SYNTHETIC BONE

Our skeletons are made of bones. Bone is made by living cells called oesteoblasts. Bones move, support and protect various organs in your body. They come in different shapes and sizes but all are light, strong and hard.

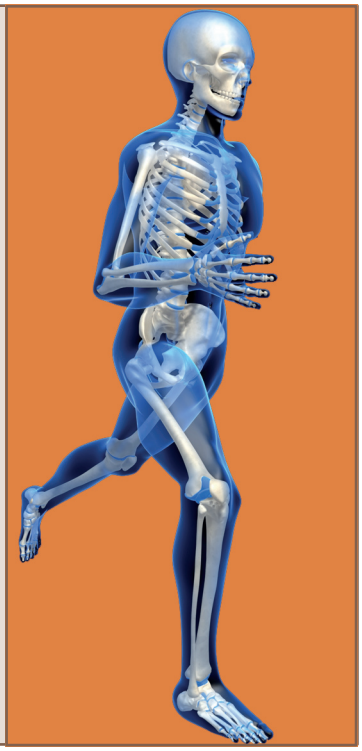
The inside of a bone has a honeycomb structure which helps explain why bones are strong and hard, yet light.

Bone is a natural composite material. It contains hydroxyapatite (a mineral), collagen (a protein) and water.

'Synthetic' hydroxyapatite can be made in the laboratory. It has two important applications when it comes to repairing damaged bones and joints:

- surfaces of metallic implants are coated with it – the body 'sees' a natural substance rather than metal and 'accepts' it, allowing the bone to mend
- holes or defects in bone can be filled with hydroxyapatite – it becomes part of the bone structure and reduces healing times.

You will investigate bone and make some synthetic hydroxyapatite.



EQUIPMENT

investigating bone

- two chicken bones, cleaned to remove any flesh
- beaker large enough for one of the chicken bones
- 1 mol dm^{-3} hydrochloric acid
- balance that weighs to the nearest 0.01 g
- eye protection

making hydroxyapatite

- solid calcium hydroxide, $Ca(OH)_2$ [IRRITANT]
- 0.3 mol dm^{-3} phosphoric(V) acid, $H_3PO_4(aq)$ [IRRITANT]
- Universal indicator paper
- 2 x 400 cm^3 beakers
- dropping funnel
- glass rod for stirring, but preferably a magnetic or mechanical stirrer
- tripod, gauze and Bunsen burner
- filter paper and funnel
- watch glass
- eye protection

SAFETY NOTES

Wash your hands thoroughly after handling the chicken bones or chemicals. Wear eye protection.

METHOD 1: INVESTIGATING BONE

The composition of chicken bones

1. Measure the mass of a small chicken bone.

   mass of bone (hydroxyapatite + collagen + water) = _____ g

2. Measure its volume.

   volume of bone (hydroxyapatite + collagen + water) = _____ cm^3

3. Heat it in an oven at 60 °C overnight to remove the water. Let it cool and measure its mass.

   mass of bone (hydroxyapatite + collagen) = _____ g

4. Heat the dried bone strongly in a furnace or very hot oven to remove the collagen. Let it cool and measure its mass.

   mass of bone (hydroxyapatite) = _____ g

The role of hydroxyapatite

1. Put a second chicken bone into a beaker and cover it with 1 mol dm^{-3} hydrochloric acid. Leave it for 1-2 days. This removes the hydroxyapatite from the bone. Left any longer, the collagen will also be removed. Use forceps or tongs to remove the bone from the acid. Wash and dry it gently.
2. Describe the difference in the bone before and after hydroxyapatite is removed.

RESULTS

Use your results to work out:

- the density of chicken bone
- the ratio of the masses of hydroxyapatite, collagen and water in chicken bone.

EXPLANATIONS

Explain the role of hydroxyapatite in bone.

Look at these values for the densities of parts of bone:

part of bone	hydroxyapatite	collagen	water
density / g cm^{-3}	3.2	1.0	1.0

You worked out the density of bone and the ratio by mass of the three parts.

- If bone was simply a mixture of the three components, what would you expect its density to be?
- Explain the difference between your 'expected' density and the value you found by measurement.

METHOD 2: MAKING HYDROXYAPATITE

1. Measure 100 cm^3 of distilled water into a 400 cm^3 beaker. Bring it to the boil and leave to cool to about 30 °C.
2. Weigh out 3.7 g of calcium hydroxide and tip it into the cool distilled water. Stir the mixture vigorously, preferably using a magnetic or mechanical stirrer.
3. Use a dropping funnel to add 100 cm^3 of 0.3 mol dm^{-3} phosphoric(V) acid drop by drop at about one drop per second. This should take about 10 minutes. Adding the phosphoric(V) acid too quickly may make the solution acidic if the reaction with calcium hydroxide is slow. Check the pH occasionally to make sure the solution remains alkaline.
4. Heat the final reaction mixture so that it boils gently for about 25 minutes. Every five minutes, add distilled water to keep the total volume of the mixture about 200 cm^3. Cover with a watch glass or cling film and leave overnight.
5. Weigh a filter paper and fold it so that it is fluted (your teacher will help you). Put the fluted filter paper in a filter funnel and filter the reaction mixture. Wash the solid (hydroxyapatite) with a little distilled water.
6. Place the filter paper on a watch glass and put them in an oven at about 40 °C for 3-4 hours. Increase the temperature of the oven to 80 °C and leave for another two hours.
7. Allow the filter paper and hydroxyapatite to cool to room temperature. Weigh them and calculate the mass of hydroxyapatite obtained.

RESULTS

mass of calcium hydroxide = _____ g

mass of hydroxyapatite (actual yield) = _____ g

The theoretical yield of hydroxyapatite from 74 g calcium hydroxide is 100 g.

- Calculate the theoretical yield from the mass of calcium hydroxide you used.

_____

_____

$$\text{percentage yield of hydroxyapatite} = \frac{\text{actual yield of hydroxyapatite} \times 100}{\text{theoretical yield of hydroxyapatite}} \%$$

- Calculate the percentage yield of hydroxyapatite in your preparation.

_____

_____

EXPLANATIONS

The word equation for the reaction used to make hydroxyapatite is:

calcium hydroxide + phosphoric(V) acid → hydroxyapatite + water

The chemical formulae of reactants and products are:

calcium hydroxide $Ca(OH)_2$ phosphoric(V) acid H_3PO_4

hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ water H_2O

1. Write a balanced symbol equation.
2. Using the balanced equation and these relative atomic masses:

   H 1 O 16 P 31 Ca 40

   Explain why the theoretical yield of hydroxyapatite from 74 g calcium hydroxide is 100 g.

HEALTH AND SAFETY

A risk assessment must be made before starting any practical work. Chicken bones can be contaminated with salmonella and other diseases. Wash your hands thoroughly after handling the bones. Warn students not put their fingers in their mouths during the experiment. Sterilise the clean bones by leaving them in domestic bleach overnight, followed by a thorough washing with cold water before they are used. Protective clothing and eye protection should be worn.

THE INVESTIGATION

In one of the videos, students watch an operation in which Jody is fitted with a Taylor Spatial Frame to help repair her damaged leg. The operation involves breaking the bone and re-setting it in the appropriate position. In another sequence, artificial bones are described.

Chicken bones

Bone is a natural composite material. Students investigate its structure and composition. They work out the proportions by mass of hydroxyapatite, collagen and water. They also compare the density of bone with the densities of three components.

This is based on an activity from the Royal Society of Chemistry resource *Inspirational chemistry – resources for modern curricula* (ISBN: 978-0-85404-399-6).

Making hydroxyapatite

Hydroxyapatite is a calcium phosphate mineral. It can be synthesised chemically and is used to:

- coat surfaces of metallic implants so that the body 'sees' a natural substance rather than metal and 'accepts' it, allowing the bone to mend
- fill holes or defects in bone, becoming part of the bone structure and reducing healing times.

Students make a sample of hydroxyapatite from a neutralisation reaction between calcium hydroxide and phosphoric(V) acid. They may compare it with the hydroxyapatite obtained from chicken bones.

The preparation is on the paper *Calcium apatite prepared from calcium hydroxide and orthophosphoric acid* (A. Osaka, Y. Miura, K. Takeuchi, M. Asada, K. Takahashi, Journal of Materials Science: Materials in Medicine, 1991, 2, 51).

RESULTS

Chicken bones

Typically bone has a density of 1.9 g cm^{-3}. Bones are usually about 70% hydroxyapatite and 30% collagen (by dry mass). The water content tends to vary.

An approximate composition (percentage by mass) might be: 63% hydroxyapatite, 27% collagen and 10% water. If a bone of this composition were simply a mixture of the three components, its expected density would be the weighted average of the three parts:

$$\text{weighted average of the three parts} = \frac{(63 \times 3.2) + (27 \times 1.0) + (10 \times 1.0)}{100} = 2.39 \text{ g cm}^{-3}$$

The density of bone is much less than the weighted average of the three parts. This is because of the honeycomb structure.

Making hydroxyapatite

$$\text{theoretical yield of hydroxyapatite} = \frac{\text{mass of calcium hydroxide x 100}}{74}$$

The percentage yield of hydroxyapatite will vary. The reaction goes to completion and a 100% yield might be expected. However, the product may contain small quantities of other calcium phosphates and it is likely that some product will be lost during the various transferring stages.

Explanations:

1. Balanced symbol equation:
   $10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O$
2. Using the relative atomic masses:
   relative formula mass $Ca(OH)_2 = 74$
   relative formula mass $Ca_{10}(PO_4)_6(OH)_2 = 1000$
   and from the equation:
   10 mol $Ca(OH)_2$ gives 1 mol $Ca_{10}(PO_4)_6(OH)_2$
   1 mol $Ca(OH)_2$ gives 0.1 mol $Ca_{10}(PO_4)_6(OH)_2$
   74 g $Ca(OH)_2$ gives 1000 → 10 = 100 g $Ca_{10}(PO_4)_6(OH)_2$

SUGGESTED SEQUENCE

This is a straightforward exercise. Students might work in pairs and the results of the whole class compared, with students asked to comment on the spread of results obtained.

Time required: Chicken bones

About 60 minutes spread over three sessions.

Time required: Making hydroxyapatite

Spread over three sessions:

- about 60 minutes to get to the end of step 4
- about 45 minutes to get to the end of step 5 (after which the product is left to dry in an oven)
- about 15 minutes to complete step 7.

TECHNICIAN EQUIPMENT LIST

per pair

Investigating bone

- two chicken bones, cleaned to remove any flesh
- beaker large enough for one of the chicken bones
- 1 mol dm^{-3} hydrochloric acid
- balance that weighs to the nearest 0.01 g
- eye protection

Making hydroxyapatite

- solid calcium hydroxide, $Ca(OH)_2$ [IRRITANT]
- 0.3 mol dm^{-3} phosphoric(V) acid, $H_3PO_4(aq)$ [IRRITANT]
- Universal indicator paper
- 2 x 400 cm^3 beakers
- dropping funnel
- glass rod for stirring, but preferably a magnetic or mechanical stirrer
- tripod, gauze and Bunsen burner
- filter paper and funnel
- watch glass
- eye protection

TECHNICIAN NOTES

Chicken bones

Use a knife to remove as much flesh as possible. Place bones in a pan of water and sodium carbonate. Simmer. Remove remaining flesh with an old brush. Return to pan and simmer again until bones are clean. Wash. Immerse in domestic bleach for at least 30 minutes. Wash.

Making hydroxyapatite

The distilled water used in step 1 may be boiled in advance to reduce the amount of dissolved air.

This is quite a sophisticated method and students are likely to require supervision/guidance, in particular:

- arranging the dropping funnel to deliver the 0.3 mol dm^{-3} phosphoric(V) acid drop by drop. If necessary show students how to check pH by taking a drop of the reaction mixture on a glass rod and touching on a piece of indicator paper
- using a magnetic or mechanical stirrer (if these are not available one student will need to stir the mixture continuously with a glass rod)
- making a fluted filter paper.

When the product is being oven-dried the temperature will need to be increased after 3-4 hours from 40 °C to 80 °C. It should be left at this temperature for another 2 hours, but leaving it longer will do no harm.