H2 Biology • RI Prelim 2023

PAPER 4

(a) You will need to carry out a serial dilution of the 10.00% protein solution, **P**, to reduce the concentration by half between each successive dilution.
You will need to prepare four concentrations of protein solution in addition to the 10.00% protein solution, **P**.
After the serial dilution is completed, you will need to have 5 cm^3 of each concentration available for use.

(i) In the space below, draw a table to show how you will prepare the serial dilution. [3]

Final concentration of protein solution/%	Concentration of protein solution used/%	Volume of protein solution used/cm^3	Volume of **W**/water used/cm^3
10.00	10.00	10.0	0.0
5.00	10.00	5.0	5.0
2.50	5.00	5.0	5.0
1.25	2.50	5.0	5.0
0.63	1.25	5.0	5.0

1. **C** – Headings and units with correct final concentration of protein solution and concentration of protein solution used;
2. **V** - correct volumes of protein solution and water (**W**) used;
3. **P** - concentration recorded to 2 dp and volume recorded to 1 dp ;

(ii) Record your results in an appropriate table. You may use the same symbols for more than one test-tube. [2]

protein concentration/%	symbol
10.00	+++++
5.00	++++
2.50	+++
1.25	++
0.63	+
0.00	-

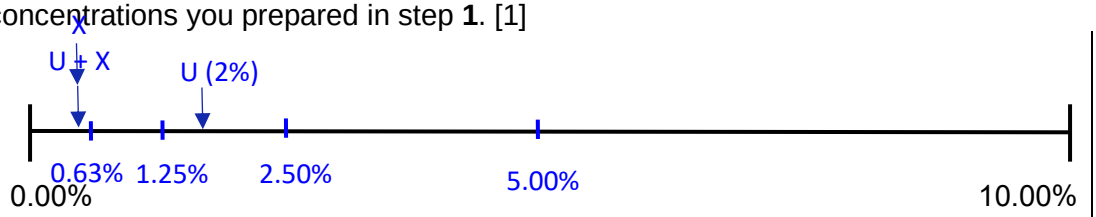
1. **H** - correct heading with units for independent variable (protein concentration/%) correct heading for dependent variable (symbol);
A: colour intensity, colour after 1 min, etc (instead of symbol) in heading

R: concentration of P in heading

2. **P** - records readings for all concentrations with symbols with decreasing trend;

(iii) Fig. 1.1 shows a scale of protein concentrations used in this investigation. The position for 0.00% and 10.00% are shown on the scale.

Complete the scale in Fig. 1.1 by showing the positions of the protein concentrations you prepared in step **1**. [1]



correct positions of protein concentrations;

(iv) Use your results in **(a)(ii)** and **(a)(iii)** to estimate the protein concentration of **U**, **X** and **U+X**. Show your estimates on Fig. 1.1 by drawing labeled arrows (↓) at the correct positions on the scale. [2]

1. U correctly labeled at 1.25%<U<2.5%
   A: U=1.25 or U=2.5
   R: if U is greater than 2.5
2. Both X and U+X labeled at 0%<X<0.63% and 0%<U+X<0.63%
   R: if X or U+X is pointing to 0

(v) Using your result in **(a)(iv)**, estimate the concentration of protein in **U** over a period of 24 hours and state the possible medical condition of the patient indicated by **U**. Show your working in the space provided. [2]

1. Correct calculation
   10.00% ➔ 1000mg
   2.00% ➔ 1000/10 X 2 = 200 mg
   (A: range of values between 125 to 250mg)
   (ECF: if U in a(iv) is incorrect)
2. Correct medical condition - Urinary tract infection or kidney tubular disease

(vi) Solution **X** was extracted from the human gastrointestinal tract.

Suggest the identity of solution **X** and explain the basis of your suggestion. [3]

1. X is an enzyme/protease/pepsin/peptidase. (A gastric juice containing protease)
2. which digests the proteins into amino acids;
3. Thus after X was added the concentration of U decreased but it did not reach 0 due to the presence of X which is an enzyme that is protein in nature and hence a slight purple colouration was noted when the biuret test was conducted on both U+ X and X.

(b) **(i)** Outline how a colorimeter is set up so as to obtain a correct measurement of the colour intensity of a coloured solution placed in the cuvette. [2]

*any **two** from:*

1. set wavelength to use**;**
2. set colorimeter (absorbance) to zero/calibrate**;**
3. using water (to calibrate colorimeter)**;**

(ii) State the limitation of the experiment described in part **(a)**, that can overcome by the use of the colorimeter. [1]

1. human perception/interpretation of colour intensity is subjective and this affects the determination of the colour intensity;
2. colorimeter enable objective, numerical determination of colour intensity;

R: determine colour

(iii) Plot a calibration curve using the data given, on the grid in Fig. 1.3.

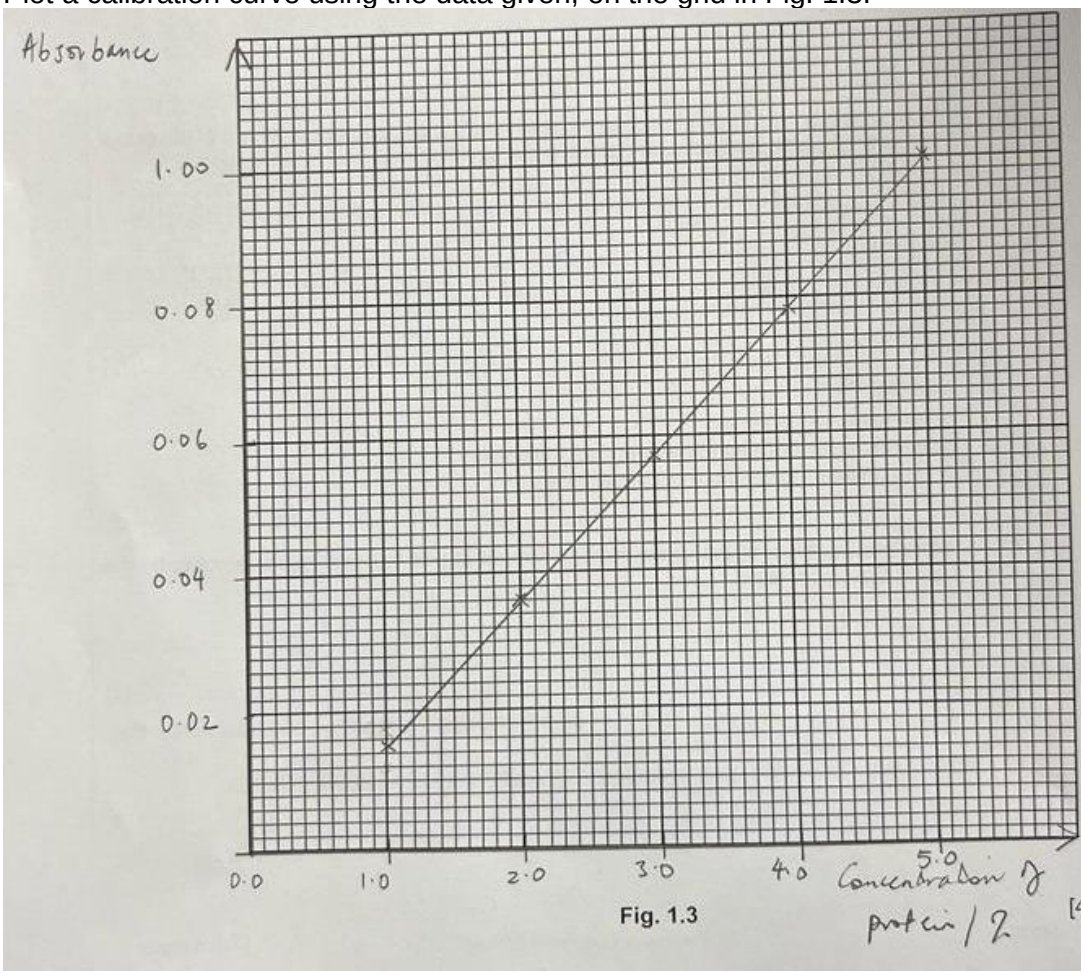


Fig. 1.3

Fig. 1.3 [4]

1. **S**cale : 2/3 of graph provided
2. **A**xes : correct with units (x-axis: protein concentration/% and y-axis: absorbance/ arbitrary units)
   Penalise for: No intervals markings, No 0.0 (origin)
3. **P**ts : all 5 points correctly plotted using X
4. **L**ine : smooth curve/line passes all pts
   Penalise for extrapolation

(iv) Describe how the student could modify the previous experiment to determine the concentration of protein in sample **R**.
*Do **not** repeat any detail of how the standard protein solutions are prepared, the biuret test and how the colorimeter would be used.*
Your method should be set out in a logical way and be detailed enough to let another person follow it. [5]

1. Using a 10cm^3 syringe, add 5 cm^3 of distilled water to 5 cm^3 of sample R; This is a 2x dilution. (compulsory point) A other dilutions e.g. 1 in 2, 1 in 4, etc (must match with pt 5)
2. Conduct biuret test on the diluted sample R from step 1;
3. Transfer the diluted sample R after the biuret's test into a cuvette, measure the absorbance of the solution using the colorimeter;
4. Use the absorbance of the diluted sample R, read off the calibration/standard curve to find the concentration of protein;
5. Multiply the concentration of protein by the dilution factor of 2 (must match with point 1) to obtain the protein concentration in undiluted sample R;

[Total: 25]

2(a) Compare the morphology of **W1** and **W2** in the space below. [3]

Differences

	Point of comparison	**W1**	**W2**
1	number of seed	There are less seeds.	There are more seeds.
2	hair on skin	There are less hair on skin/shorter hair on skin. R no hair A smoother	There are more hair on skin/ longer hair on skin.
3	colour of flesh	The flesh is yellow.	The flesh is green.
4	size of middle core	The middle core is smaller.	The middle core is larger.
5	shape of core/middle	The shape is circular.	The shape is oval.
AVP	colour of skin	The skin yellowish brown in colour.	The skin darker brown in colour.
AVP	shape of cross section of fruit	The shape is circular.	The shape is oval.

Max 2

Similarities:	
6	Both have black seeds/flesh/skin;
7	Both have a pale middle core.
8	Both have hair.

9	Both have brown skin.
10	Both have numerous seeds arrange around the centre core/middle of the fruit

Max 2

(b) **(i)** Cut a 5mm thick transverse slice of specimen **W1** on the cutting board. The slice should contain a full complement of seeds and should not be taken from the extreme ends of the fruit.

Blot dry the cut surface with a paper towel. Cover one cut surface of the slice with bromocresol green, **G**. Leave it to stain for one minute. **G** stains proteins blue.

In the space provided, draw a large, detailed diagram of the cut surface of the slice as shown by the shaded area in Fig. 2.1.

You are required to use a sharp pencil for drawings.

Your drawing should show details of the arrangement of different regions and their correct shapes and proportions. Indicate where the highest concentration of protein is present. [4]

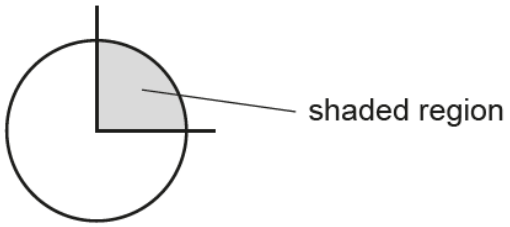
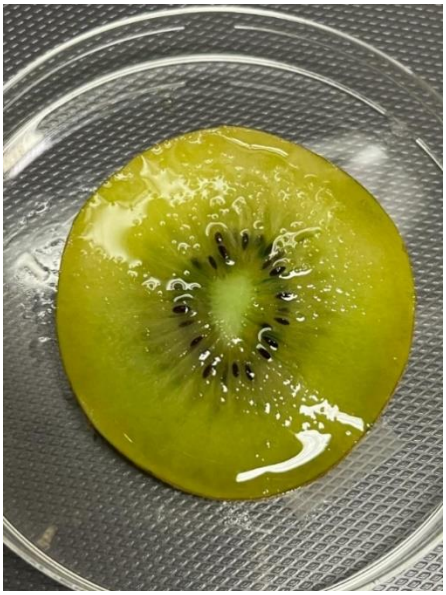
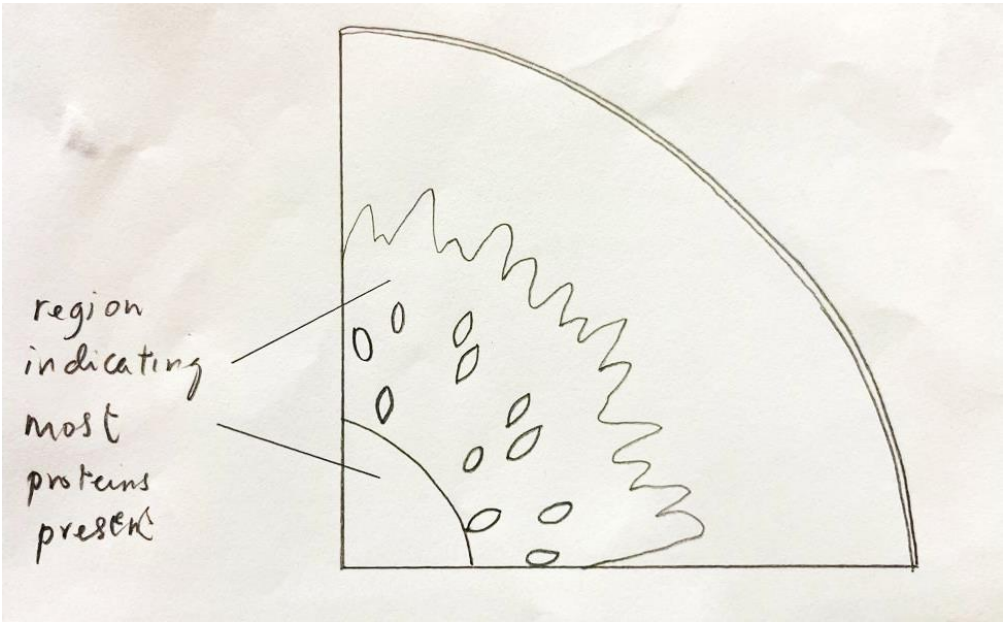


Fig. 2.1

1. Scale : detailed diagram of appropriate size (>3/4 space given) + no shading;
2. Proportion: Correct proportion of layers including correct proportions of core compared to the diameter of the fruit, and skin, size of the seeds;
3. Accuracy : skin, seeds, locules + correct section drawn;
   R if draw LS
4. Annotations : blue green core/locules indicating highest concentrations of proteins present;





(ii) You are required to estimate the total number of seeds that were present in the fruit from which **W1** was taken.

- Cut the 5mm slice of **W1** into equal quarters.
- Place one of these quarters on the cutting board and, using the forceps, squeeze out all of its seeds.

Count the number of seeds present: ……6 - 26……....

Repeat this for another quarter.

Count the number of seeds present: ……6 - 26…… [1]

(iii) Plan how you would estimate the number of seeds in the whole fruit, taking into consideration and explaining any allowance that you may think is necessary. [4]

1. Find the mean of the number of seeds by adding the 2 samples and divide by 2;
2. Estimate the number of seeds in a slice by multiplying the mean by 4;

3a. Show how they derive 10 slices

3. Fig 2.2 shows that 10 slices can be obtained from the whole fruit, multiplying the number of seeds per slice by 10 would give the total number of seeds in a fruit;
4. As the region containing seeds taper at the ends, there should be less seeds at the ends and hence should be taken into consideration. Hence a good estimate would be the total number in a slice multiplied by 7/8/9 instead;

(iv) Using the method you described in **(b)(iii)** and data collected from **(b)(ii)**, estimate the number of seeds found in **W1**. Show your working clearly. [2]

1. Total number of seeds in 2 quarters 12 + 15 = 27
   Mean number of seeds in 1 quarter 27/2 = 13.5;

2. Total number of seeds in 1 slice = 13.5 x 4 = 54;
   Total number in 8 slices = 54 x 8 = 432 seeds;

(c) **(i)** Complete the calculation to find the value of *t* for the concentration of sucrose in the fruits. [2]

$$t = \frac{|23-15|}{\sqrt{\frac{4^2}{30}+\frac{3^2}{30}}}$$

= 8/0.9 [1]

t = 8.9 [1]

(ii) State the null hypothesis for **(c)(i)**. [1]

There is no significant difference (R: insignificant) in the mean sucrose concentration (R: sweetness) in the samples of W1 and W2 variety of fruit; Any difference is due to random chance.

(iii) State the conclusion that could be gathered from the *t*-test. [3]

1. At **58** degrees of freedom (v) and t = 8.9, p < 0.001; A t > 2.02 or 2.00
2. At 5% level of significance, ***p < 0.05**** so we reject the null hypothesis, H_0;
3. and conclude that mean of concentration of sucrose in W1 is ***significantly different**** than the mean concentration of sucrose in W2 and the difference is not due to chance alone;

[Total: 20]

3(a) You are given slides **S1** and **S2** in a petri dish

(i) Using the slide **S2**, determine the diameter of the field of view at high power magnification. [1]

5 stage micrometer division x 100 µM
= 500 µM

1. Correct working and correct final ans expressed nearest µM
   R mm

(ii) Calculate the volume of blood under the field of view at high power magnification. [2]

$V = \pi r^2 h$
$= \pi \times 250^2 \times 100$
$= 19\ 634\ 954\ \mu M^3$

1. Correct working using ans from part **(i)**
2. Correct final ans expressed to nearest μM^3

(b) **(i)** Place slide **S2** under higher power magnification.
Use slide **S2** to determine the dimensions of each eye piece graticule unit.
Show your working. [1]

40 eye piece graticule unit = 100µM
1 eye piece graticule unit = 100/40 µM = 2.5 µM

1. Correct working and final ans

(ii) Place slide **S1** under high power magnification.
Locate a lymphocyte and use the markings on the eye piece to measure the diameter of the:

a) nucleus (N); and [1]
4 eyepiece graticule unit x 2.5 µM = 10 µM

1. Correct working and final ans. (ecf allowed for 2.5 µM)

b) cell (C). [1]
5 eyepiece graticule unit x 2.5 µM = 12.5 µM

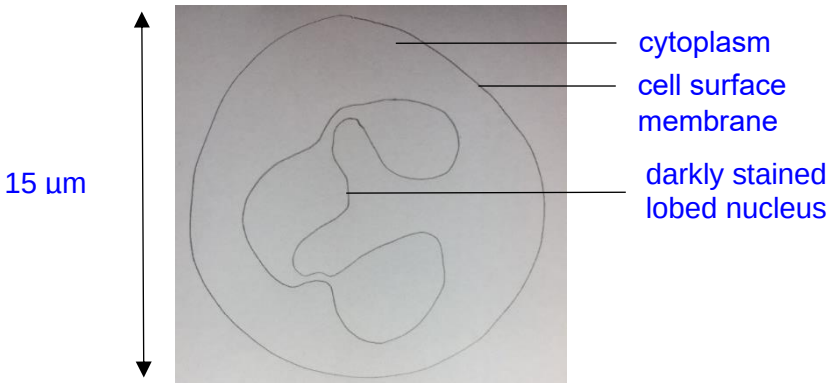
1. Correct working and final ans. (ecf allowed for 2.5 µM)

(iii) Calculate the N:C ratio of the lymphocyte, and express your answer in whole numbers. [1]

10 : 12.5 = 20: 25 = 4 : 5

1. Correct working and final ans (in whole numbers)

(c) Place slide **S1** under high power magnification.
In the space below, make a labeled drawing of a neutrophil. [3]



High power drawing (X400) of a human white blood cell (neutrophil)

1. **S**ize (use 2/3 of the space provided)
2. **A**ccuracy → Drawing shows nucleus with at least 2-5 lobes
3. **P**roportion →Size of nucleus proportional to cell
4. **L**abels → lobed nucleus, cytoplasm, cell surface membrane

[Total: 10]