

# [Lab biochemistry](https://assignbuster.com/lab-biochemistry/)

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Determination of the Kinetic constants, Km, Kcat and Vmax of yeast alcohol dehydrogenase (ADH) with three alcohol substrates (ethanol, propan ol and butan-1-ol).
Introduction
The objective of this experiment is to find the Km, kcat and Vmax values of yeast alcohol dehydrogenase. We used ethanol, propan-1-ol and butan-1-ol to carry out this experiment.
With a molecular weight of 150. 000, yeast dehydrogenase is classified as an NAD linked enzyme. There are four subunits within NAD which consist of Zn2+ ions, the purpose of which is to aid in transfereing a hydride ion from the alcohol to NAD+ .
The overall reaction equation is :
RCH2OH + NAD+  RCHO + NADH + H+
Method
As written in the lab protocol sheet
Results
Table 1
Ethanol
Time
Absorbance values for Substrate concentrations
Seconds
Minutes
0. 080
0. 040
0. 020
0. 010
0. 005
0. 000
0. 000
0. 000
0. 000
0. 000
0. 000
0. 000
10. 000
0. 167
0. 267
0. 120
0. 081
0. 073
0. 035
20. 000
0. 333
0. 369
0. 165
0. 126
0. 133
0. 061
30. 000
0. 500
0. 439
0. 264
0. 169
0. 178
0. 098
40. 000
0. 667
0. 550
0. 318
0. 217
0. 216
0. 127
50. 000
0. 833
0. 600
0. 384
0. 258
0. 252
0. 155
60. 000
1. 000
0. 725
0. 439
0. 308
0. 287
0. 178
Fig. 1
Fig 1. 1
Fig 1. 2
Fig 1. 3
Fig 1. 4
Table 2
Propan-1-ol
Time
Absorbance values for Substrate concentrations
Seconds
Minutes
0. 08
0. 04
0. 02
0. 01
0. 005
0
0. 000
0. 000
0. 000
0. 000
0. 000
0. 000
10
0. 167
0. 018
0. 041
0. 036
0. 033
0. 038
20
0. 333
0. 053
0. 061
0. 044
0. 039
0. 040
30
0. 500
0. 085
0. 085
0. 062
0. 052
0. 044
40
0. 667
0. 115
0. 111
0. 079
0. 035
0. 052
50
0. 833
0. 143
0. 137
0. 097
0. 076
0. 058
60
1. 000
0. 168
0. 159
0. 112
0. 087
0. 063
70
1. 167
0. 196
0. 184
0. 128
0. 099
0. 071
Fig 1. 5
Fig 1. 6
Fig 1. 7
Fig 1. 8
Fig 1. 9
Table of absorbance values at 340nm using different substrate concentration for Butan-1-ol and the graphs to show this data.
Table 3
Butan-1-ol
Time
Absorbance values for Substrate concentrations
Seconds
Minutes
0. 08
0. 04
0. 02
0. 01
0. 005
0
0. 000
0. 000
0. 000
0. 000
0. 000
0. 000
10
0. 167
0. 043
0. 03
0. 029
0. 027
0. 024
20
0. 333
0. 078
0. 05
0. 048
0. 038
0. 032
30
0. 500
0. 119
0. 082
0. 074
0. 048
0. 036
40
0. 667
0. 161
0. 108
0. 083
0. 057
0. 041
50
0. 833
0. 193
0. 134
0. 098
0. 066
0. 047
60
1. 000
0. 23
0. 16
0. 116
0. 076
0. 051
70
1. 167
0. 264
0. 183
0. 13
0. 083
0. 054
Fig 2. 0
Fig 2. 1
Fig 2. 2
Fig 2. 3
Fig 2. 4
The graphs depicted above have been plotted for for 0. 080, 0. 040, 0. 020, 0. 010 and 0. 005M substrate concentrations. All of these graphs have absorbance (340nm) on the y-axis and Time (min) on the x-axis. The graphs were drawn individually however due to a great deal of overlap in the data, it was better to combine it into a single graph for each alcohol as it made the interpretation of the date considerably easier.
Michaelis Menten curves
Velocity calculation:
1) The linear line equations of the absorbance vs. Time plot. provided the gradient.
2) The gradient was divided by the Enzyme co-efficient which in this case was
6220 l/mol/cm
3) The above answer was multiplied by the volume of the cuvette which was 0. 003 (3ml÷1000)
4) The above answer was multiplied with 106 to convert the volume into micro mol.
5) The answer from step 4 in µmol was divided by 0. 1 which was the amount of enzyme used.
Example for Ethanol:
1) 0. 851 ÷ 6220 = 1. 37 x
2) 1. 37 x10-4 × 0. 003 = 4. 10 x10-7
3) 4. 10 x10-7 × 106 = 0. 41
4) 0. 41 ÷ 0. 1 = 4. 1µmol min-1 ml-1
The same method was used for the velocity calculation of others. The results have been tabulated above.
Ethanol:
Table 4
Ethanol
Gradient
Enzyme co-efficient (l/mol/cm)
Cuvette (L)
Convert to micromoles (µmol)
Enzyme used (µmol min-1 ml
0. 851
6220
0. 003
1000000
0. 1
0. 502
6220
0. 003
1000000
0. 1
0. 331
6220
0. 003
1000000
0. 1
0. 356
6220
0. 003
1000000
0. 1
0. 192
6220
0. 003
1000000
0. 1
Table 5
Substrate Concentration (M)
Velocity (V)
0. 080
4. 105
0. 040
2. 421
0. 020
1. 596
0. 010
1. 717
0. 005
0. 926
Propan-1-ol:
Table 6
Propan-1-ol
Gradient
Enzyme co-efficient (l/mol/cm )
Cuvette (L)
Convert to micromoles
enzyme used
0. 174
6220
0. 003
1000000
0. 1
0. 165
6220
0. 003
1000000
0. 1
0. 116
6220
0. 003
1000000
0. 1
0. 097
6220
0. 003
1000000
0. 1
0. 080
6220
0. 003
1000000
0. 1
Table 7
Substrate Concentration (M)
Velocity (V)
0. 080
0. 839
0. 040
0. 796
0. 020
0. 559
0. 010
0. 468
0. 005
0. 386
Butan-1-ol:
Table 8
Butan-1-ol
Gradient
Enzyme co-efficient (l/mol/cm )
Cuvette (L)
Convert to micromoles
enzyme used
0. 235
6220
0. 003
1000000
0. 1
0. 159
6220
0. 003
1000000
0. 1
0. 144
6220
0. 003
1000000
0. 1
0. 093
6220
0. 003
1000000
0. 1
0. 069
6220
0. 003
1000000
0. 1
Table 9
Substrate Concentration (M)
Velocity (V)
0. 080
1. 133
0. 040
0. 767
0. 020
0. 695
0. 010
0. 449
0. 005
0. 333
The Michaelis Menten graphs are attached, fig 2. 5, 2. 6 and 2. 7.
Lineweaver Burk plots
. Since the velocity had already been calculated from the gradient values of the previous absorbance plots, they could be used in plotting the above mentioned plots.
The Graphs of were plotted by dividing the values of velocity and substrate concentrations both by 1 Now that all the info was here, it was possible to plot the Lineweaver Burk plots.
Ethanol:
Table 10
Ethanol
Substrate Concentration (M)
Velocity (V)
1/S
1/V
0. 080
4. 105
12. 500
0. 244
0. 040
2. 421
25. 000
0. 413
0. 020
1. 596
50. 000
0. 627
0. 010
1. 717
100. 000
0. 582
0. 005
0. 926
200. 000
1. 080
Fig 2. 8
Propan-1-ol:
Table 11
Propan-1-ol
Substrate Concentration (M)
Velocity (V)
1/[S]
1/V
0. 080
0. 008
12. 500
125. 000
0. 040
0. 008
25. 000
125. 000
0. 020
0. 006
50. 000
166. 667
0. 010
0. 005
100. 000
200. 000
0. 005
0. 004
200. 000
250. 000
Fig 2. 9
Butan-1-ol:
Table12
Butan-1-ol
Substrate Concentration (M)
Velocity (V)
1/S
1/V
0. 080
1. 133
12. 500
0. 883
0. 040
0. 767
25. 000
1. 304
0. 020
0. 695
50. 000
1. 439
0. 010
0. 449
100. 000
2. 227
0. 005
0. 333
200. 000
3. 003
Fig 3. 0
Eadie Hofstee
The final set of graphs that needed to be plotted were the Eadie Hofstee plots. These are plots of V/[S] against velocity. All graphs for each alcohol had a negative gradient because of the inverse relationship between the V/S and velocity.
Ethanol:
Table 13
Ethanol
Substrate Concentration (M)
Velocity (V)
V/S
0. 080
4. 105
51. 313
0. 040
2. 421
60. 525
0. 020
1. 596
79. 800
0. 010
1. 717
171. 700
0. 005
0. 926
185. 200
Fig 3. 1
Propan-1-ol:
Table 14
Propan-1-ol
Substrate Concentration (M)
Velocity (V)
V/S
0. 080
0. 839
10. 488
0. 040
0. 796
19. 900
0. 020
0. 559
27. 950
0. 010
0. 468
46. 800
0. 005
0. 386
77. 200
Fig 3. 2
Butan-1-ol:
Table 15
Butan-1-ol
Substrate Concentration (M)
Velocity (V)
V/S
0. 080
1. 133
14. 163
0. 040
0. 767
19. 175
0. 020
0. 695
34. 750
0. 010
0. 449
44. 900
0. 005
0. 333
66. 600
Fig 3. 3
Kcat Calculations
1) Step one is: 0. 1÷1000= 0. 001g/ml
2) The molecular weight of the enzyme was 150, 000g/mol
3) The 0. 001 g/ml is divided by 150, 0000. 001÷150000= 6. 67×mol/ml
4) Convert mol to µmol by 6. 67×mol/ml × = 6. 67µmol/ml
5) Kcat= Vmax÷[E]t
Kcat = 3. 48 ÷ 6. 67 = 5217min-1
1/60 5217 = 86. 95 s-1
Summary table
Vmax, Km and Kcat values for each substrate and each analytical method used have been summarized in the tables below.
Table 16

Ethanol
Propan-1-ol
Butan-1-ol
Velocity
Vmax (V)
4. 2
0. 85
1. 145
Km (M)
0. 031
0. 07
0. 015
Kcat

Lineweaver Burk
Vmax (V)
3. 48
0. 82
1. 076
Km (M)
0. 01
0. 01
0. 011
Kcat
86. 95
20. 48
26. 88
Eadie Hofstee
Vmax (V)
4. 96
0. 913
1. 25
Km (M)
0. 026
0. 01
0. 016
Kcat
123. 93
22. 81
31. 23
Lineweaver Burk
For the Lineweaver Burk method the Vmax and Km values were worked out using the following method:
Example for Propan-1-ol:
Equation of the linear line: y= 0. 007x + 1. 216
Step 1
The value 1. 216 has been taken from the linear line equation, which in this case is C in the equation.
Step 2
Km = Slope Vmax
The value for the slop was taken from the linear line equation which was the gradient. The Vmax in the above equation has already been calculated in step 1.
Eadie Hofstee
Example for Butan-1-ol:
Linear line equation: y=-62. 20x + 77. 92
Y= 0
To work out the Vmax we want “ x” to be the subject therefore:
To work out the Km the following equation was used:
The -62. 20 value was taken from the gradient of the linear line equation. This value of gradient was then by -1 because this value was part of the Eadie Hofstee equation.
Discussion
Only the first 4 plots were used in making the Absorbance vs. Time because using all the absorbance values would give a curvy orientation to the graph. The reason for this is that the enzyme sites were occupied by the substrates because of which there were no more enzymes sites where the substrate could bind. This is an indication of the Vmax meaning the all the catalytic sites have been occupied and the enzyme has become saturated with the substrate.