Proteins: we're made of them, we eat them, but can they be drugs too?

Key Stage 4 Programme

Pupil Name

Handbook Designed by Dr Gavin Marshall

Timetable and Assignment Submission

Timetable – Tutorials

Tutorial	Date	Time	Location
1	**12th Dec 2018**	**13:00**	**Bristol University**
2	**8th Jan 2019**	**13:20**	**U207**
3	**22nd Jan 2019**	**13:20**	**U207**
4	**5th Feb 2019**	**13:20**	**U207**
5	**12th Feb 2019**	**13:20**	**U207**
6 (Feedback)	**26th Feb 2019**	**13:20**	**U207**
7 (Feedback)	**12th Mar 2019**	**13:20**	**U207**

Timetable – Homework Assignments

Homework Assignment	Description	Due Date
Tutorial 1	Baseline assignment – Safety of a drug in your home	19th Dec 2018
Tutorial 2	Compare and Contrast – haemoglobin and hemocyanin	22nd Jan 2019
Tutorial 3	Protein structure – Model and explain	5th Feb 2019
Tutorial 4	Aggregation profile – calculate peaks and suggest what might have happened to the sample	12th Feb 2019
Tutorial 5	Final Assignment – Report on viability of new drug.	26th Feb 2019

Assignment Submission – Lateness and Plagiarism

Lateness	
Submission after midnight on 9th August	10 marks deducted
Plagiarism	
Some plagiarism	10 marks deducted
Moderate plagiarism	20 marks deducted
Extreme plagiarism	Automatic fail

Uni Pathways – Pupil Feedback Report

Grade	Marks	What this means
1st	70+	Performing to an excellent standard at A-level
2:1	60-69	Performing to a good standard at A-level
2:2	50-59	Performing to an excellent standard at GCSE
3rd	40-49	Performing to a good standard at GCSE
Working towards a pass	0-39	Performing below a good standard at GCSE
Did not submit	DNS	No assignment received by The Brilliant Club

Lateness	
Any lateness	10 marks deducted
Plagiarism	
Some plagiarism	10 marks deducted
Moderate plagiarism	20 marks deducted
Extreme plagiarism	Automatic fail

Name of RIS teacher	Dr Gavin Marshall		
Title of Assignment	Drug suitability report		
Name of Pupil			
Name of School	Berkeley Green UTC		
ORIGINAL MARK / 100		FINAL MARK / 100	
DEDUCTED MARKS		FINAL GRADE	

If marks have been deducted (e.g. late submission, plagiarism) the PhD tutor should give an explanation in this section:

Knowledge and Understanding
Critical Evaluation/Problem Solving
Structure and Presentation
Resilience Comment

Contents

Course Rationale	**P5**
Mark Scheme	**P6**
Glossary of Keywords	**P8**
What happens when our drugs go bad?	P9
Can proteins be drugs?	P11
What is a protein?	P12
Aggregation and protein stability	P14
Can we keep proteins stable?	P17
Reflection Tutorial	P20
Appendices	**P24**

Course Rationale

Proteins exist in all living things, they make up a great deal of our bodies, from our hair to the molecules that move oxygen through our blood, but can they also be made into drugs? We will investigate how proteins might be beneficial drugs, and why their inherent instability makes it very hard to use them for drugs.

Students will learn the importance of having stable drugs and the risks in using unstable ones. Real world protein drugs will be used as examples of what can go wrong and make the drug go off.

Students will then learn some techniques for investigating and improving the stability of protein drugs using real experimental data. We will assess the stability of some protein drugs and make an assessment on their value.

Finally students will create a scientific report which assesses the viability of a new drug mixture, in exactly the same manner as a scientist would create a report; either recommending the development of the new drug, or suggesting that it is a poor candidate.

Mark Scheme

Skills	1st (70-100)	2:1 (60-69)	2:2 (50-59)
Knowledge and Understanding	• All content included is relevant to the general topic and to the specific question/title • Good understanding of all the relevant topics. • Scientific terms are used accurately throughout • Clear justification on how the content included is related to the specific issues that are the focus of the assignment	• Most of the content included is relevant to the general topic and to the specific question/title • Good understanding of most the relevant topics • Scientific terms are frequently but not always used accurately. • Adequate justification on how the content included is related to the specific issues that are the focus of the assignment	• Some of the content included is relevant to the general topic and to the specific question/title • Good understanding on some of the relevant topics but occasional confusion on others. • Scientific terms are used mostly accurately with occasional confusion. • Some justification on how the content included is related to the specific issues that are the focus of the assignment
Critical Evaluation/ Problem solving	• Moved beyond description to an assessment of the value or significance of what is described • Evaluative points are consistently explicit/systematic/reasoned/justified • Effective critiques on the reliability of sources provided • Consistently demonstrate clear, analytical and logical steps to solving problems	• Mostly description but some assessment of the value or significance of what is described • Evaluative points are mostly explicit/systematic/reasoned/justified • Some evidence of critiques on the reliability of sources provided • Some examples of solving problems but not consistently clear, analytical and logical	• Only description with minimal assessment of the value or significance of what is described • Evaluative points are at times explicit/systematic/reasoned/justified • Limited evidence of critiques on the reliability of sources provided • Demonstrate clear steps to solving problems but not consistently analytical or logical

Structure and Presentation	• Ideas are presented in paragraphs and arranged in a logical structure that is appropriate for the assignment • The introduction clearly and succinctly outlines how the report will deal with the issues • The conclusion summarises all the main points clearly and concisely • All calculations, formulas and methods are clearly structured, clear to follow and correct • Tables and graphs are effectively constructed including appropriate headings, units and scales. • All sources are referenced correctly in an agreed format	• Ideas are presented in paragraphs and arranged in a structure that is mostly appropriate for the assignment • The introduction adequately describes how the report will deal with the issues • The conclusion summarises most of the main points clearly • Calculations, formulas and methods are mostly structured, clear to follow and correct • Most tables and graphs are well constructed • Most sources are referenced correctly in an agreed format	• Ideas are presented in paragraphs and arranged in a structure • The introduction mentions how the report will deal with the issues • The conclusion summarises some of the main points clearly • Calculations, formulas and methods are not always structured, clear to follow and correct. • Some tables and graphs are well constructed but contains some errors • Some sources are referenced correctly in the agreed format with occasional errors

Subject Vocabulary

Word	Definition	In a sentence
Particle	A small piece of material.	A particle of dust was floating in the air
Aggregate	The combination of small particles into larger ones.	
Particle size distribution	The relative amount of particles at each size.	
Protein	A molecule made of amino acids. Many different shapes and sizes of protein can be formed in a living thing.	
Stable	Will not change in time, unless there is an outside force.	
Peak	The point on a graph representing the highest value	
Formulation	A mixture of ingredients	

Tutorial 1 – Is this drug still safe to eat?

What is the Purpose of Tutorial 1?

- To understand the importance of getting the drug you expect.
- To be able to describe some effects of taking degraded drugs
- To be able to calculate the LD50 and therapeutic index of a drug

Humans have been using pharmaceutical drugs for centuries, with varying levels of success. One key factor in whether a drug will work or no is the stability of that drug. This is why all modern drugs have expiry dates. But what really happens to a drug as it expires, and is it still safe to eat?
One common problem is that some of the drug may have degraded, leaving it ineffectual.

What might happen if you:

Got too high dosage of the drug?	Got too low a dosage of the drug?

Stability Profiles of Drug Products Extended beyond Labeled Expiration Dates

ROBBE C. LYON,[1] JEB S. TAYLOR,[1] DONNA A. PORTER,[2] HULLAHALLI R. PRASANNA,[1] AJAZ S. HUSSAIN[3]

[1]Division of Product Quality Research, Center for Drug Evaluation and Research, Food and Drug Administration, HFD-941, White Oak, Life Sciences Building 64, 10903 New Hampshire Avenue, Silver Spring, Maryland 20993-0002

[2]Division of Field Science, Office of Regional Operations, Office of Regulatory Affairs, Food and Drug Administration, Rockville, Maryland 20857

[3]Vice President & Global Head of Biopharmaceutical Development, Sandoz, 506 Carnegie Center, Princeton, New Jersey 08540

Received 6 January 2006; accepted 17 March 2006

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20636

ABSTRACT: The American Medical Association has questioned whether expiration dating markedly underestimates the actual shelf life of drug products. Results from the shelf life extension program (SLEP) have been evaluated to provide extensive data to address this issue. The SLEP has been administered by the Food and Drug Administration for the United States Department of Defense (DOD) for 20 years. This program probably contains the most extensive source of pharmaceutical stability data extant. This report summarizes extended stability profiles for 122 different drug products (3005 different lots). The drug products were categorized into five groups based on incidence of initial extension failures and termination failures (extended lot eventually failed upon re-testing). Based on testing and stability assessment, 88% of the lots were extended at least 1 year beyond their original expiration date for an average extension of 66 months, but the additional stability period was highly variable. The SLEP data supports the assertion that many drug products, if properly stored, can be extended past the expiration date. Due to the lot-to-lot variability, the stability and quality of extended drug products can only be assured by periodic testing and systematic evaluation of each lot. © 2006 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 95:1549–1560, 2006

Keywords: shelf life; drug stability; shelf life extension program; SLEP; expiration date

In order to get the correct dosage, scientists calculate the minimum and maximum dosages which a drug will work for. This is called the therapeutic window, and we will calculate it for paracetamol below.

Data:
To determine the toxicity, and calculate the LD 50 Rats were given paracetamol until adverse effects were recorded. Half of all the rats died once a total dosage of 0.31g of paracetamol was given. The average weight of a rat is 400g. Calculate the LD50.

Toxic dose 150mg/kg active dose 1g/60kg – what is the therapeutic window?

Homework - Baseline assignment

Research a common medicine that is in your family home. Write a paragraph on the safety of the drug.

1. Compare the intended effects and side effects.
2. Find the LD50 and calculate what the lethal dose would be for yourself.
3. Decide if the drug should be sold or not, justify your judgement.

Tutorial 2 – Can proteins be drugs?

What is the Purpose of Tutorial 2?

- To be able to list some ways our bodies use proteins like drugs
- To be able to recall some proteins in our bodies
- To be able to describe the value of insulin to our health.

Insulin
Insulin is responsible for the absorption of carbohydrates (such as sugars) from our blood into our tissues. Diabetic people do not produce enough insulin in their bodies and so need to take it artificially in order to absorb sugars. Without it their blood sugar levels increase and energy levels decrease until they are unable to function.

Haemoglobin
Haemoglobin is the protein that carries oxygen around in our blood. It bonds to oxygen using an iron atom in the protein. The oxygen is able to easily bond and release from the protein allowing us to transport it to different cells in the body.

Oxytocin
Often called the cuddle hormone, oxytocin is the chemical released in humans which is creates feelings of attachment in our brain chemistry. The release of oxytocin is triggered by many stimuli including childbirth, physical touch and sexual arousal. As a mood altering hormone, oxytocin has been suggested for use as a drug to help alter human perception.

Hemocyanin
Hemocyanin is the oxygen carrier found in arthropods (e.g. scorpions) and crustaceans (e.g. snails). Unlike haemoglobin, which is transported in red blood cells, hemocyanin is large enough to be transported directly within the blood of the animal, making it one of the largest proteins ever discovered at 8MDa.

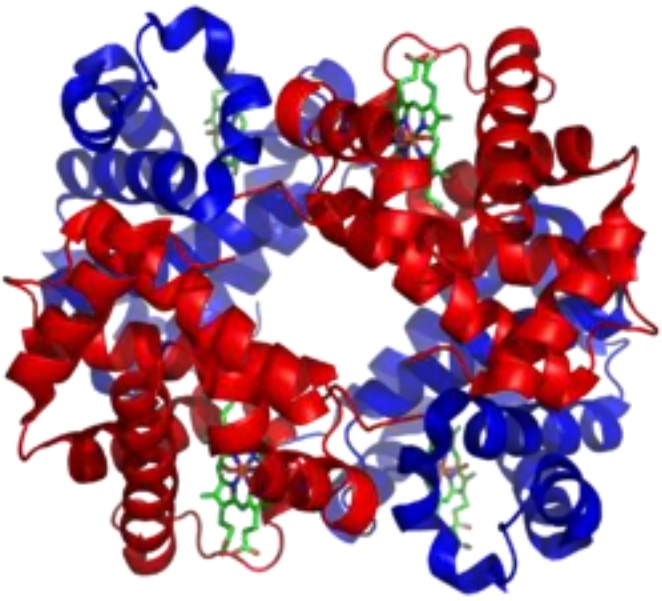
Insulin and haemoglobin are common proteins in our body, discuss the use of them in our bodies.

Activity 2:
Calculate how much insulin a diabetic patient might need in a day.

Homework:
Haemoglobin and Haemocyanin: Research these proteins further online. Draw a table to compare and contrast them.

Figure

Tutorial 3 – What is a protein



What is the Purpose of Tutorial 3?

- To describe the primary structure of a protein
- To be able to create a computer model of a common protein
- To understand the importance of primary, secondary, tertiary and quaternary structures to a protein shape.

Theory:
Proteins are large molecules found in, and made by, all living things. They are used for many of the core functions of living things, comprising the physical structure of most cells, creating DNA and allowing us to breathe. At the molecular level, proteins are comprised of a bonded amino acids, linked together into long chains, called the **primary structure** of the protein.

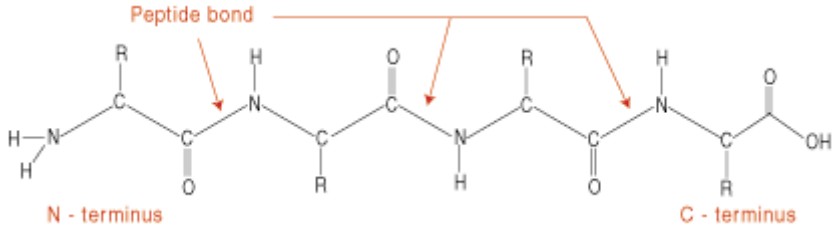


Fig 1. A polypeptide chain.

Unlike most simple molecules, however, proteins are rolled up into complex shapes which allow them to perform all their weird and wonderful functions. At the smallest level, they fold into their **secondary structure**. This is comprised of many shapes, of which most look like springs, or flat sheets.

A series of these springs and sheets are then assembled into the final structure of the protein allowing it to fit into the right place for its particular job. This final structure is called the **tertiary structure**. Most proteins form into ball like tertiary structures, however some, such as the keratin which makes up our hair. This forms long thin strands as required for hair.

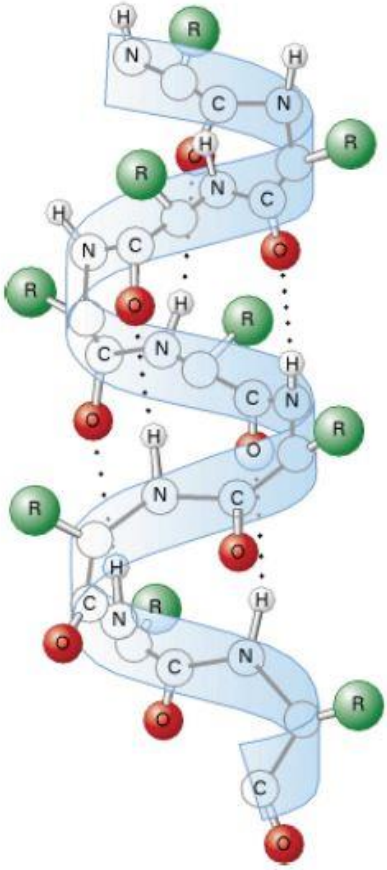


Fig 3. Beta sheet – a flat shaped protein structure.

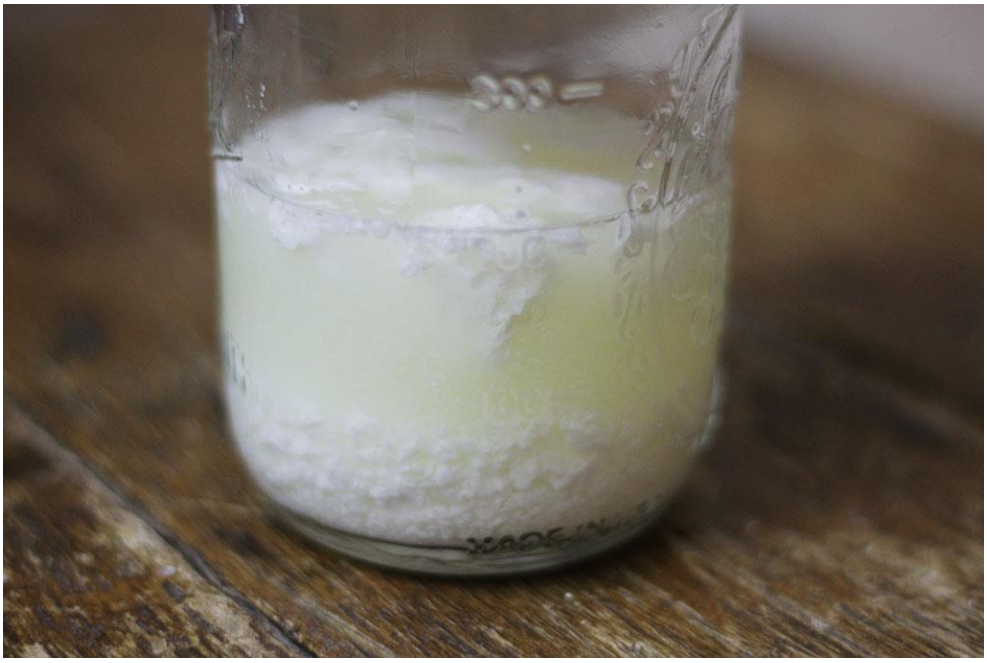
Fig 2. Alpha helix – A spring shaped protein structure

Finally if multiple protein strands work together to perform their function, then this group of proteins can collectively be called the quaternary structure. Proteins with complicated roles, such as haemoglobin, often have quaternary structures. Haemoglobin is a group of four individual proteins working together.

Activity:
Use modelling software to create a 3D image of a protein. Identify key shapes and comment on the structure of the protein. Print a 2D version.

Homework:
Pick a protein which you find interesting. Use an online modelling software to plot the structure of the protein. Whilst looking at this structure, note any strange or important shapes you see. Write a few sentences about what these shapes are and how they might be crucial to the function of the protein.

Tutorial 4 – Protein aggregation and stability



What is the Purpose of Tutorial 4?

- To understand the process of aggregation and what this means for formulation
- To be able to interpret a particle size distribution, finding the mean particle size and the range of sizes in the sample
- To be able to draw a conclusion about whether a broad or narrow distribution may be preferable for a medicine.

Aggregation is the process by which small things stick together into larger things. This often leads to very large aggregates forming and then settling to the bottom. Some common examples of aggregation are the clumping of protein in off milk and the settling of salad dressing into layers over time.

Why might this aggregation be a problem?

A particle size distribution

A particle size distribution is a plot showing how many particles of any particular size are found within a sample. For instance if in a sample all the particles were exactly 10 nanometres (nm) across the distribution would be a single peak at 10nm. However, for real world samples, normally many sizes of particle are found in a sample.

Examine the particle size distribution below. What is this telling us about the size of particles within the sample? Fill in the table below.

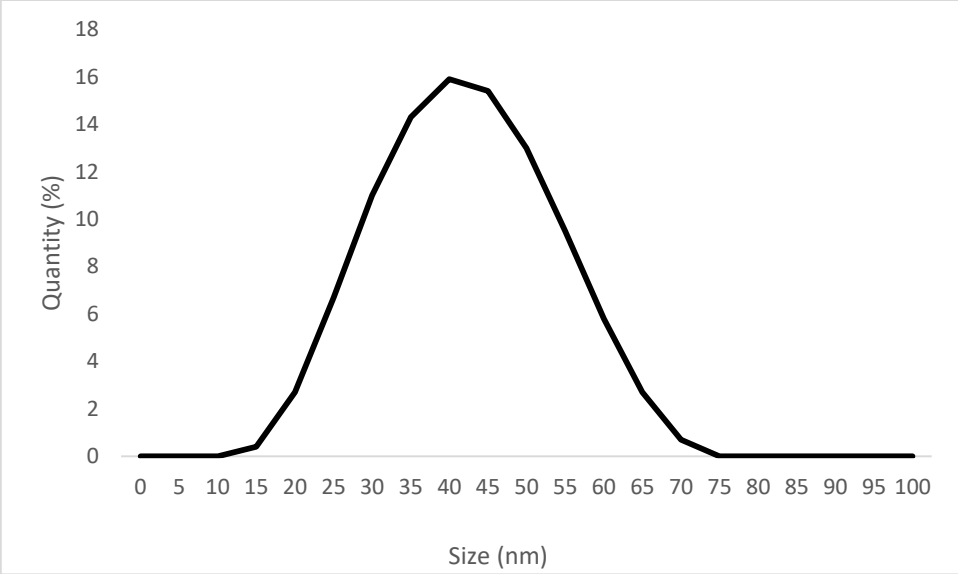


Figure 1: particle size distribution of hemocyanin in a water solution

Feature	Value	Significance
Peak (nm)		
Width (nm)		

Stability

When a drug is stored for a long period of time, sometimes its particle size distribution might change. Look at the below particle size distribution and decide if you think the average particle size has changed.

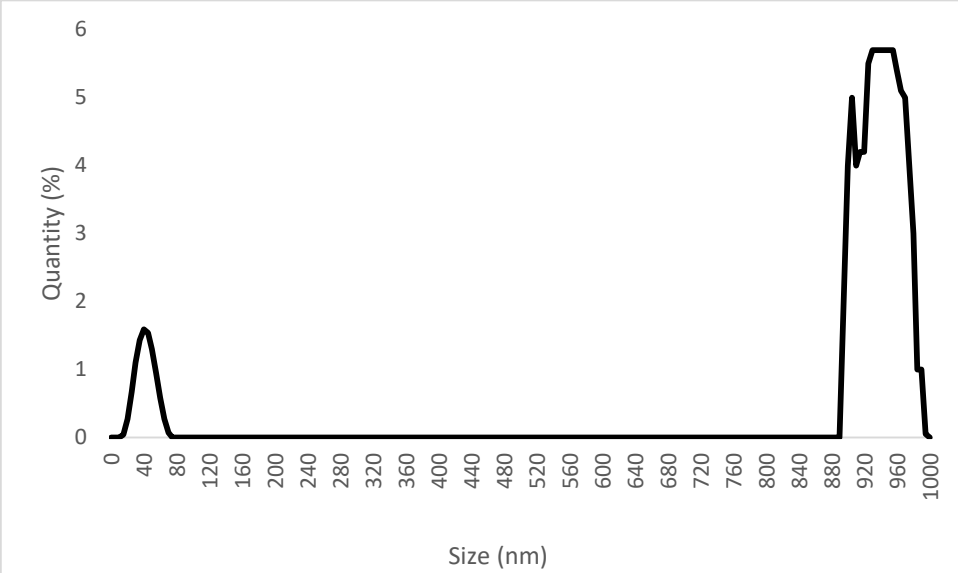


Figure 1: particle size distribution of hemocyanin after 6months storage in water solution

What do you think happened to this sample? Is this a good or a bad change?

Homework assignment:

- Using the data above, calculate the peak positions and width of any peaks on the particle size distribution. (note: there may be multiple peaks).

- Suggest what may have happened in this sample since the initial test.

- Suggest one thing that could have been done to reduce the level of aggregation in this sample.

Tutorial 5 – Can we make proteins stable?

What is the Purpose of Tutorial 5?

- List some common methods for making proteins more stable
- Use stability data to evaluate if a protein is stable
- Draw conclusions about the life time of a protein based on its stability data.

When proteins are unstable, they often aggregate as they are damaged. This is because the same forces which make a protein fold can make proteins attract each other. However, in order for proteins to function properly they must not be aggregated, as aggregated proteins won't be transported through the body properly and may have changed physical properties. Therefore our bodies create conditions that maintain the stability of the protein and avoid aggregation. How can we mimic this behaviour to help keep our protein drugs stable?

Protein scientists therefore start by creating conditions similar to those found inside the animal in order to try and stabilise the protein. We can often also make further improvements, which may not be ideal inside the animal, but do improve protein stability.

Changes to the protein concentration, acidity (pH), amount of salt (Ionic strength) and viscosity of the sample affect stability. Below is some data on the stability of hemocyanin in various formulations. Consider which ones have the highest stability. Do you think a similar trend would be observed with other proteins?

Sucrose Concentration (mg mL^{-1})	T_m (°C)	Activation Energy (kJ mol^{-1})
0.1	75.03	472
1	75.11	530
10	74.96	545
100	75.09	557
Sol Lim	75.15	371

Here differing buffering agents have been added to the protein formulation. The temperature at which the protein unfolds changes based on this formulation.

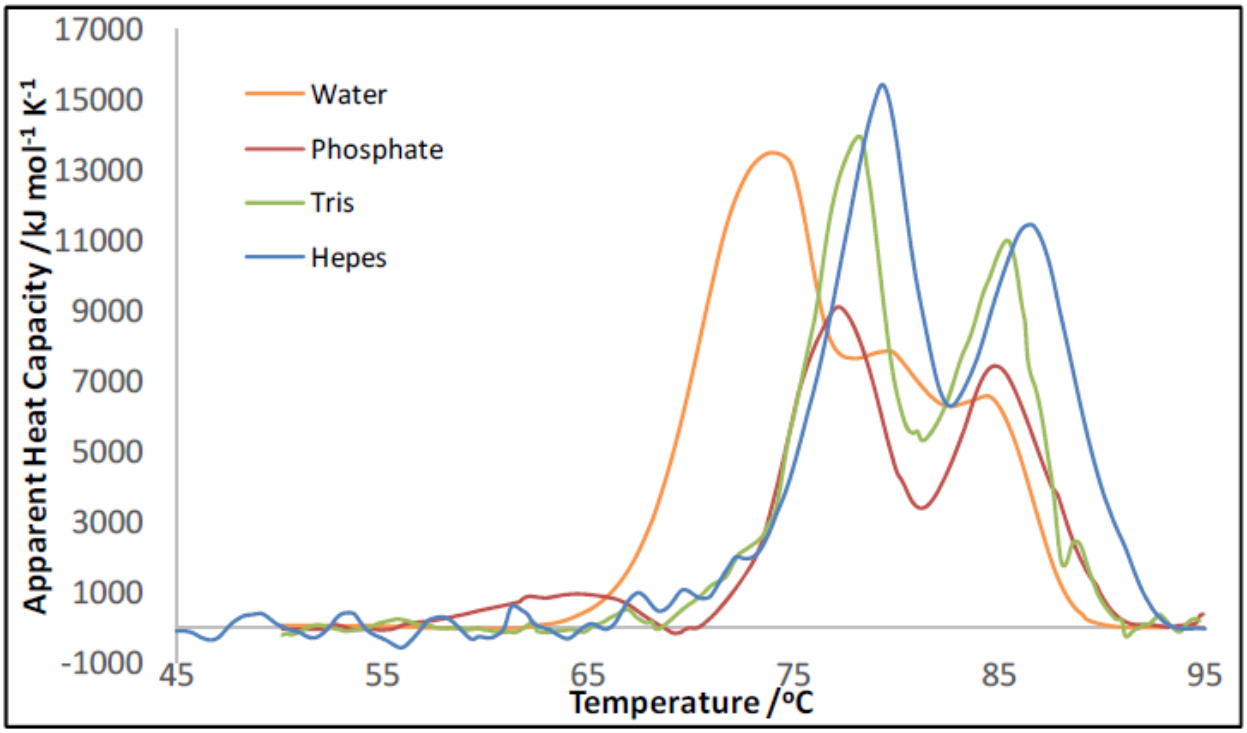


Figure 5-2 DSC *traces of Haliotis Rubra hemocyanin in buffers which promote stability*

Which buffer promotes the highest stability?

Would all of these buffers be suitable?

When making a new protein formulation, protein scientists must test many conditions in order to find the most stable formulation. For some small simple proteins, a basic formulation may work, where there is no need to test different combinations, but for complex proteins such as hemocyanin, it is difficult to predict what conditions will work before we test them.

Final Assignment

You represent a large pharmaceutical company looking to invest in new drug candidates. A research team has come to you with some data on a new stabilised drug they have developed based on foetal bovine albumin protein. You must prepare a report for your CEO detailing what this drug is, how it was stabilised, and if the new stable formulation should be allocated £10million in funding for commercial development. You will need to explain what proteins are and the specific structure of proteins, as your CEO is a businessperson and has never studied protein chemistry.

In your report you must

1. Describe the chemical structure of the protein drug, including a brief description of protein structures.
2. Explain how proteins may be used as drugs, citing existing commercial protein drugs.
3. Explain what the scientists have done to stabilise the protein.
4. Analyse the data from the research team (found below) and calculate the important variables about the stability of the protein. This will include the Therapeutic window, particle size, % of protein stable after 6 months, % of protein stable after 6 months when stabilised, and % increase in stability.
5. Decide if the drug should be given the £10million pounds funding for commercial development and justify your decision.

Your report should be 1000 words long and include calculation of the five key variables on protein stability listed above.

Tutorial 6 – Feedback tutorial

What is the Purpose of Tutorial 6?

- To receive feedback on final assignments.
- To share examples of best practice with the other pupils in your group.
- To write targets for improvement in school lessons.
- To reflect on the programme including what was enjoyed and what was challenging.

Final assignment feedback

What I did well…	What I could have improved on…
•	•
•	•
•	•

My target for future work is…

Tutorial 7 – Feedback tutorial

What is the Purpose of Tutorial 7?

- To receive feedback on final assignments.
- To share examples of best practice with the other pupils in your group.
- To write targets for improvement in school lessons.
- To reflect on the programme including what was enjoyed and what was challenging.

Final assignment feedback

What I did well…	What I could have improved on…
•	•
•	•
•	•

My target for future work is…

Reflecting on Uni Pathways

What did you most enjoy about Uni Pathways?
•   •   •

What did you find challenging about the programme?	How did you overcome these challenges?
• • •	• • •

Appendix 1 – Referencing correctly

When you get to university, you will need to include references in the assignments that you write, so we would like you to start getting into the habit of referencing in your Brilliant Club assignment. This is really important, because it will help you to avoid plagiarism. Plagiarism is when you take someone else's work or ideas and pass them off as your own. Whether plagiarism is deliberate or accidental, the consequences can be severe. In order to avoid losing marks in your final assignment, or even failing, you must be careful to reference your sources correctly.

What is a reference?

A reference is just a note in your assignment which says if you have referred to or been influenced by another source such as book, website or article. For example, if you use the internet to research a particular subject, and you want to include a specific piece of information from this website, you will need to reference it.

Why should I reference?

Referencing is important in your work for the following reasons:

- It gives credit to the authors of any sources you have referred to or been influenced by.
- It supports the arguments you make in your assignments.
- It demonstrates the variety of sources you have used.
- It helps to prevent you losing marks, or failing, due to plagiarism.

When should I use a reference?

You should use a reference when you:

- Quote directly from another source.
- Summarise or rephrase another piece of work.
- Include a specific statistic or fact from a source.

There are a number of different ways of referencing, and these often vary depending on what subject you are studying. The most important to thing is to be consistent. This means that you need to stick to the same system throughout your whole assignment. Here is a basic system of referencing that you can use, which consists of the following two parts:

1. **A marker in your assignment**: After you have used a reference in your assignment (you have read something and included it in your work as a quote, or re-written it your own words) you should mark this is in your text with a number, e.g. [1]. The next time you use a reference you should use the next number, e.g. [2].
2. **Bibliography**: This is just a list of the references you have used in your assignment. In the bibliography, you list your references by the numbers you have used, and include as much information as you have about the reference. The list below gives what should be included for different sources.

   a. **Websites** – Author (if possible), title of the web page, website address, [date you accessed it, in square brackets].

   E.g. Dan Snow, 'How did so many soldiers survive the trenches?', http://www.bbc.co.uk/guides/z3kgjxs#zg2dtfr [11 July 2014].

   b. **Books** – Author, date published, title of book (in italics), pages where the information came from.

   E.g. S. Dubner and S. Levitt, (2006) *Freakonomics*, 7-9.

   c. **Articles** – Author, 'title of the article' (with quotation marks), *where the article comes from* (newspaper, journal etc.), date of the article.

   E.g. Maev Kennedy, 'The lights to go out across the UK to mark First World War's centenary', Guardian, 10 July 2014.

Notes

Notes

Notes

Notes

Proteins: we're made of them, we eat them, but can they be drugs too?

Key Stage 4 Programme

researchersinschools.org