AMYLASE ACTIVITY – A MICROSCALE APPROACH IN BIOLOGY

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ABSTRACT

I have worked as a Technician at CLEAPSS for a number of years. CLEAPSS is an advisory service, providing support and advice for all aspects of practical work in schools and colleges across England, Wales and Northern Ireland. Our aim is to promote safe and effective practical work in Science, Design &Technology and Art. We run training for science teachers and laboratory technicians, both in the UK and abroad, and we write guidance for our members in line with any relevant legislation regarding safety and the environment. Part of our work at CLEAPSS is to look at particular practicals that pose problems and through our contacts with teachers and technicians, as well as exam boards and other specialist organizations, we are in a unique position to gather information. We have developed a protocol, using information from these various sources as well as through trialing in our laboratory, to look at amylase activity and the effect of pH in particular. This microscale method is simple to carry out, requires minimal resources and gives reliable results. *[African Journal of Chemical Education—AJCE 9(3), November 2019]*

INTRODUCTION

The microscale approach has been shown to have many benefits for Chemistry and is an effective teaching method for a number of reasons: there is less load on students' working memory and students can work individually, rectifying mistakes quickly, pupils seem to concentrate more on the observations and there is greater control of the class as experiments can usually be carried out sitting down, the procedures can be carried out in less time than more traditional procedures, allowing more time for discussion of the subject and it's applications.

From a logistical point of view work can often take place in a non-laboratory room (although a bucket of water can be useful!). These practicals are safer, using fewer solutions and lower concentrations than traditional methods, and they are easier to prepare and clear away with simple disposal – most chemicals can be wiped off with a paper towel which can then be placed in a bowl of water or straight into the bin.

The question is can this approach be used to make practical experiments in biochemistry more accessible?

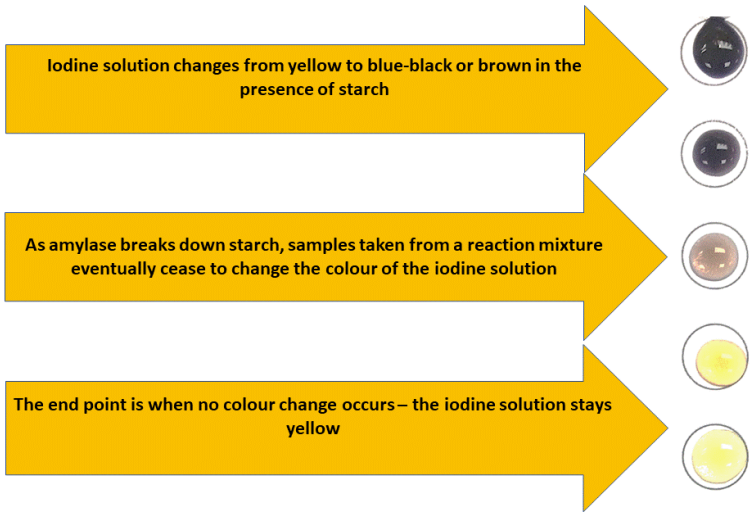
One topic that has always struck fear into the hearts of many technicians (and I suspect, teachers as well) is enzyme practical work. Enzymes are biological catalysts. They control chemical reactions and are notoriously problematic for practical work because they are affected by a number of variables: temperature, pH, concentration of substrate, enzyme and product, possible effects of cofactors, source of the enzyme and even the age of the enzyme.

The enzyme amylase provides a good model for studying enzyme activity and it can be used to investigate the impact of different conditions. In addition, amylase is often used for assessment activities.

BACKGROUND TO THE PROCEDURE

Amylase catalyses the hydrolysis of the glycolytic bonds in starch, breaking it down to produce smaller chain molecules such as glucose and maltose.

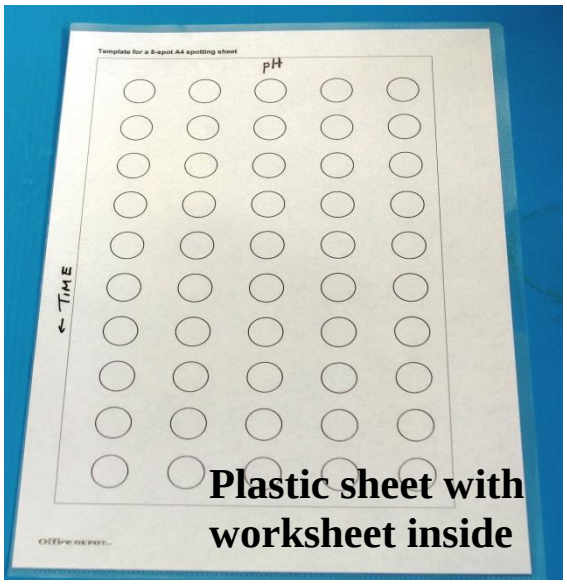
We can detect the presence of starch using Iodine solution which turns blue / black in the presence of starch.



As the starch is broken down, the iodine no longer gives this colour change.

When all the starch has gone we have reached the endpoint of the reaction.

Previous methods have used test tubes or expensive dimple tiles to investigate amylase activity. These only allow a few samples to be tested at a time and comparison between test runs is difficult. The microscale method uses a customizable worksheet placed inside a simple and cheap plastic wallet (obtainable from office suppliers). Drops of Iodine solution are placed on



the plastic surface of the wallet. The reaction mixture of enzyme and substrate is mixed in a test tube and drops of this are added at intervals to the drops of iodine on the plastic sheet. The time to reach endpoint (when all the starch has been broken down and no color change occurs) under different conditions can be investigated.

The plastic sheet is a cheap replacement for dropping tiles and allows a larger number of results to be obtained, easily displayed and compared side by side. In addition, low concentrations and minimal amount of chemicals are needed, typically using only 1 - 2 ml of each component per test run.

Amylase can be purchased from a number of suppliers but human salivary amylase is an extremely reliable (and free!) source of the enzyme and can be used safely providing students only work with their own saliva and correct hygiene procedures are followed. This also gives an opportunity to look at basic hygiene as a separate teaching topic in the classroom.

The following protocol has been published by CLEAPSS and is available to our members on our website, along with a number of other related documents. Normally this sheet is behind a password, but CLEAPSS has given permission to publish it in this Journal.

Investigating the effect of pH on amylase digestion

Why do this?

This procedure demonstrates that the activity of the enzyme amylase is affected by changes in pH.

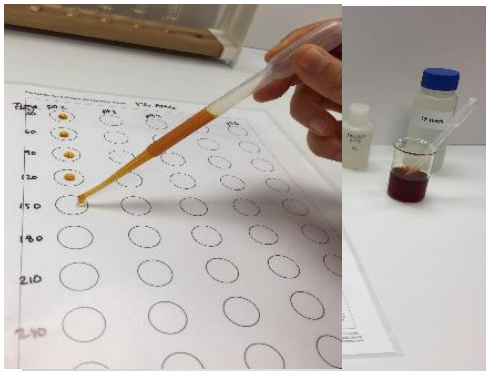
Method with control measures (including Personal Protective Equipment, PPE)

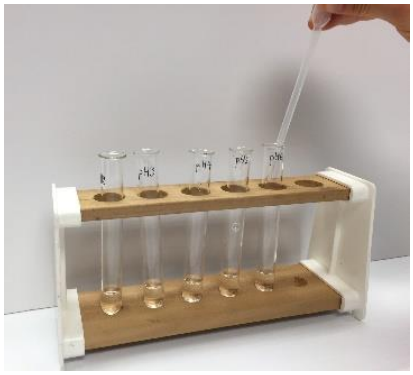
Note that enzyme powders can cause sensitisation if inhaled.

Wear eye protection when using buffer solutions.

Procedure

1. Label each test tube with a different pH and fill the first tube with 1 ml of the corresponding buffer solution
2. Add 1 ml of 1% amylase into the first tube and mix
3. Label each column of the spotting sheet with the different pHs and number each row with the time starting with 0 and every 30 seconds thereafter.
4. Place a drop of iodine solution in each 'spot' of the first column on the spotting sheet.





5. When ready add 1ml of 1% starch solution to the test tube. Mix well, immediately start the timer and take your first sample by placing a drop of the mixture using a dropping pipette onto the 0 spot for the corresponding pH. Avoid contaminating the pipette with the iodine.
6. Return the remainder of the liquid in the pipette to the test tube
7. At 30 second intervals place a drop of the sample of the mixture onto the next iodine spot
8. Continue until the sample no longer produces a colour change on the iodine spot. This is the end point for the test. You may need to add more iodine spots. You may decide to stop the test if there is no change after a set time period e.g. 10 minutes.
9. Repeat steps 1-8 for the other pHs to be tested

Disposal

- Wipe the spots off the sheet with paper towels and bin, then wipe clean with wet paper towels
- Flush away the unused enzyme and starch with lots of water

Disposal notes:

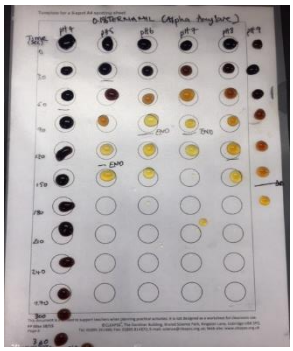
- If saliva is used as a source of amylase any equipment used with saliva needs to be immersed for 10 minutes in a 1% solution of Virkon (a multipurpose disinfectant) before washing up

This document is intended to support teachers when planning practical activities. It is not designed as a worksheet for classroom use.

Expected observations/results

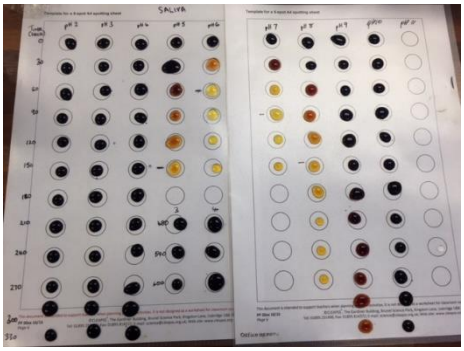
Iodine turns blue-black in the presence of starch. As amylase hydrolyses the starch into sugars the iodine spot will change colour. The reaction will continue until all the starch has been broken down and the iodine spot remains the same colour after the sample has been added. This is the end point.

In trials conducted at CLEAPSS, we obtained the following results.



Termamyl (alpha amylase) has an optimum pH of 7 so the range of pH4 – pH9 was tested.

The results of testing 0.1% Termamyl (alpha amylase) showed that the action of Termamyl worked fastest at pH6 & pH7 in 90 seconds. Below pH5 and above pH9 the enzyme action was inhibited.



Salivary amylase has a broad spectrum of pH use so the range pH2 – pH10 was tested.

The results of testing 12.5% salivary amylase showed that the action of salivary amylase worked fastest at pH6 in 60 seconds. Below pH5 and above pH9 the enzyme action was inhibited by the pH.

Science notes

Different enzymes will require different starting concentrations and produce different end points. It is best to trial the enzyme beforehand.

Adding amylase to the buffer first introduces the enzyme to the different conditions before any substrate is added. This will allow any effects of pH to take action immediately.

Suggested apparatus and materials

- 0.1 - 1% amylase
- 1% starch
- 0.01M Iodine solution
- Buffers adjusted to produce a suitable range of pH solutions
- Small vials or test tubes
- Spotting sheet in a file pocket or laminated (template provided)
- Stop clock or timer
- Plastic dropping pipettes
- Permanent marker
- Beaker of water for rinsing

Apparatus and materials notes

- Adjust the concentration of amylase between 0.1 – 1% to suit the time frame or activity of the amylase
- Enzyme should be made just before use
- Find out the optimum pH from the enzyme information sheet and choose at least 2 pH units either side of the optimum
- Saliva can be used as a source of amylase. Dilute to 10 – 15%
- Spotting tiles & microwell plates can be used instead of the spotting sheet
- A glass rod can be used for transferring the sample to the iodine spot. This will need to be cleaned between sampling
- Ensure that the reagents have reached much the same temperature before use
- Iodine evaporates from spots quite quickly at room temperature, so carry out one pH trial at a time

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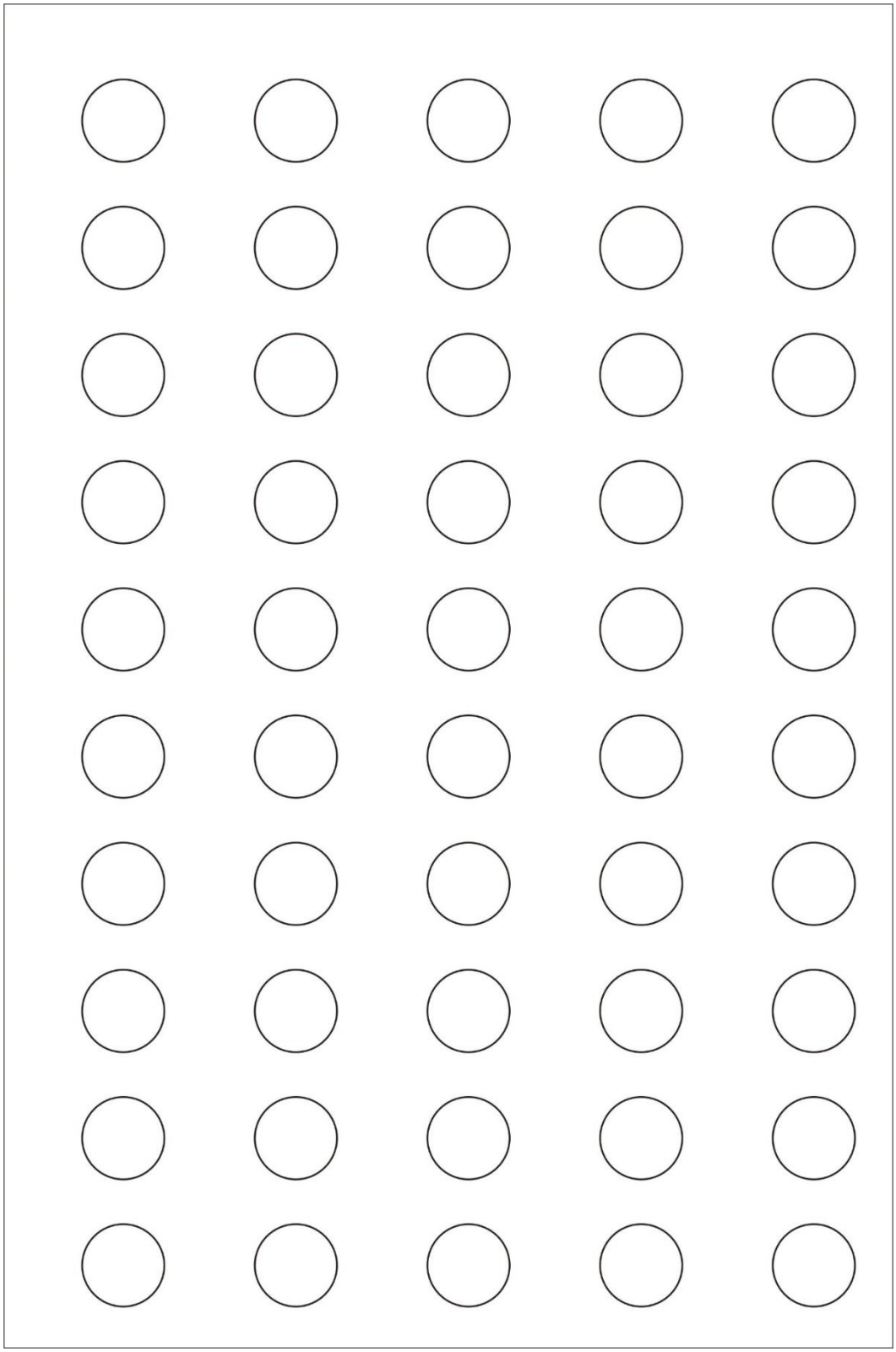
Template for a 5-column, A4 spotting sheet

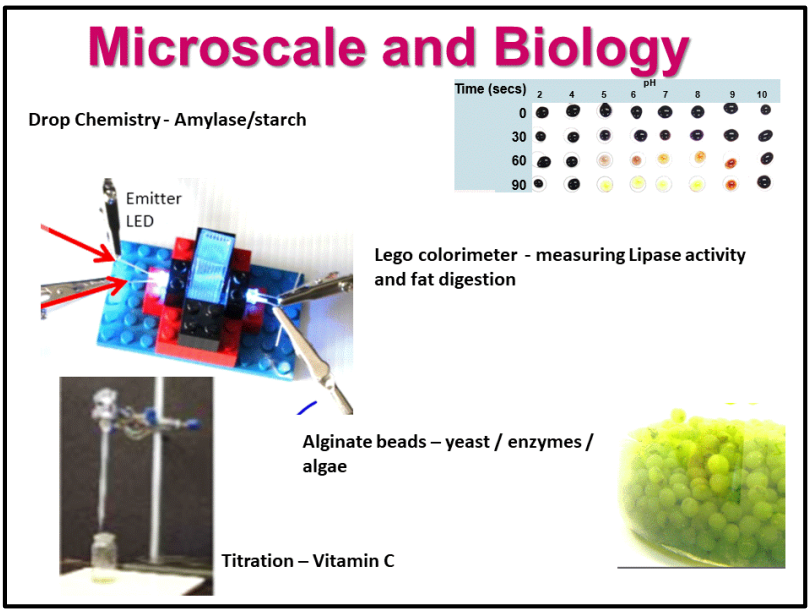


DISCUSSION

As mentioned previously one of the most effective and free amylases is that found in saliva. Previous trials used a dilution of between 10 – 15%, however my saliva needed diluting to 4% to give results in a measurable timescale. This illustrates the need for trialling enzyme experiments generally, whatever the source, as the concentrations needed to give reliable results within a class timescale will vary considerably. This drop method does allow you to try out different concentrations quickly. Under optimal conditions for the amylase you would look to get an endpoint within 2-3 minutes of adding the amylase to the starch. Less than optimal conditions may take 4-7 minutes and the extreme conditions may not reach endpoint at all (at least not within a set time limit, e.g. 10 minutes, when the experiment could be terminated)

CLEAPSS advisors have looked at the safety aspects of using human saliva and the guidance given is that as long as proper hygiene is followed then the risk of spread of infection is low. Students should only use their own saliva and rinse out their own equipment which can then be placed in a solution of sodium chlorate(I), NaOCl, for 30 minutes or in 1% Virkon disinfectant for 10 minutes. Equipment can then be washed in hot soapy water. Hands should be washed afterwards and the bench disinfected. As a separate teaching topic this use of human saliva could be used to teach aspects of hygiene and infection control as well as digestion.

SUMMARY



As shown previously, drop chemistry lends itself to biological studies but there are other Microscale Chemistry techniques that might also be adapted. Determination of Vitamin C levels by titration with DCPIP could be carried out using the microscale gravimetric method – an alternative to the traditional titration method - involving small balances for weighing and accurate Berol dropping pipettes.

Microscale chemistry already borrows from biology with its use of Petri dishes so perhaps other small scale techniques could have applications in chemistry. In certain biological experiments sodium alginate is used to make beads of immobilised yeast / enzymes / algae. This microscale technique uses small quantities that can be reused. One possible use would be to immobilise catalase in yeast or potato and use the beads to produce oxygen from hydrogen peroxide instead of using manganese oxide. This would be a much greener option.

In the past we have looked at an idea from Norway using Lego bricks and LEDs to make a simple colorimeter.[1] Working with our Physics colleagues to understand how LEDs generate a low voltage when light shines on them, we have used the colorimeter in Chemistry applications to measure concentrations of solutions (e.g. copper chloride) There may be applications in Biochemistry e.g. action of lipase on the breakdown of fats in a milk suspension or the measurement of glucose with Benedict's solution or DNSA.

Increasingly we see a blur between the lines of the Chemistry / Biology / Physics disciplines. Microscale techniques have a lot to recommend them. With microscale you don't need a lot of expensive resources and relatively simple techniques can be adapted. With a bit of imagination, collaboration and ingenuity we can borrow techniques from one discipline to develop ideas in another.

REFERENCES

1. Asheim, J., Kvittingen, E.V., Kvittingen, L., and Verley, R. (2014). A Simple, Small-Scale Lego Colorimeter with a Light-Emitting Diode (LED) Used as Detector. *J. Chem. Educ.* 91(7), 1037-1039; https://doi.org/10.1021/ed400838n

Information from several CLEAPSS publications have also been used in this article. These documents are only available to our members on the CLEAPSS website and are password protected however we may be able to answer questions on and factors of this document that may cause safety concerns.

ACKNOWLEDGEMENTS

Firstly, I would like to thank Mrs. Marié du Toit and other organizers for inviting me to present at the 10th International Symposium on Microscale Chemistry, Potchefstroom, South Africa, a truly inspiring event.

I would also like to thank CLEAPSS and its Director, Steve Jones, for allowing me to attend the conference and also for their willingness to support the trip and share the work we do at CLEAPSS.

Finally, I would like to thank Bob Worley for all his help, support and encouragement over the years in the world of Microscale Chemistry.

Both Bob and CLEAPSS have encouraged me and given me the confidence to use my imagination and ingenuity as a Technician.