Investigating effect of temperature on the activity of lipase

This practical gives you a chance to:

* investigate how lipase activity changes with temperature
* consider how indicators can help us to follow chemical reactions.

### Procedure

SAFETY: Keep the phenolphthalein solution away from naked flames.

Wear eye protection and quickly rinse any splashes of enzyme solution or sodium carbonate from the skin.

Make sure you know what to do if a thermometer is broken.

### Investigation

1. Label a test tube with the temperature you will be investigating.
2. Add 5 drops of phenolphthalein to the test tube.
3. Measure out 5 cm3 of milk using a measuring cylinder (or syringe) and add this to the test tube.
4. Measure out 7 cm3 of sodium carbonate solution using another measuring cylinder (or syringe) and add this to the test tube. The solution should now be pink.
5. Place a thermometer in the test tube. Take care as the equipment could topple over.
6. Place the test tube in a water bath and leave until the contents reach the same temperature as the water bath.
7. Remove the thermometer from test tube and replace it with a glass rod.
8. Use the 2 cm3 syringe to measure out 1 cm3 of lipase from the beaker in the water bath for the temperature you are investigating.
9. Add the lipase to the test tube and start the stopclock/ stopwatch.
10. Stir the contents of the test tube until the solution loses its pink colour.
11. Stop the clock/ watch and note the time in a suitable table of results.

****

1. Plot a graph of time taken for the reaction to occur against temperature.
2. You can convert this to a rate of reaction graph by calculating 1 ÷ time for each of the temperatures. (If any tubes have not reacted in the time taken, this is a rate of zero.)

**QUESTIONS**

1. When fat breaks down, what is produced
2. Use this information to explain why the phenolphthalein changes colour.
3. What is the effect of temperature on the time taken for lipase to break down the fat in milk?
4. Why does the temperature affect the action of lipase in this way?
5. What is the difference between a ‘time taken’ and a ‘rate of reaction’ curve for this investigation?
6. Why is it necessary to break down fat in the digestive system?
7. Use other sources of information to find out about:
* bile salts and their effects on digestion of fats
* what happens to the fatty acids and glycerol once they have been absorbed from the digestive tract.

**ANSWERS**

1. When fat breaks down, fatty acids and glycerol are produced.
2. The fatty acids lower the pH of the mixture which changes the colour of the phenolphthalein from pink to colourless.
3. Increasing temperatures from 0 ºC to around 45 ºC will reduce the time taken for the lipase to break down the fat in milk. Over this temperature, the time taken will increase, or perhaps the lipase will not work at all.
4. Temperature affects the action of lipase this way because increasing temperatures (up to around 40 ºC) increase the rate of reaction, by increasing the collision rate between the enzyme and substrate molecules (as in any chemical reaction). The highest rate of reaction is at the *optimum* temperature for the enzyme. The rate of reaction then reduces as temperature increases until, at some point, the reaction stops altogether. This is because at high temperatures (usually over 45 ºC), the protein structure of the enzyme is denatured by heat. The molecule loses its shape and the enzyme is de-activated.
5. A ‘time taken’ curve and a ‘rate of reaction’ curve show similar patterns, but one is an upside-down version of the other.
6. It is necessary to break down fat in the digestive system to make it easier to absorb through the membranes of the gut and also to make it soluble enough to transport in the blood.
7. Bile salts *emulsify* fats, which means they make it easier to form an emulsion of tiny droplets of fat suspended in water. A fatty emulsion will not separate quickly. This increases the surface area of fat exposed to enzymes in solution and increases the rate of digestion.

When fatty acids and glycerol have been absorbed from the digestive tract, they are transported through the lymphatic system and enter the bloodstream at the sub-clavian vein (underneath the collar bone).