2.00, 5.00, 10.00, 15.00 and 20.00 g, (it does not have to be exactly 1.00 g, etc., but you should weigh each quantity to two decimal places e.g. 1.03 g) of D-sucrose in distilled water in 6 separate 100 mL volumetric flasks. Make up to exact volume. Ensure you mix them thoroughly - concentrated sugar solutions are quite viscous. (If you can see swirling “oily” patterns, it is NOT well mixed!).

Measure the rotation of each solution in a 1 dm tube in the digital polarimeter. A demonstrator will show you how to do this. The instruments are in the instrumentation lab (the lab you walked through on the way in). Fill the tube with distilled water and set it in place in the polarimeter. Zero the instrument. Refil the tube with the first sucrose standard. Take the optical rotation reading. Repeat for each of the 5 sucrose solutions, making sure you rinse out the tube with several portions of each solution before finally filling it for measurement. Finally, measure the rotation for the solution of the pharmaceutical formulation.

Once you have all the optical rotation values for your solutions, you can plot a calibration graph and calculate the sucrose concentration of the pharmaceutical formulation (see results sheet).

2. Estimation of Ascorbic Acid by Redox Titration

Using a weighing boat weigh about 2.00 g of potassium iodide (it does not need to be exactly 2.00 g, but you should weigh it to 2 decimal places).

Transfer to a 100 mL volumetric flask and dissolve in a small volume of distilled water. Do not take too much water – about 2 mL is enough. If you take too much, the iodine will not dissolve in the next step.

Using a weighing boat accurately weigh about 1.3000 g of iodine (it does not need to be exactly 1.3000 g, but you should weigh it to 4 decimal places).

NOTE:- Iodine stains very badly, so please take extra care not to spill it on the benches or balances…

Add the iodine to your potassium iodide solution and mix thoroughly until all the iodine has dissolved (you will need to look carefully since it is a dark brown colour). Add about 50 mL distilled water and mix. Check that all the iodine has dissolved, then make up to final volume with distilled water and mix thoroughly. This is your iodine solution for titration. Fill a 50 mL burette with this solution, ready for the titration.

Using a large weighing boat, weigh about 12.00 g of the pharmaceutical formulation. It does not need to be exactly 12.00g, but you should record the weight to two decimal places. Transfer this to a 100 mL volumetric flask using a powder funnel to help you. Add about 70 mL distilled water and shake until all the powder has dissolved. Make up to final volume with distilled water, then mix again.

Transfer 25 mL of the pharmaceutical formulation solution into a 100 mL conical flask using a bulb pipette and filler (ask if you do not know how to use the filler). Add 10 mL of dilute sulphuric acid solution, followed by a squirt of starch indicator solution (use a Pasteur pipette). Titrate this solution using your iodine solution, recording the initial and final volume readings on the results sheet. Due to the dark colour of the iodine solution, you may find it more reliable to read the top rather than the bottom of the meniscus in the burette (a rare situation where the former may be preferable and lead to more consistent results).

You need to continue adding iodine solution until you obtain a definite blue colouration that persists for at least 30-60 seconds. As you approach the end point you will find that you get a blue colour initially, but that it quickly fades as the iodine is removed from the starch complex and reacts with the residual ascorbic acid.

Repeat the titration a further two times.

From the titration volumes and the weights of reagents used, you can now calculate the ascorbic acid content (see results sheet).

DO NOT DISPOSE OF EXCESS IODINE SOLUTION DOWN THE SINK – POUR IT INTO THE WASTE BOTTLES PROVIDED.

3. Estimation of Paracetamol by HPLC

Using a weighing boat, accurately weigh (4 decimal places) about 0.5000 g of pharmaceutical formulation and transfer it to a 250 mL volumetric flask. Add about 200 mL distilled water and shake thoroughly until all the solid has dissolved. Make up to volume with distilled water and mix again. This is your **sample solution**.

Using a weighing boat, accurately weigh (4 decimal places) about 20 mg (0.0200 g) of paracetamol and transfer it to a 250 mL volumetric flask. Add about 200 mL distilled water and shake thoroughly until all the solid has dissolved. Make up to volume with distilled water and mix again. This is your **standard solution**.

Take two 100 mL volumetric flasks and label them **"sample"** and **"sample + standard"**.

Using a 10 mL bulb pipette, transfer 10 mL of sample solution into each flask. Using a 10 mL bulb pipette, transfer 10 mL of standard solution into the flask labelled "sample + standard". Make each flask up to volume with distilled water and mix thoroughly.

Take your two flasks to the HPLC instrument. The instruments are in the instrumentation lab (the lab you walked through on the way in). A demonstrator will show you how to analyse your samples. Once you have the printed chromatograms and the peak area integration information, you can calculate the paracetamol content of the mixture (see results sheet).

Final Calculations

Once you have calculated the amount of each compound in the pharmaceutical formulation, you need to calculate the composition of the mixture in terms of its %w/w (i.e. the fraction by weight of each component of the sample, expressed as a percentage). Please ensure that you transfer your results to the final summary sheet before you hand the individual experiment sheets in for marking, since you will need these values for the final calculations.