$$n_{PNP\ made} = (C_{PNP})(V_{final\ assay})$$
$$n_{PNP\ made} = (1.14\ x\ 10^{-5}M)\ (2.5 \cdot 10^{-3}\ L)$$
$$n_{PNP\ made} = 2.9\ \cdot 10^{-8}M$$
$$n_{PNP\ made} = 2.9\ \cdot 10^{-2}\ umol$$

$$\%conv. = \frac{n_{PNP\ made}}{n_{PNPP\ (theory)}} \times 100\%$$

$$\%conv. = \frac{2.9\ \cdot 10^{-2}\ umol}{1\mu mol} \times 100\%$$

$$\%conv. = 2.85\%$$

Therefore, tube 2 contained 2.9 $\cdot 10^{-2}$ umol of PNP at concentration $1.14\ \cdot 10^{-2}$mM with a percent conversion of 2.85%

<u>Concentration of acid phosphatase</u>

$C_{Acid\ p.\ inital}$ = 0.5 mg/mL
$V_{Acid\ p.\ inital}$ = 0.4 mL
V_{final}= 5.0 mL

$$C_{Acid\ P.} = \frac{(C_{Acid\ p.\ initial})(V_{Acid\ p.\ inital})}{V_{final}}$$

$$C_{Acid\ P.\ total} = \frac{(0.5mg/mL)(0.4mL)}{5.0mL}$$

$$C_{Acid\ P.\ total} = 0.04mg/mL$$

Therefore, the concentration of acid phosphatase is 0.04 mg/mL.

Comment [Dk4]: Good job!! 10/ 10

R6

Table 5. Kinetic parameters obtained from experiments 2 and 3. V_{max} and K_M values are shown for the control and inhibitor reactions. V_{max} and K_M can be calculated by using the equations of the linear trendlines on the Hanes plot in Figure 2, the slope of the trendlines correspond to $1/V_{max}$, and the x-intercepts to $-K_M$. The control tubes contained variable amounts of diluted PNPP solution in water, 50 µL of 1.0 M Na-Acetate (pH 5.7), 50 µL of 0.5mg/mL acid phosphatase and 2.0 mL of 0.5 M KOH. The inhibitor contained the same solutions as the control but it also contained 50 µL of a 6.0 mM solution of unknown inhibitor #10.

Comment [Dk15]: 3.5/ 3.5

Method	Control		Inhibitor	
	V_{max} (µmol/min)	K_M (M)	V_{max} (µmol/min)	K_M (M)
Hanes	$8.2 \cdot 10^{-3}$	$1.9 \cdot 10^{-4}$	$8.5 \cdot 10^{-3}$	$6.6 \cdot 10^{-4}$

The type of inhibiton that is observed in the Hanes plot is competitive inhibition. Competitive inhibition is when the V_{max} is unaffected but the amount of substrate needed to achieve it increases; which is graphically visible in Figure 2 by an increase in K_m by factor of 6. As can be seen from Table 5, the V_{max} was calculated to be $8.2 \cdot 10^{-3}$ µmol/min for the control and $8.5 \cdot 10^{-3}$ µmol/min for the inhibitor. This is a 1% difference in V_{max} which is within the allowed 25% cutoff range. The K_M was calculated to be $1.9 \cdot 10^{-4}$M for the control and $6.6 \cdot 10^{-4}$ M for the inhibitor. The difference in K_M is 29%, which is outside the 25% cutoff range. Since the V_{max} was unaffected and the K_M increased the 6.0 mM solution of unknown inhibitor #10 is a competitive inhibitor as it shares the characteristics of completive inhibition.