Experiment 20

Consumer Chemistry: Vitamin C in Fruit Juices

The Task

The goal of this experiment is to determine the concentration of vitamin C in a range of different fruit juices (fresh and preserved) using titration and to rank these sources of vitamin C.

Skills

At the end of the laboratory session you should be able to:

- use a pipette correctly,
- use a pipette filler safely,
- manipulate a burette and carry out a quantitative titration properly,
- weigh a sample accurately,
- understand and utilise error analysis.

Other Outcomes

- You will extract vitamin C from a number of types of fresh fruit and bought juices.
- You will present a conclusion concerning the ranking of different sources of vitamin C that includes clear reference to their interpretation of the error analysis.
- You will explore the stability of vitamin C in solution and relate this to differences between fresh and preserved juices.

The Assessment

You will be assessed on your ability to use a burette correctly. See Skills 4.6 & 4.8. Make sure you remove the little plastic funnel before you begin any titration.

Introduction

Vitamin C is a water-soluble compound that is essential for life. It is involved in many processes in the human body, including: the production of collagen in the connective tissue; the synthesis of dopamine, noradrenaline and adrenaline in the nervous system; and the synthesis of carnitine, which is important in the transfer of energy to the cell mitochondria.

A deficiency in vitamin C causes scurvy, a disease that affected sailors in the 16th - 18th Centuries. It was discovered that fresh fruit, *e.g.* limes and oranges, or sauerkraut (preserved cabbage) provided the sailors with protection from scurvy.

In Australia and New Zealand, the recommended daily intake (RDI) of Vitamin C is 60 mg. The Nobel-prize winning scientist, Linus Pauling (1901 – 1994), believed in regular mega doses of vitamin C, but this is still regarded as unorthodox in conventional medicine. Vitamin C is often used as an antimicrobial and antioxidant in foodstuffs. It was first isolated in 1928 and in 1932 it was proved to be the agent which prevented scurvy (hence its scientific name of "ascorbic acid", which literally translates as "anti-scurvy acid"). Its structure was determined in 1933 and confirmed by total synthesis soon after.

Enantiomers are isomers that are mirror images of each other, a concept dealt with in detail in E15. Vitamin C is the L-enantiomer of ascorbic acid, as shown in Figure 1. (See Skill 13 if you can't understand the stick structures used.) Ascorbic acid is a stable solid that does not react with air, however, it is rapidly oxidised on exposure to air and light when in aqueous solution. The product of this oxidation is dehydroascorbic acid, as shown in Figure 2.

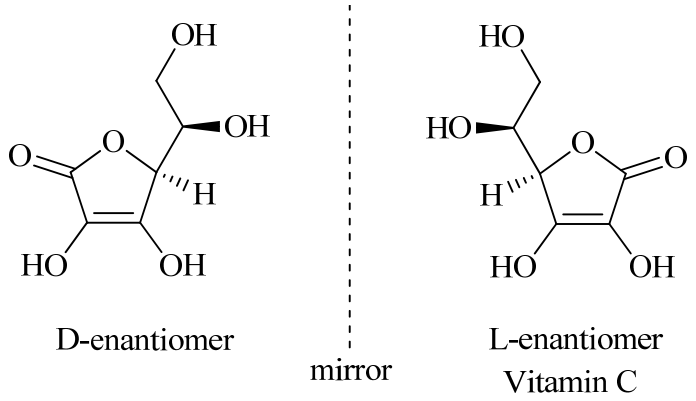

Figure 1: The two enantiomers of ascorbic acid.
(D and L are specialised labels used in sugar chemistry.)

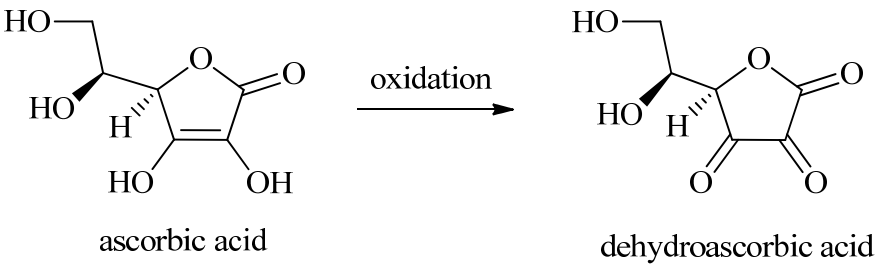

Figure 2: The oxidation of ascorbic acid to dehydroascorbic acid.

Redox reactions

You will come across the terms oxidation and reduction in the lecture course. These two processes are the opposite of each other and always accompany each other - as one species is oxidised, another is reduced - and the combined reaction is called a redox process. In organic chemistry, it is convenient to think of oxidation as a decrease in the number of H atoms in a molecule and reduction as an increase in the number of H atoms.

Vitamin C the antioxidant

In biological systems, reactive oxidants are often produced from metabolic processes. They have the ability to react with other molecules (*e.g.* DNA), thus damaging the cell. The body protects its cells by utilising another group of molecules called antioxidants (to which vitamin C belongs) to reduce (and hence detoxify) the oxidants. This experiment uses this phenomenon in a reduction/oxidation (redox) titration, where vitamin C reduces the orange solution of iodine to the colourless iodide ion as shown in Figure 3.

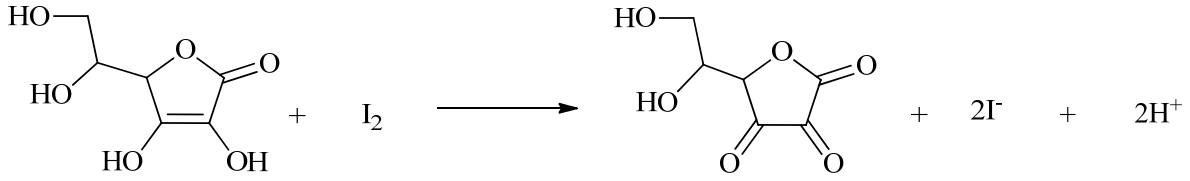

Figure 3: The reaction that is occurring in your titration today.

Volumetric analysis

Volumetric analysis (see Skill 4) is a technique that employs the measurement of volumes to determine quantitatively the amount of a substance in solution. In any reaction between two or more species, the reaction equation shows the stoichiometric ratio of reacting species. Take, for example, the reaction being investigated today, that between solutions of ascorbic acid and I_2. The reaction equation, as shown in Figure 3, tells us that 1 mol of I_2 reacts with 1 mol of vitamin C.

Indicators

I_2 forms a blue complex in the presence of starch (Vitex) and hence can act as its own indicator. Whilst the ascorbic acid is in excess, the orange I_2 which is being added from the burette is being reduced and is decolourising. As soon as all the ascorbic acid present has been oxidised, the added I_2 will no longer be reduced and, due to the Vitex that has been added, the solution becomes pale blue.

Standardisation

In this experiment, you will perform a series of redox titrations. In the course of these titrations you will become familiar with the technique of titration and the calculations associated with volumetric analysis. Read the Skills section on volumetric analysis (Skill 4) before your practical session. An example titration (not the one you will perform) and the relevant calculations can be found in Appendix 20.1.

Safety

Chemical Hazard Identification

dilute aqueous iodine solution – low toxicity, irritant

dilute sodium thiosulfate solution - non-hazardous

0.2 M acetic acid- non-hazardous

Vitex - non-hazardous

Risk Assessment and Control

Moderate risk.

Pipettes are easily broken resulting in dangerous jagged glass edges, especially if the pipette filler is used incorrectly. Read Skill 4.2 & 4.3 for its correct use and practise the techniques described before proceeding.

Waste Disposal

All of the solutions used today can be washed down the sink with water. Solid waste should be thrown in the bin.

Experimental

This experiment is to be carried out individually.

NOTE: Failure to follow the correct procedures as explained in Skill 4 will result in wildly inaccurate results. The following notes do NOT give a full description of the techniques.

Part A Standardisation of I_2

A solution of iodine can be standardised by titration against a known concentration of sodium thiosulfate according to the following equation.

$$I_2(aq) + 2S_2O_3^{2-}(aq) \rightarrow 2I^-(aq) + S_4O_6^{2-}(aq)$$

A solution of sodium thiosulfate has already been prepared for you. The exact concentration is written on the label. You are going to use this solution to determine the exact concentration of the I_2 solution used for determining the concentration of vitamin C in your fruit.

(A1) Collect about 200 mL of a solution of I_2 in a clean dry stoppered 250 mL conical flask. Prepare the burette for titration (see Skill 4.6) and as shown by your demonstrator, by washing with water and then three times with a small amount of the I_2 solution. Remember to restopper the iodine solution in your 250 mL flask.

(A2) In a clean and dry 250 mL conical flask collect 120 mL of the sodium thiosulfate solution. Record the exact concentration of the solution in your logbook.

(A3) Pipette (see Skill 4.3) a 25.00 mL aliquot of the sodium thiosulfate solution into a clean 250 mL conical flask. Add a half a Ni spoonful of Vitex reagent and 10 drops of 0.2 M acetic acid. Titrate with the I_2 solution (Skill 4.8) until you get a permanent colour change for at least 30 seconds. The endpoint is a light blue colour. Record your initial and final volumes in your logbook. This is your rough titration.

(A4) Pipette another 25.00 mL aliquot of sodium thiosulfate into a clean conical flask, add the Vitex and acetic acid as in step (A3) and titrate with the I_2 solution, slowing down to drop by drop and then split drops about 2 mL before the rough titration volume. Record your initial and final volumes to 2 decimal places in your logbook.

(A5) Repeat step (A4) until you have 3 concordant titre values that are within 0.1 mL of each other.

(A6) In your logbook, calculate the concentration of your I_2 solution. A sample calculation is given in Appendix 20.1.

Part B Vitamin C Determination

Your demonstrator will allocate you a fresh juice OR a preserved juice.

Fresh fruit juice preparation

Begin with step (B6) if you have been allocated a preserved juice.

(B1) Weigh 2 clean and dry Petri dishes on the top loading balance (Skill 3.1A). Record their masses in your logbook.

(B2) In duplicate, accurately weigh about 5 g of a fruit available on the side bench using a top loading balance. Record the exact masses in your logbook.

(B3) For each sample, cut the fruit into small pieces, place it in the microcloth and squeeze the fruit juice into a clean 250 mL conical flask via a funnel.

(B4) Rinse the cloth twice with an additional 5 - 10 mL of deionised water. Squeeze the water through the cloth and allow the filtrate to mix with that from step (B3).

(B5) Use a spatula to scrape the remaining pulp onto the pre-weighed Petri dish and reweigh. Record the mass in your logbook. Add half a Ni spoonful of Vitex and 10 drops of 0.2 M acetic acid. Continue with step (B10).

Preserved fruit juice

(B6) Weigh a 10 mL measuring cylinder on the top loading balance (see Skill 3.1A) and record its mass in your logbook.

(B7) Measure 5 mL of fruit juice in the pre-weighed 10 mL measuring cylinder and re-weigh it.

(B8) Place the fruit juice into a clean 250 mL conical flask and dilute with 20 mL of deionised water. If necessary filter off any pulp using the microcloth and rinse with deionised water into a clean 250 mL conical flask. Add a spatulaful of Vitex and 10 drops of 0.2 M acetic acid.

(B9) Repeat steps (B6) - (B7) to obtain a duplicate sample.

(B10) Fill the burette with the standardised I_2 solution and titrate your fruit juice until a permanent pale blue colour persists for at least 30 seconds. Record your initial and final volumes of I_2 in your logbook.

(B11) In your logbook, calculate the mass of vitamin C in mg per g of fruit or mg per mL of fruit juice for your assigned sample. [A sample calculation has been given in Appendix 1.]

(B12) Write your answer in the table on the board. You need to record all of the class results in your logbook for the group discussion at the end of your laboratory session.

(B13) The RDI (recommended daily intake) of vitamin C in Australia and New Zealand is 60 mg per day. In your logbook, calculate what mass of your fresh fruit (or volume of your preserved fruit juice) you need to consume 60 mg of vitamin C?

Part C Statistical analysis of results

Your Question: Which fruit provides the most vitamin C per gram of juice?

(C1) Collect on a white board everyone's measurements of mg of ascorbic acid per gram of juice for each different fruit. Each student should contribute two separate values, one for each of their two samples.

(C2) Calculate the mean value of mg of ascorbic acid per gram of juice (we'll call this the *mean vitamin C content*) for each fruit. **Record these values in your logbook.**

(C3) Which fruit juice has the biggest mean vitamin C content?

In the real world, what we want to know is which fruit is most likely to provide us with the highest vitamin C content. Is that the same as your answer in (C3)? Not quite! What if the mean value has been skewed by just one or two very high readings for one particular sample? (It may have been an extraordinary piece of fruit or the experimenter may have made an error.) In general, measurements don't give an exact number, but rather a distribution of numbers. When we try and use the information contained in this distribution of numbers in some real world problem, we need to consider what bit of the distribution is the most relevant.

Consider the two fruits that had the highest mean vitamin C content. For the sake of this discussion we'll call them fruits A and B, but you, of course, will know what they are from your data. If fruit A had the higher mean vitamin C content, what is the probability, if we were to analyse new samples, that fruit A would again have a higher vitamin C content than fruit B? Here's how we can estimate that probability from the group data on the white board. (The statistical method used is called *the sign test for two medians*.)

(C4) Draw a Table in you logbook in which you list all the group measurements for fruit A down the left hand side. Across the top you list all the group measurements for fruit B. The result should be a grid with m_A x m_B boxes (where m_A is the number of measurements for fruit A and m_B for fruit B). Now draw a cross, X, in every box in your Table for which the A reading is bigger than the B reading. (See the example Table below.) The probability that fruit A wins the next comparison with fruit B is equal to the fraction of boxes in your Table with a cross in them. Calculate that fraction and record it in your logbook.

		Fruit B values					
		6.0	5.4	5.8	5.2	5.9	6.3
Fruit A values	6.2	X	X	X	X	X	
	6.9	X	X	X	X	X	X
	5.6		X		X		
	7.1	X	X	X	X	X	X
	5.1						
	6.3	X	X	X	X	X	

Table 1: Example of the data table described in the text. The probability that fruit A would have the higher reading in the next sample from this example is 24/36 = 0.67.

Has this analysis changed your recommendation regarding the fruit that provides the most vitamin C? (Note that if your probability is less than 0.5 then it means you are more likely to find juice B with the higher vitamin C content.)

Appendix 20.1: Example of Calculations

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Part A: Standardisation of I_2

Results [thiosulfate ion] = 2.040×10^{-3} M (from bottle)

Table 1: Titration results for the standardisation of I_2

	1 (rough)	2	3	4
Initial volume (mL)	0.20	1.50	2.00	2.50
Final volume (mL)	34.00	35.20	35.60	36.20
Titre (mL)	33.80	33.70	33.60	33.70

Average titre = 33.67 (3 concordant values ≤ 0.10 mL)

The clear aliquot solution changed to a light blue colour.

Calculations

Reaction: $I_2(aq) + 2S_2O_3^{2-}(aq) \rightarrow 2I^-(aq) + S_4O_6^{2-}(aq)$

Therefore 1 mol of I_2 reacts with 2 mol of $S_2O_3^{2-}$

The standard thiosulfate solution is 2.040×10^{-3} M. That is:

1.000 L of 2.040×10^{-3} M $S_2O_3^{2-}$ solution contains 2.040×10^{-3} mol of $S_2O_3^{2-}$ ions.

$\therefore$ 25.00 mL of 2.040×10^{-3} M $S_2O_3^{2-}$ solution contains 2.040×10^{-3} mol $L^{-1} \times 0.025$ mol

$= 5.100 \times 10^{-4}$ mol of $S_2O_3^{2-}$ ions.

Therefore 33.67 mL of unknown I_2 solution contains $\frac{5.100 \times 10^{-4}}{2}$ mol of I_2.

Therefore 1000 mL of unknown I_2 solution contains $\frac{5.100 \times 10^{-4} \times 1000}{2 \times 33.67}$ mol of I_2.

$= 7.574 \times 10^{-4}$ mol of I_2.

That is, the I_2 solution is 7.574×10^{-4} M.

Part B: Vitamin C Determination

Results Fruit selected: orange

Mass of sample 1 orange juice = 4.85 g Mass of sample 2 orange juice = 4.90 g

Table 1: Titration results for determination of vitamin C in orange juice

	Sample 1	Sample 2
Mass of juice	4.85 g	4.90 g
Initial volume (mL)	1.50	0.05
Final volume (mL)	22.73	21.54
Titre (mL)	21.23	21.49

The endpoint colour was a purplish pink.

$[I_2] = 7.574 \times 10^{-4}$ M (from Part A)

Calculations

Reaction: $I_2(aq) + \text{ascorbic acid} \rightarrow 2I^-(aq) + \text{dehydroascorbic acid}$

Therefore 1 mol of I_2 reacts with 1 mol of ascorbic acid

Molar mass of ascorbic acid ($C_6H_8O_6$) = 176.12 g mol^{-1}

Sample 1

21.23 mL of 7.574×10^{-4} M I_2 solution contains $7.574 \times 10^{-4} \times 0.02123$ mol of I_2

Therefore 4.85 g of orange juice contains $7.574 \times 10^{-4} \times 0.02123$ mol of Vit C.

Therefore 4.85 g of orange juice contains $7.574 \times 10^{-4} \times 0.02123$ mol $\times$ 176.12 g mol^{-1}

$= 2.831 \times 10^{-3}$ g = 2.832 mg of Vit C.

Therefore content of Vit C in fresh orange juice = $\frac{2.832 \text{ mg}}{4.85 \text{ g}}$ = 0.58 mg/g

Sample 2

21.49 mL of 7.574×10^{-4} M I_2 solution contains $7.574 \times 10^{-4} \times 0.02149$ mol of I_2

Therefore 4.90 g of orange juice contains $7.574 \times 10^{-4} \times 0.02149$ mol of Vit C.

Therefore 4.90 g of orange juice contains $7.574 \times 10^{-4} \times 0.02149$ mol $\times$ 176.12 g mol^{-1}

$= 2.866 \times 10^{-3}$ g = 2.866 mg of Vit C.

Therefore content of Vit C in fresh orange juice = $\frac{2.866 \text{ mg}}{4.90 \text{ g}}$ = 0.58 mg/g

Therefore the average content of vitamin C in fresh orange juice is:

$\frac{0.58 + 0.59}{2}$ = 0.59 mg/g