Enzyme inhibition

Threonine is an essential amino acid for birds and mammals. There is much interest in the industrial synthesis of this amino acid, largely by conversion of Aspartate via a multi-step pathway in Escherichia coli. Your goal is to investigate the effects of inhibitors, which may be present in feed waters, on the last metabolic step in which O-phospho-homoserine (OPH) is converted to Threonine by the enzyme threonine synthase (TS):

You examine the effects of two inhibitors: cadmium and 2-amino-5-phosphonovaleric acid (APB).

	K_I (nM)	inhibition mechanism
cadmium	100	non-competitive
APB	100	competitive

Assume standard conditions of:

- OPH is present at a concentration of 1 mM.
- K_m for the interaction of OPH with TS is 0.5 mM.
- At this concentration of OPH, the reaction proceeds at a rate of 300nM/min in the absence of inhibitor.
- These questions explore two of the enzyme kinetics inhibition equations:
  Competitive: V = Vmax*s / (Km*(1 + i/Ki) + s)
  Non-competitive: V = Vmax / (1 + i/Ki) * s / (Km + s)

Find:

1) The maximum reaction rate (Vmax) associated with this reaction.
- Vmax=(300nM/min)*(1mM+0.5mM)/(1mM)=450nM/min

2) The reaction rate expected if cadmium OR APB are present at 200 nM.
- Cadmium: non-competitive, V = Vmax / (1 + i/Ki) * s / (Km + s) =100nM/min
- APB: competitive: V = Vmax*s / (Km*(1 + i/Ki) + s) = 180nM/min

3) The concentration of cadmium that reduces the reaction rate by 90%.
- It comes down to the multiplicative term in the non-competitive case, 1/(1+i/Ki) set that equal to 0.1 (90% inhibition). 0.1 = 1/(1+i/100 nM). Then i = 900 nM

4) One day, you find that Threonine production has been decreased by 10% (to 270 nM/min). Analysis of the feed waters indicates the presence of APB. Your goal is to bring the production rate back to 300 nM /min by increasing the concentration of OPH. What concentration should you raise OPH to?
- Find the concentration of APB that would produce that drop in reaction, using the rate equation for competitive inhibition. i = 1/3 K_I = 33.33 nM.
- Now, find the concentration of s that would bring the rate back to 300 nM. Use the same competitive inhibition rate equation, with i=33.33 nM and V = 300 nM/min. With that, [OPH] = 1.33 mM.