BIOC2004

Biomolecular Structure and Function

PRACTICAL REPORTS 2015/2016

Student Name: Feng Geng

PRACTICAL REPORT FOR
BIOC2004: BIOMOLECULAR STRUCTURE & FUNCTION EXPERIMENT 1

NAME: _____

1. In this crude protein purification protocol, what is the role of ammonium sulphate in the first step? (1 mark)

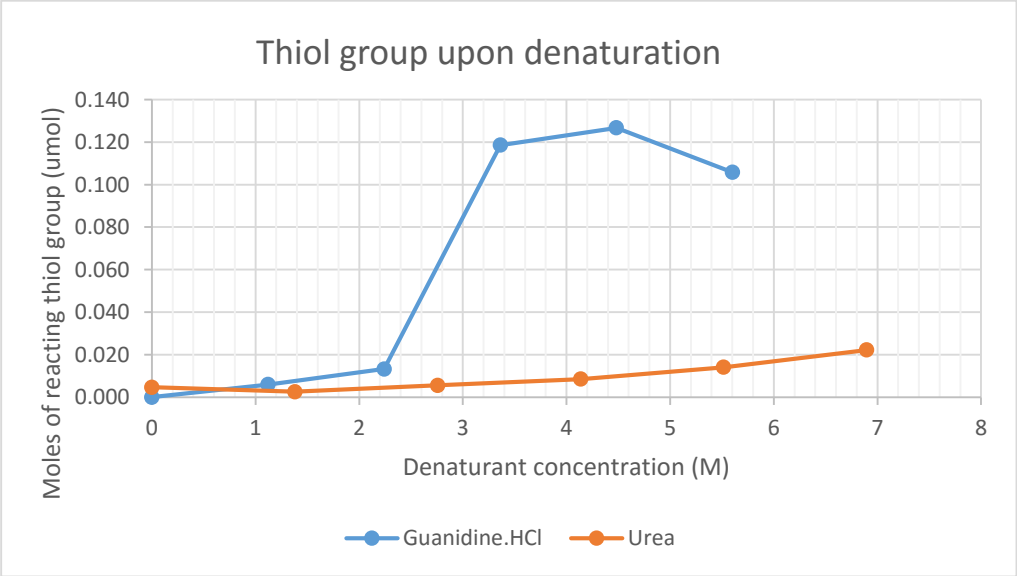
   Addition of 100% ammonium sulphate at volume equal to egg white suspension increase the ionic strength of suspension, thus increase the solubility of proteins by surrounding them with ions of opposite net charge. This ensure the solubilisation of ovalbumin.

2. In the second step of purification, why is the ovalbumin precipitate formed at pH 4.6? (1 mark)

   In literature ovalbumin has a pI of 4.6, at which pH the protein will carry a net charge of zero, thus unable to form a stable solvent shell upon solvation and dispense energy via electrostatic interaction with the counter salt ion. In this way, the solubility of the protein decreases reflecting the decreased of free energy change of solvation.

3. Calculate the moles of thiol group for each sample. The molar extinction coefficient of the nitromercaptobenzoate ion is 1.36 x 10^4 $M^{-1}cm^{-1}$. Plot the number of moles of reacting thiol group against the concentration of the denaturant.
   (3 marks)

Denaturant	**Guanidine-HCl**						**Urea**						
Test Tube No	1	2	3	4	5	6	7	8	9	10	11	12	13
Amount of thiol group(umol)	0.000	0.006	0.013	0.119	0.127	0.106	0.005	0.003	0.006	0.009	0.014	0.022	0.000
Denaturant Conc.	0.00	1.12	2.24	3.36	4.48	5.60	0.00	1.38	2.76	4.14	5.52	6.90	0.00



4. Estimate the total titre of thiol groups from your graph and calculate the number of free cysteine residues per molecule of ovalbumin. The molecular weight of ovalbumin is 45,000. (2 marks)

Ovalbumin stock concentration= 5mg/mL
Ovalbumin working amount=5mg.mL^{-1}*0.4mL/45kg.mol^{-1}=0.0444umol

Reacting thiol group: (0.119+0.127)/2=0.124 (Denaturation by urea is low, either ovalbumin is not sensitive to urea or the urea used is oxidised over time, thus the guanidine result is used and the last point is deemed abnormal possibly due to unexpected interaction at low water content and excluded)
Thiol/cysteine per molecule=0.124/0.0444=2.8 around 3.

To account for the approximation of A_{280}-protein relation.
Literature extinction coefficient for ovalbumin is 30,590 cm^{-1}M^{-1}, thus A_{280} of 1 indicates 1/(1cm*30.59cm^{-1}.mM^{-1})*45g.mmol^{-1}=1.47g/L=1.47mg/mL
Reactive cysteine residues after correction=0.124/0.0444/1.47=1.90 around 2.

However, such correction is not completely legitimate since other protein may express high A_{280}.

5. What does your data tell you about the number of interior and surface cysteine residues? (1 mark)

An absorbance of 0.000 at non-denaturing environment indicates that all three of the cysteine residues are interior and not surface.
Acknowledged from Uniport#P01012, ovalbumin contains 6 cysteine residues. Inspection of its structure reveal 4 thiol group and 1 disulphide bond. With an experimental thiol number of 3, it is likely that another cysteine is involved in disulphide bond thus undetectable.
Overall, we confirm all 3 reactive cysteines are interior but no information about the disulphide cysteines.

6. When you determined protein concentration of your sample you made dilution of 1:10. If you have diluted your sample 1:22 and you obtained A_{280}=0.52 what would have been the molar concentration of your sample? (2 marks)

A_{280}=0.52 at 1:22 dilution indicates c_m=0.52*22=11.44mg/mL=11.44g/L
c_v=11.44g.L^{-1}/45kg.mol^{-1}=0.254mM

PRACTICAL REPORT FOR

BIOC2004: BIOMOLECULAR STRUCTURE & FUNCTION EXPERIMENT 3

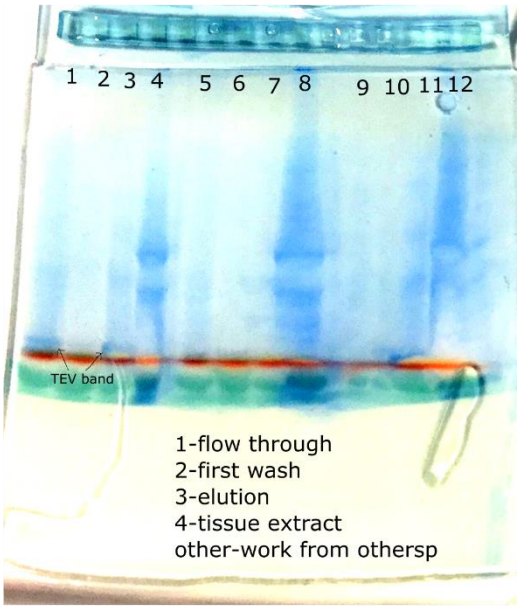
NAME: Feng Geng

1. Sketch the results, or attach the photo of the results of your SDS-PAGE gel after the staining. Describe your results with respect to the content of the four samples that you have loaded on the gel.

   (4 marks)

TEV protease (TEVp) has a molecular weight of 23kDa.

To identify the cyan stain beyond the red frontline and the redline itself, I took an online search, and confirm the cyan stain is bromophenol blue that runs around protein of 3-5kDa. The chemical nature of the red frontline remains untackled but its functional is most possibly to indicate the resolution range of the gel. For Amersham ECL 10% acrylamide gel it should indicate a molecular weight of 20kDa.



1.The flow-through contain more protein with a bigger molecular weight in its upper half, as compared to the first wash and the elution, and shows a similar composition to the tissue extract. Interestingly, our tissue extract seems to contain no TEV at 23kDa position but defective color development can't be ruled out unless Western Blot against TEV is performed. The flow-through also contains unexpected 23kDa protein, possibly unbound TEV due to incomplete binding or his-tagging.
2.The first wash (20mM imidazole) contains little protein, possibly due to at whatever molecular weight, thus the wash appears to be ineffective. Little TEV has been washed off.
3.The elution contains mostly TEV, but also impure proteins of larger MW.
4. Mammalian tissue extract shows 2 unknown abundant bands with unknown size.

Overall, our operation of IMAC did not seem to purify the His-tagged TEVp from the impurities by specific bonding between imidazole ring and nickel ion. However, the recombinant *E. coli* cell extract do shows a distinct profile from mammalian tissue extract, in that TEVp is greatly enriched.

2. If you were to start from the same stock solutions as those that you used to prepare 'wash' and 'elution' buffer (Table 1 in the experiment description), how would you prepare 30 ml of a buffer with the following composition: 50 mM Tris-HCL, 125 mM NaCl, 100 mM Imidazole, 15 % glycerol, 2 mM BME? Show your calculations and present your results in tabular fashion.

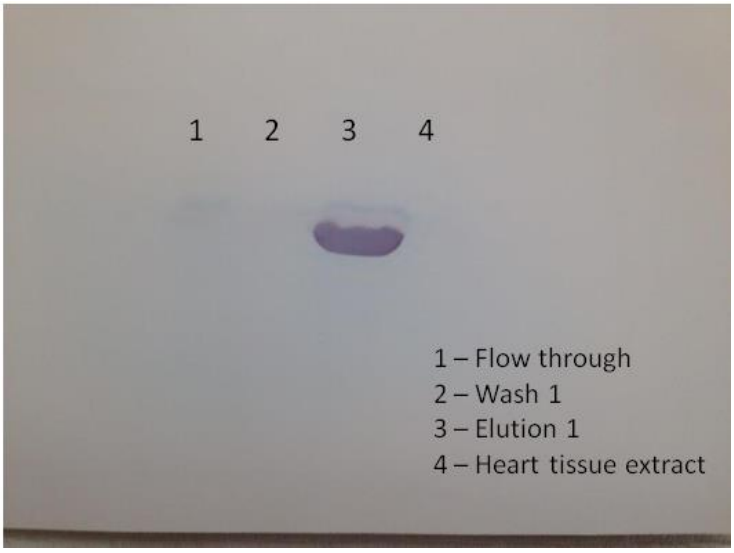
   (3 marks)

Buffer recipe:

Stock solution	Conc. for buffer B	Volume for 30mL of Buffer B
1M Tris-HCl	**50mM**	**50mM*30mL/1000mM=1.5mL**
2.5M NaCl	**125mM**	**125mM*30mL/2500mM=1.5mL**
1M imidazole	**100mM**	**100mM*30mL/1000mM=3.0mL**
50% glycerol (?/v)	**15% (?/v)**	**15%*30mL/50%=9.0mL**
1M BME	**2mM**	**2mM*30mL/1000mM=0.06mL**
H2O	**Not specified**	**30-1.5-1.5-3.0-9.0-0.06=14.94mL**

3. Sketch and explain the results of the Western Blot Analysis following the development of the stain.

(3 marks)



On the Western blot, the majority of coloring develops at the elution lane, which indicates activity of antibody-conjugated horseradish peroxide and thus antibody epitome, his tag. As compared to the comassie blue staining, the coloring here is dense and more contrasted, thus a better evidence of presence of His-tagged TEVp.

PRACTICAL REPORT FOR

BIOC2004: BIOMOLECULAR STRUCTURE & FUNCTION EXPERIMENT 4

NAME: Feng Geng

CALCULATIONS

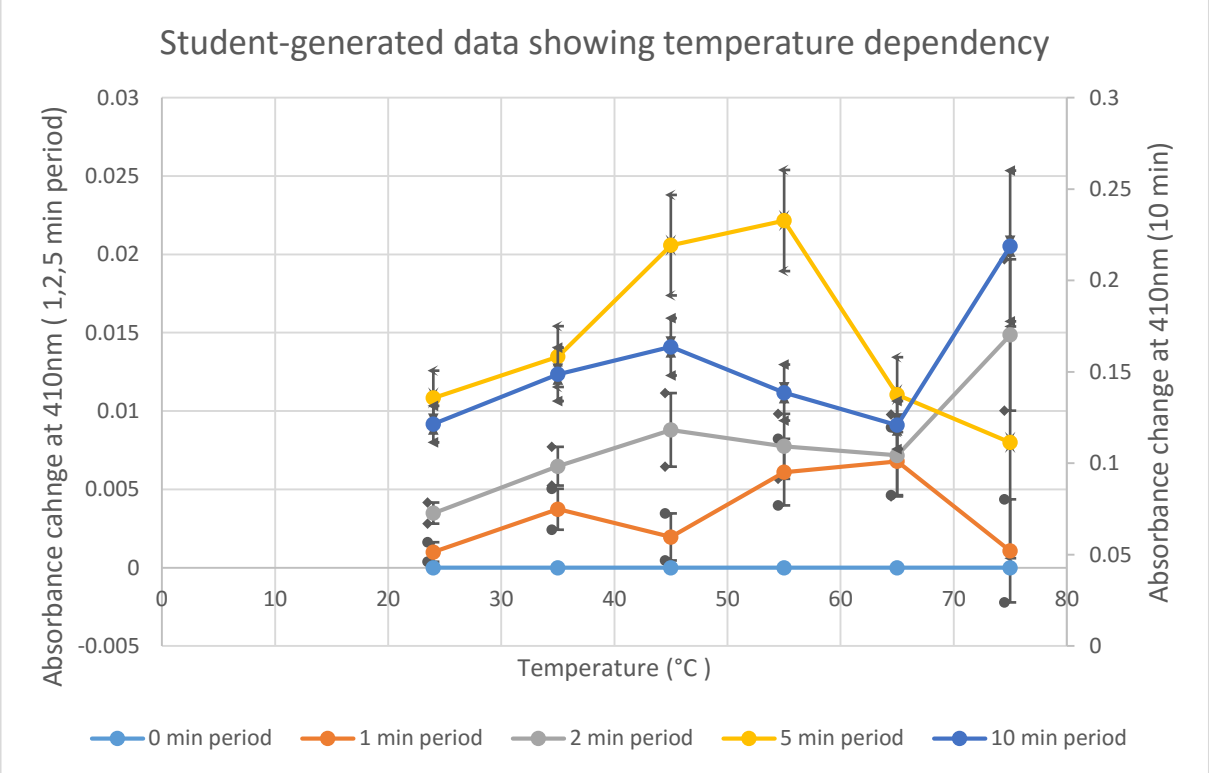
A. Temperature Optimum

1. Average the duplicate reaction and control values, subtract the averaged control values from the averaged reaction values (in Excel).

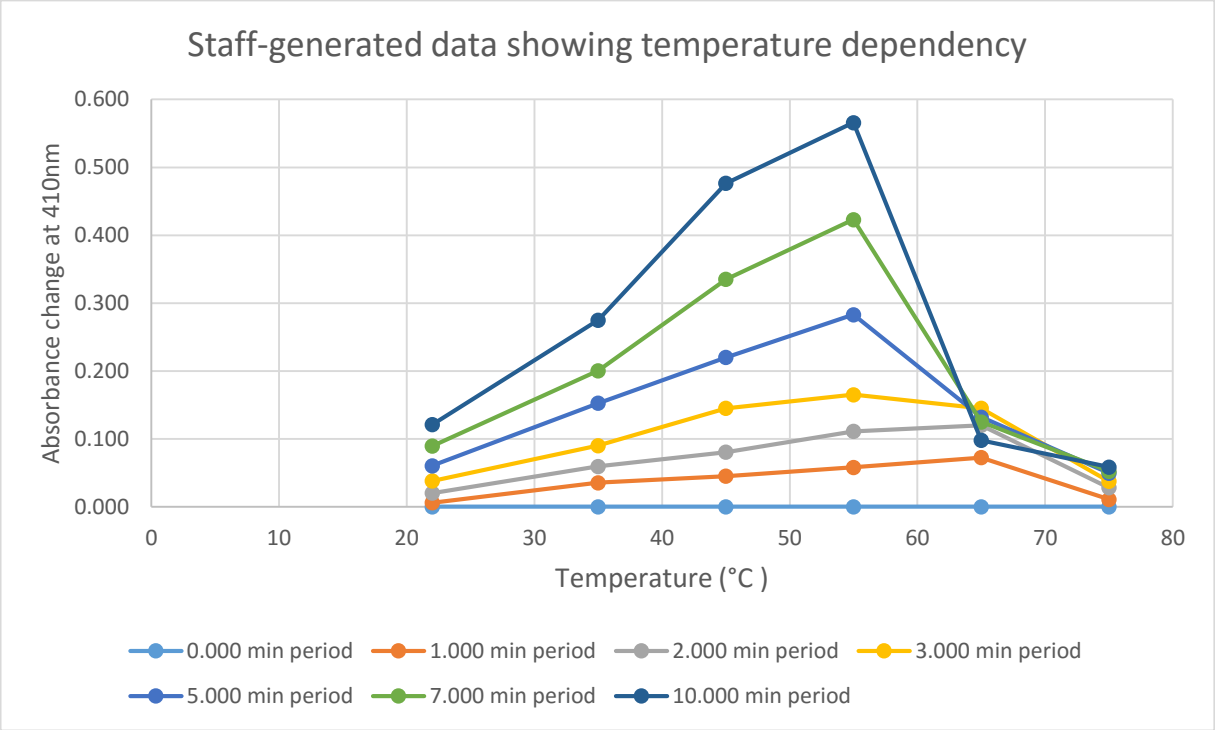
   I took a slightly modified approach. The reasoning is that control absorbance differs between data, and it is really the deviation of reaction absorbance from the control absorbance that we are measuring. Thus I generated a reaction-control subtraction for each set of data and averaged them, and calculated the standard error (SEM) associated with this average..
   In addition, histograms and scatter diagram showing the distribution of the result of subtraction are plotted for several temp-period combination to rule out outliers.

2. Generate a "temperature optimum curve" for 1 min and 10 min incubation periods by plotting the absorbance change versus the reaction temperature for these incubation times (1 and 10 minutes).

   Temperature dependency curves were plotted for both classs generated data and staff generated data



SEM is plotted as error bar



Error bar is too small to show on this graph

3. From the curves, estimate the apparent T_{opt} values for 1 min and 10 min assay time.

From the student-generated data, the errors at 75 degrees are too big .Referring to time-dependency (or reaction progress) curve at 75C, the enzyme activity variation is completely mess, possibly due to a small sample size at 75C (n=7). Thus we exclude the 75C points from subsequent analysis.

Assay Time (min)	Estimated Topt (℃, student data)	Estimated Topt (℃, staff data)
1	65	65
2	45	65-55
3		55-65
5	55	55
7		55
10	45	55

In both data, the optimum reaction time is decreased as assay time increases. Possibly due to denaturation built up over longer assay time.

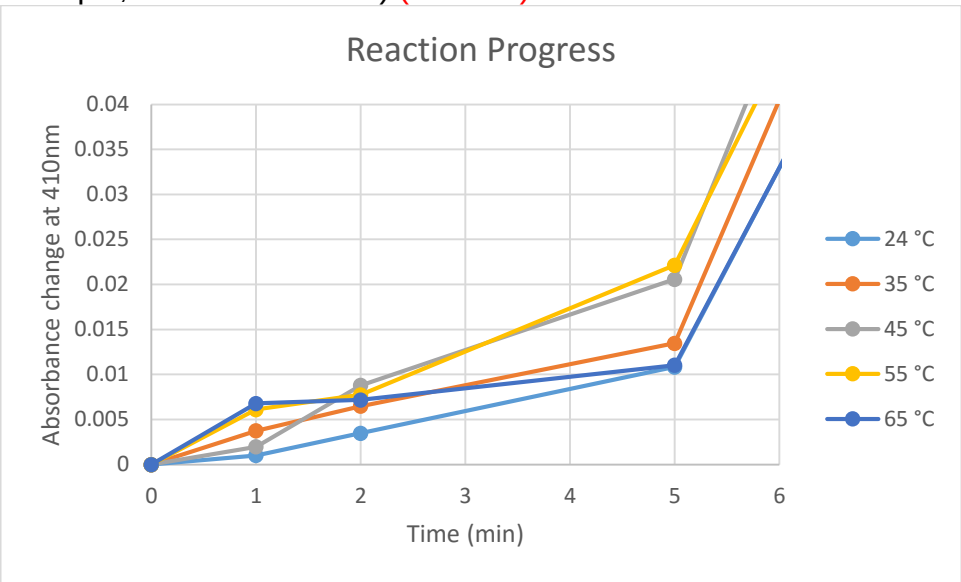
(2 marks, 1 mark for each incubation time.)

PRACTICAL REPORT FOR
BIOC2004: BIOMOLECULAR STRUCTURE & FUNCTION EXPERIMENT 4

NAME: Feng Geng

B. Arrhenius Plot and Activation Energy

1. For each temperature, plot a reaction progress curve (absorbance change versus time) and determine the initial rate (ΔA_{410}/minute) of the reaction. (This can be done, for example, in Microsoft Excel) (2 marks)



Points for 10 minute are too high and irrelevant for calculating initial rates thus not shown here.

Depending whether the curve is roughly linear for the first 4 points or curved, slope across the first 4 points or the first point is calculated as initial rate.

Temperature(°C)	Initial Rate as A410 change (min-1)	Associated error
24	0.001000	0.00062
35	0.003238	0.000618
45	0.004117	0.000641
55	0.006100	0.002128
65	0.006792	0.002162
75	n/a	n/a

2. Convert these initial rate values into μmoles product produced per minute/ml enzyme using the Beer-Lambert Law. The dissociated o-nitrophenolate ion (ONP) has an extinction coefficient at 410nm (pH 10) of 2.13 x 10^4 M^{-1} cm^{-1}. (2 marks)

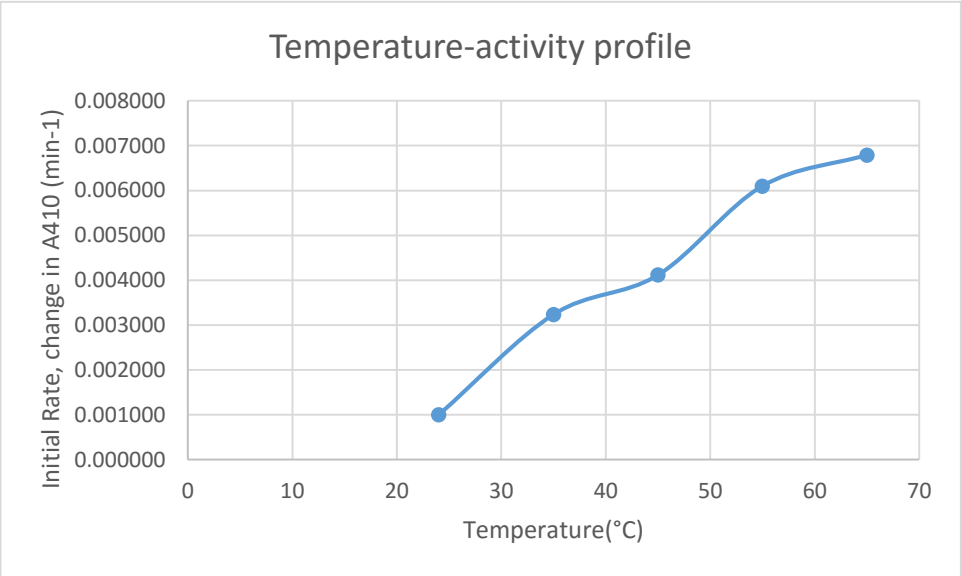
o-nitrophenyl-galactoside ——β-galactosidase——> o-nitrophenol + galactose

Calculate ONP production from absorbance. Vonp=Vabs/(2.13E4M^{-1}.cm^{-1}*1cm)*10^6 uM.M^{-1}

Temperature(°C)	Initial Rate as A410 change (min-1)	Associated error	Initial rate as ONC production (uM.min-1)	Associated error
24	0.001000	0.00062	0.046948	0.029128
35	0.003238	0.000618	0.151995	0.028994
45	0.004117	0.000641	0.193271	0.030111
55	0.006100	0.002128	0.286385	0.099887

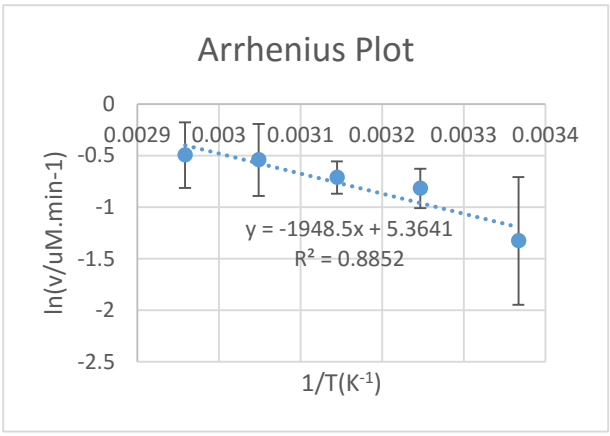
65	0.006792	0.002162	0.318858	0.10148
75	n/a	n/a	n/a	n/a

3. Plot this data as a temperature: activity profile (reaction rate versus T °C) and also in the Arrhenius form (ln v versus 1/T (Kelvin)). From the latter, determine the slope of the linear portion of the curve and calculate the Activation Energy of the reaction.
(slope = - E_a/R where R is the universal gas constant) (4 marks)



Arrhenius equation: $v = Ae^{-Ea/RT}$ → $\ln(v) = -E_a/R * 1/T + \ln(A)$

1/T(K-1)	ln(v/uM.min-1)	Associatied error
0.003367	1.380	0.620
0.003247	1.544	0.191
0.003145	1.653	0.156
0.003049	1.740	0.349
0.002959	1.813	0.318
n/a	n/a	n/a



$-E_a/R = -1948.5K$

Estimated activation energy: $E_a = 1948.5K * R = 1948.5K * 8.314 J.K^{-1}.mol^{-1} = 16.2 kJ.mol^{-1}$