

### 13. Experiments with enzymes – the effect of enzyme concentration on reaction rate

#### Student Sheet

In this experiment, you will investigate the effect of lipase concentration on the rate of hydrolysis of **glycerol triethanoate** (triacetin)

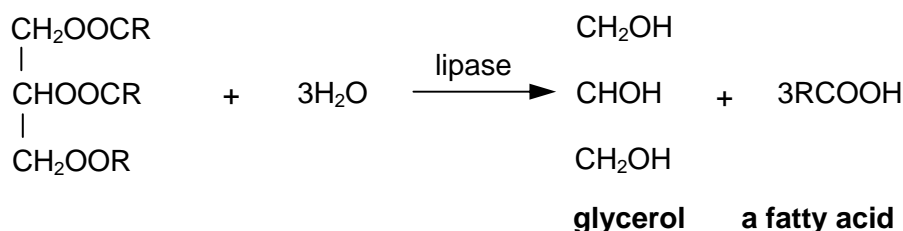
#### Intended lesson outcomes

By the end of this exercise you should be able to:

- handle small volumes of liquid
- deduce organic structures
- perform mole calculations
- analyse and evaluate your results

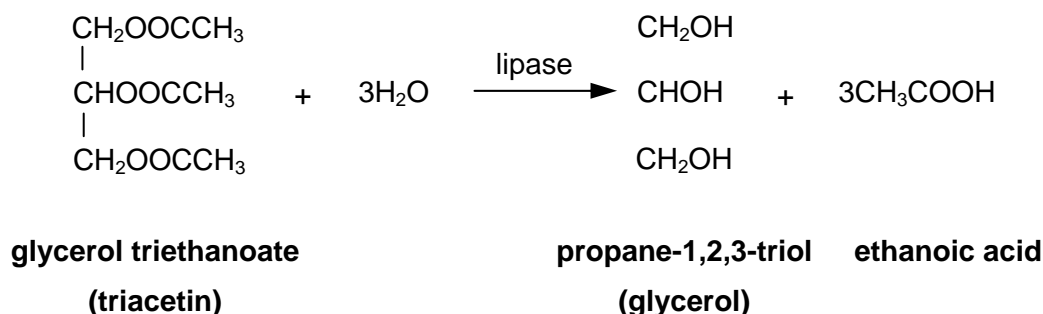
#### Background information

**Lipase** is an enzyme that breaks down, hydrolyses, the lipids present in animal fats and vegetable oils into propane-1,2,3-triol (glycerol) and long-chain carboxylic acids (fatty acids). These acids can be detected by following the drop in pH of the solution as the enzyme releases them. A general reaction scheme for this process is shown below; the size of the **R** group, and its level of saturation, will depend on the lipid used



Olive oil is often used as a substrate in testing for lipase, with the oleic acid released being titrated with sodium hydroxide solution. Oleic acid,  $\text{C}_{17}\text{H}_{33}\text{COOH}$ , is a mono-unsaturated fatty acid.

In this exercise, glycerol triethanoate (triacetin) is used as the substrate. Lipase breaks this substrate down into glycerol and ethanoic acid as shown below.





The ethanoic acid produced lowers the pH of the solution. This change can be detected by using a suitable acid-base indicator, in this case bromocresol purple which changes from purple at high pHs to yellow at lower pH values. Sodium carbonate is added to the triacetin to ensure that the pH is high enough at the start of the experiment. Only after the sodium carbonate has all reacted with the liberated ethanoic acid will the ethanoic acid concentration rise and the pH value fall. The reaction time is measured from the initial point of mixing the solutions until the end-point of the indicator is reached.

**Questions on background information**

- 1 How can you tell from its formula that oleic acid is likely to be mono-unsaturated?
- 2 Suggest an alternative structure for  $C_{17}H_{33}COOH$  in which the alkyl group is fully saturated.
- 3 A sample of glycerol triethanoate was completely hydrolysed by lipase. The ethanoic acid formed required  $17.5\text{ cm}^3$  of aqueous sodium hydroxide of concentration  $0.100\text{ mol dm}^{-3}$  for complete neutralisation. Calculate the number of moles, and hence the mass, of glycerol triethanoate in the sample.

**Safety**

Materials safety data sheets should be consulted so that the correct action can be taken in event of a spillage and/or accident.

	You must wear eye protection throughout this experiment
	Sodium carbonate is an <b>irritant</b>

**Procedure**

1. Add the triacetin solution, distilled water and sodium carbonate solution to the numbered test tubes as shown in the table below.

test tube	triacetin / $\text{cm}^3$	water / $\text{cm}^3$	sodium carbonate / $\text{cm}^3$	lipase / $\text{cm}^3$
1	1.00	4.30	0.50	0.20
2	1.00	4.25	0.50	0.25
3	1.00	4.20	0.50	0.30
4	1.00	4.10	0.50	0.40
5	1.00	4.00	0.50	0.50
6	1.00	3.90	0.50	0.60
7	1.00	3.80	0.50	0.70
8	1.00	3.50	0.50	1.00

2. Add 4 drops of bromocresol indicator to each test tube.
3. Add the lipase solution and start the clock. **Warning:** tubes 6, 7 and 8 are very quick!
4. Shake the tubes and continue to shake them every 30 seconds. This is necessary to ensure that the triacetin and lipase solution are continually mixed otherwise the triacetin will separate out.
5. Record the time when each solution turns yellow in the table below.
6. Calculate the value for  $1/t$  for each experiment; this represents the reaction rate of each experiment.
7. Multiply the reaction time, **t**, by the appropriate conversion factor shown in the table below.
8. Plot the reaction time, **t**, against the volume of lipase used in **Graph 1**.
9. Plot the reaction rate,  **$1/t$** , against the volume of lipase used in **Graph 2**.

## Appendix 2

10. Plot the **weighted reaction time** against the volume of lipase used in **Graph 3**. Calculate the weighted reaction time by using the following relationship.

$$\text{weighted reaction time} = \text{actual reaction time} \times \text{conversion factor}$$

**Results**

test tube	reaction time 't' / s	1/t / s <sup>-1</sup>	conversion factor	weighted reaction time / s
1			0.20	
2			0.25	
3			0.30	
4			0.40	
5			0.50	
6			0.60	
7			0.70	
8			1.00	

**Questions on results**

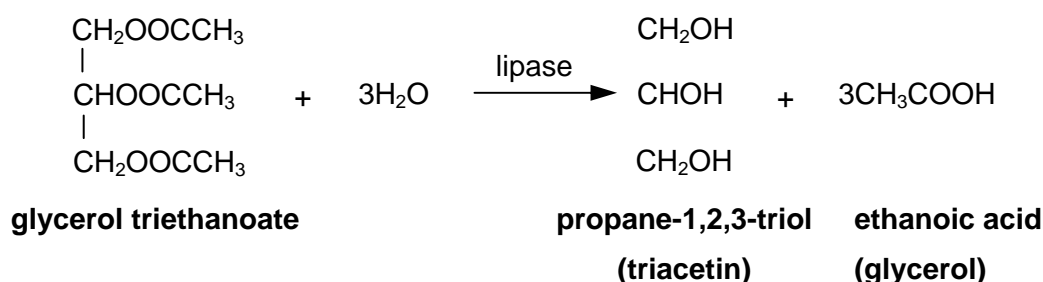
- 1 What sort of curve is produced in **Graph 1**? Is it exponential?
- 2 How does the rate of reaction change with increasing enzyme concentration? (See **Graph 2**)
- 3 If the reaction time is proportional to the enzyme concentration then a plot of the weighted reaction time against the volume of lipase used should give a straight line. By considering **Graphs 2 & 3**, deduce the rate order with respect to the lipase concentration.

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#### Teachers' Notes

This is a relatively straightforward rates exercise in which the effect on the rate of an enzyme catalysed reaction of varying the concentration of the enzyme is investigated. The volumes involved are quite small, so the use of syringes rather than pipettes or burettes is recommended if available.

In the exercise, glycerol triethanoate (triacetin) is used as the substrate. Lipase breaks this substrate down into glycerol and ethanoic acid as shown below.



The ethanoic acid produced lowers the pH of the solution. The rate is determined by measuring the time needed to reduce the pH to a value determined by mid-point colour of a chosen indicator. In this case the indicator is **bromocresol purple**, which changes from purple at high pH values to yellow at lower pH values. Sodium carbonate is added to the triacetin to hold the pH high enough so that the colour remains purple. Only after the sodium carbonate has all reacted with the liberated ethanoic acid will the acid concentration rise and the pH value fall sufficiently low to trigger the colour change in the indicator. The reaction time is measured from the initial point of mixing the solutions until the end-point of the indicator is reached.

It might prove useful to revise the basic rates theory from the AS course prior to doing this exercise. Great emphasis should be placed on the importance of accurate volume measurements, as an error of a single drop in measuring the volume of the lipase solution will have significant consequences in terms of experimental accuracy. This point is best made by calculating the percentage error when, for example, 1 drop (around  $0.05\text{ cm}^3$ ) too much/too little lipase solution is added in **Experiment 1** (where the required lipase volume is only  $0.20\text{ cm}^3$ ).

The questions provide an opportunity to consolidate AS knowledge. Also, this exercise could be performed as a practice exercise before tackling the urease planning exercise.

#### Answers to questions on background information

- 1 The formula  $\text{C}_{17}\text{H}_{33}$  is two hydrogen atoms short of the formula of an alkyl group ( $\text{C}_n\text{H}_{2n+1}$ ), and this corresponds to the presence of one  $\text{C}=\text{C}$  bond.
- 2 The missing 2 hydrogen atoms could signify the presence of a cyclic **R** group.
- 3 moles  $\text{NaOH} = 17.5 \times 10^{-3} \times 0.100 = 1.75 \times 10^{-4}\text{ mol}$   
 moles  $\text{CH}_3\text{COOH} = 1.75 \times 10^{-4}\text{ mol}$   
 moles glycerol triethanoate  $= 1.75 \times 10^{-4} \div 3 = 5.83 \times 10^{-4}\text{ mol dm}^{-3}$   
 $M_r (\text{C}_6\text{H}_{14}\text{O}_6) = 182$   
 mass glycerol triethanoate  $= 5.83 \times 10^{-4} \times 182 = 0.106\text{ g}$

**Answers to questions on results**

The answers provided by the students provide valuable information about their understanding of what analysis and evaluation involves. It is suggested that a set of results, which are flawed in some way, are analysed and evaluated by the whole class. The likelihood is that their understanding will be significantly broadened and misconceptions can be dealt with.

**Technical information**

Requirements per student/group

**Apparatus**

- eight test tubes (labelled 1 to 8) and rack
- distilled/deionised water
- three 1 cm<sup>3</sup> syringes and one 5 cm<sup>3</sup> syringe, or equivalent pipettes/ graduated pipette
- stopclock

**Materials**

- Access to 5% lipase solution (this should be freshly prepared and filtered).
- Access to 0.5% sodium carbonate solution.
- Access to **Bromocresol purple** solution (as provided).
- Access to **Triacetin** (as provided).

**Safety**

The main points are included on the Student Sheet but it is the teacher's responsibility to ensure that a full risk assessment is carried out prior to the practical session. MSDS sheets should be consulted so that the correct action can be taken in event of a spillage and/or accident.