Assay of Alcohol Dehydrogenase (ADH)

Laxman Manjhi 2019BB10034

Ratnesh Kumar Sharma 2019BB10047

BACKGROUND

Alcohol dehydrogenase (ADH) is a family of enzymes that oxidize primary or secondary alcohols to aldehydes or ketones.

ADH uses NAD+ or NADP+ as coenzyme which is reduced during reaction.

R-CH2-OH can be converted to R-CHO + NADH + H+ in a reaction catalyzed by ADH.

NADH has strong UV absorbance at 340 nm, while oxidized NAD+ has little to no absorbance at this wavelength.

Absorbance of solution at 340 nm increases as NADH is formed during reaction.

Reaction can be monitored by starting with a mixture of ethanol, NAD+, and enzyme in buffer and measuring absorbance until equilibrium is established.

Method

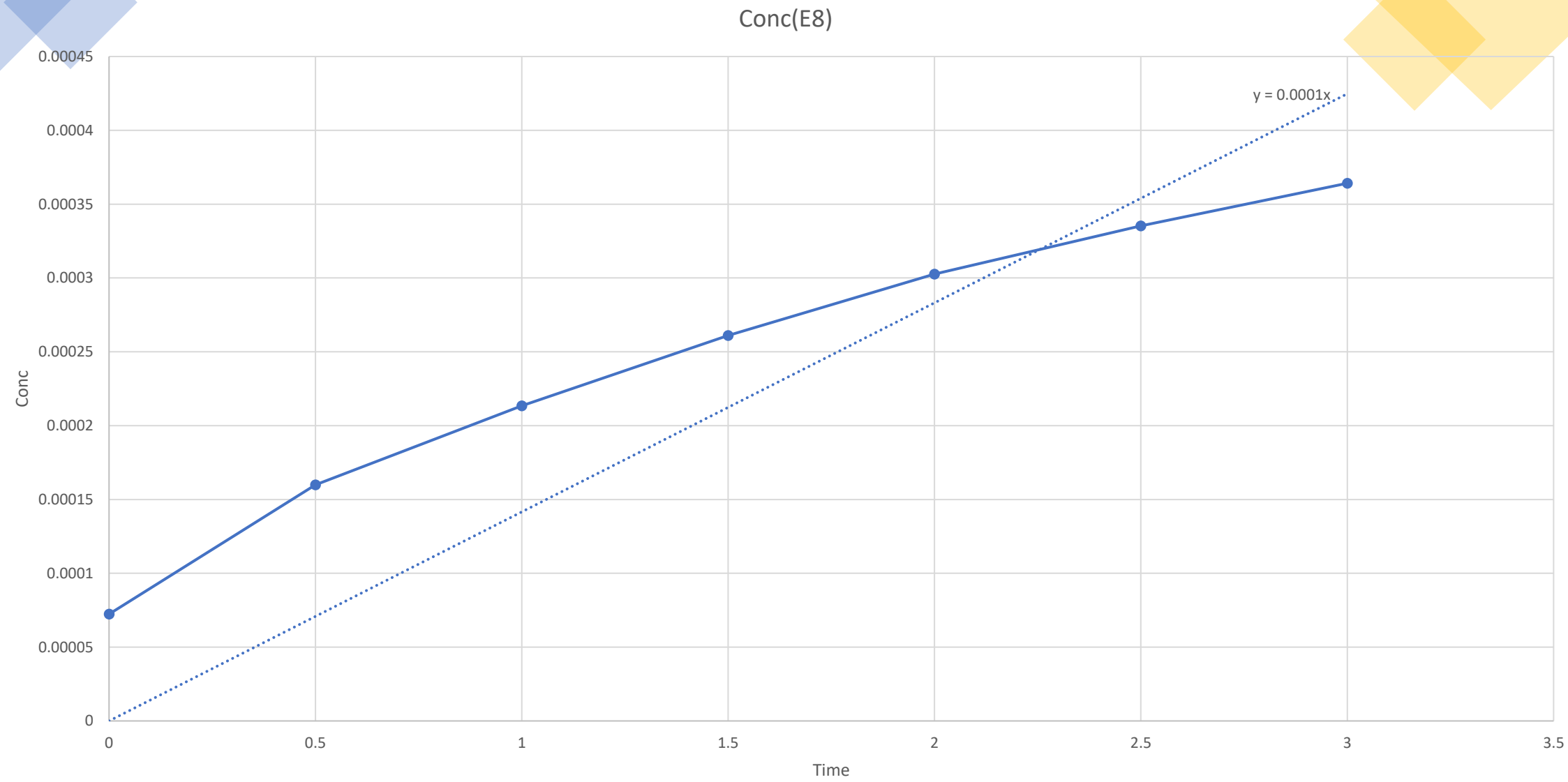
Prepare	Prepare two reaction systems: Test and Blank
Test	Test: 0.6 ml Phosphate buffer + 0.2 ml NAD+ + 0.1 ml Enzyme (Final Reaction Volume = 0.9 ml)
Blank	Blank: 0.75 ml Phosphate buffer + 0.2 ml NAD+ + 0.1 ml Enzyme (Final Reaction Volume = 1.050 ml)
Calibrate	Calibrate the Blank in a UV spectrophotometer at 340nm using 1 ml of the solution
Start	Start the reaction by adding 0.15 ml of alcohol to the Test and measuring the absorbance at 340 nm
Take	Take readings at every 30 seconds for 2-3 minutes and calculate the unit activity of the enzyme using the change in absorbance per unit time (dA/dt).

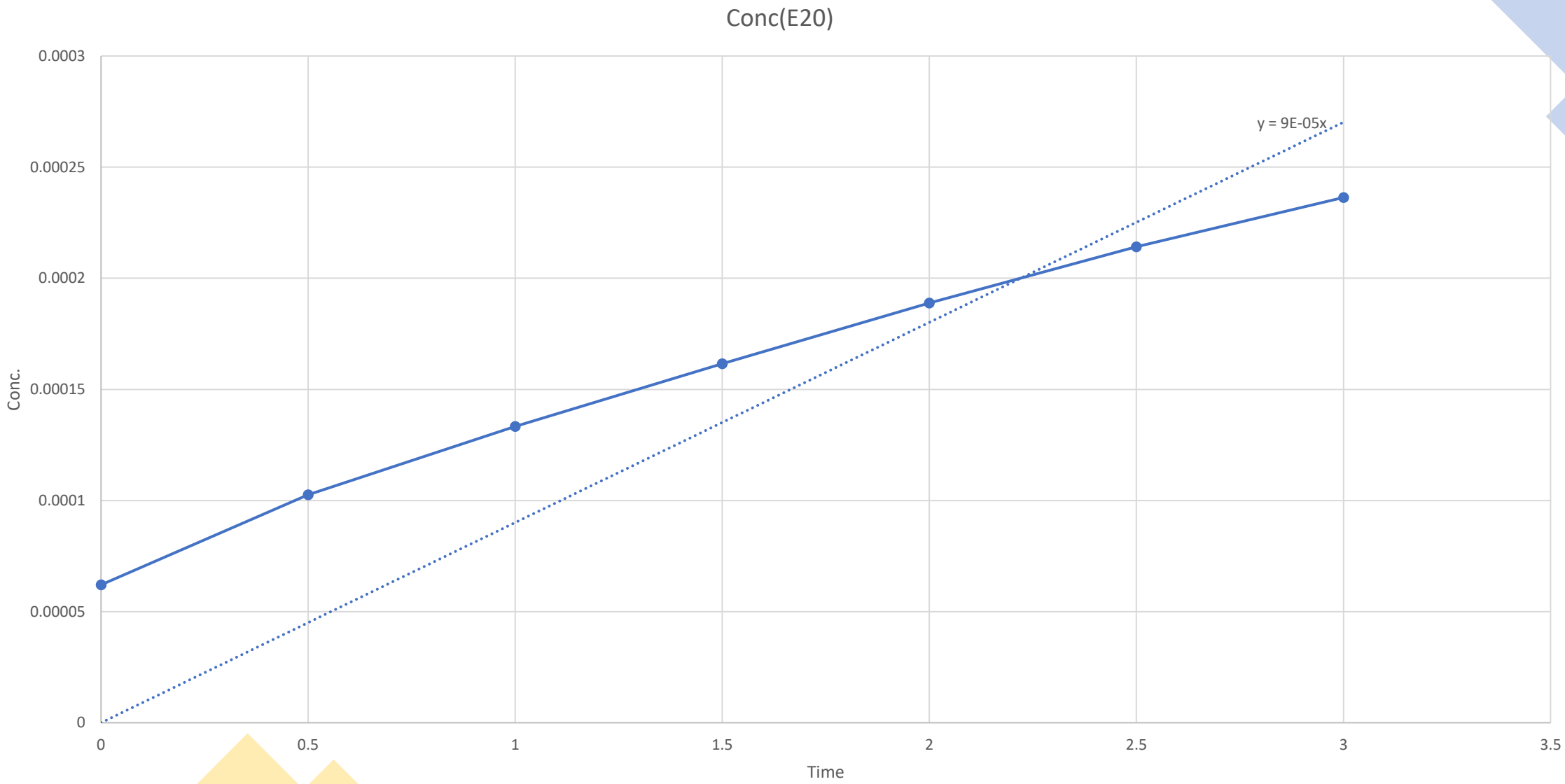
Observation

Time (sec)	Abs(8)	Abs(20)
0	0.45	0.386
30	0.995	0.638
60	1.328	0.829
90	1.624	1.005
120	1.882	1.175
150	2.086	1.332
180	2.265	1.47

Time vs concentrations of enzymes

Time (min)	Conc(E8) in M		Time(min)	Conc(E20) in M
0	7.23473E-05		0	6.20579E-05
0.5	0.000159968		0.5	0.000102572
1	0.000213505		1	0.00013328
1.5	0.000261093		1.5	0.000161576
2	0.000302572		2	0.000188907
2.5	0.00033537		2.5	0.000214148
3	0.000364148		3	0.000236334


Conc(E8)
y = 0.0001x
Conc
Time
0.00045
0.0004
0.00035
0.0003
0.00025
0.0002
0.00015
0.0001
0.00005
0
0
0.5
1
1.5
2
2.5
3
3.5


Conc(E20)
0.0003
0.00025
0.0002
0.00015
0.0001
0.00005
0
Conc.
y = 9E-05x
0
0.5
1
1.5
2
2.5
3
3.5
Time

Calculation and result

Activity of enzyme(8) = 0.0001 * 10^6 = 100 µmol min^{-1}

Activity of enzyme(20) = 0.00009 * 10^6 = 90 µmol min^{-1}


Thank you