**Investigating the Hydrolysis of Fats and Oils**

**Background**

Fats and oils are esters of triglycerols and fatty acids. Hydrolysis can break down a fat or oil and release the triglycerol and fatty acids. The acids can be separated and identified and this information can be used to identify the original fat or oil.

**Practical Techniques**

You will need to find out about volumetric analysis (titrations) and how to make up accurate solutions. You also need to find out about using a pH meter.

**Where to start**

An enzyme called lipase catalyses the hydrolysis of the fats and oils. When the hydrolysis occurs the fatty acids will be released and the acidity of the reaction mixture will rise. An alkali can be added to the reaction mixture to neutralise the fatty acids. The rate at which the alkali needs to be added can be used as a measure of the rate of the hydrolysis.

Plan an experiment to hydrolyse an oil and measure the rate of hydrolysis.

**Possible Investigations**

- Investigate the effect of changing the concentration of the enzyme on the rate of the reaction.
- Investigate the effect of changing the concentration of the oil on the rate of the reaction.
- Investigate the effect of changing the concentration and/or type of the detergent on the rate of the reaction.
- Investigate the effect of changing the temperature on the rate of the reaction.
- Investigate the effect of changing the type of oil on the rate of the reaction.
- Investigate the effect of changing the pH at which the experiment is run at.
Sources of Information

- Shipton M., *Fats and Oils*, Unilever Educational Booklet: Advanced Series,
- Denby D., Investigating Enzymes, *Chemistry Review*,
- Thorpe A., Experimental error and error analysis: just how good are those results, *Chemistry Review*, November 2001
**Teachers' Notes**

**General**

The Unilever Fats and Oils Booklet is an excellent source for this investigation and has **full experimental details** for the starter experiment. Students can find it initially difficult to carry out the technique but with practise, clear results can be obtained. Each experiment can take up to 1 hour.

**Chemical Principles**

Esters, hydrolysis, kinetics

**Essential Equipment**

Burette, pH meter, magnetic stirrer

**Essential Chemicals**

sodium hydroxide, solid lipase, calcium chloride, sodium lauryl sulphate, glycerol triethanoate (or triethanoyl glycerol or triacetin)

**Safety**

No risk assessment has been given. It is essential that students prepare a detailed risk assessment before they start. Teachers must be satisfied that this is suitable for the proposed investigation.
Starter Experiment Sheet - Investigating hydrolysis of fats and oils

A basic procedure is given below to measure the rate of hydrolysis is given below.

Prepare the following solutions

- 0.01 mol dm\(^{-3}\) sodium hydroxide solution (placed in a burette)
- 0.5g solid lipase (the enzyme) in 10 cm\(^3\) of water – add 0.1g calcium chloride to activate
- 5% sodium lauryl sulphate detergent

You will need to think about how much of each solution to prepare. This will depend on how much of the solution is used in each experiment and how many experiments you do (including any repeats).

You will also need glycerol triethanoate (or triethanoyl glycerol or triacetin) – this is the oil that you will hydrolyse.

Take the enzyme solution and carefully adjust the pH to 8, using drops of the sodium hydroxide solution.
Place equal volumes of the oil and the detergent solution into a beaker and shake vigorously. This is now an emulsion of the oil in water. Carefully adjust the pH to 8 using drops of the sodium hydroxide solution.
Place 20 cm\(^3\) of the emulsion and another 10 cm\(^3\) of the detergent into a beaker and stir continuously with a magnetic stirrer.
Place a pH electrode into the mixture and measure the pH. Carefully adjust the pH to 8 using drops of the sodium hydroxide solution.

Add 2 cm\(^3\) of water to the mixture and carefully adjust the pH to 8 using drops of the sodium hydroxide solution and start a stop clock.
You are now ready to start taking readings. At regular time intervals add the sodium hydroxide to re adjust the pH to 8. (It may be difficult to reach exactly 8 so you may have to approximate). Continue for about 5 minutes.

You now need to repeat the experiment by substituting 2 cm\(^3\) of enzyme for the water.

You can plot a graph of volume of alkali added against time and the gradient will give an initial rate of reaction in cm\(^3\) of 0.01 mol dm\(^{-3}\) of alkali per second. You can convert this into moles of acid produced per second.