Secondary alcohol reductase mediates the conversion of 2-propanol to acetone in the presence of NAD. One International Unit "IU" is defined as the amount of enzyme required to produce 1.0 μmole of acetone or NADH in one minute. A 0.50 mL solution containing this enzyme ("original sample") is found to have a total protein concentration of 4.1 mg/L. When 0.25 mL of a 2-propanol solution and 0.25 mL of a NAD solution are mixed together with this sample and placed in a spectrophotometer, the absorbance of the solution increases by 0.47 Absorbance Units (AU) in 1 minute. The molar extinction coefficient of NADH is 6.22 AU·L/mmol. *(23 pts)*

1) What is the enzyme activity in the ***original sample*** (IU/mL)?
2) What is the specific enzyme activity in the ***original sample***?
3) If the K_M of the particular enzyme is known to be 4 mM 2-propanol, what should be the 2-propanol concentration in the 0.25 mL solution prior to mixing the two solutions together? Explain.

A biologist places substrate (S) with 1.33 mg/L of purified enzyme having a molecular weight of 42,800 in several beakers of product-free water. Relatively quickly, samples are taken and analyzed for product (P) and the rate of product formation is calculated (dP/dt). The following table presents the results. *(24 pts)*

S (mmol/L)	dP/dt (mmol/Ls)
15	7.6
30	11.8
90	18.2

1) Assuming Michaelis-Menten kinetics applies, find the value of the Michaelis-Menten constant, K_M.
2) Find the value of V_{MAX} of this experiment.
3) If the enzyme has two active sites, find the value of k_{CAT}.
4) If 5 μg of the same enzyme is added to 100 mL of water containing 7 mmoles of substrate, how much time is required before one-half of the substrate is consumed?