Assignment Part 1: (Homework Assignment)

The 18th Amendment, which took effect on January 17, 1920, outlawed the sale and consumption of alcohol in America and resulted in a sharp increase in the production of moonshine. To meet demand, and maximize their revenues, some moonshiners lowered their standards, to produce more liquor which was sometimes of lesser quality.

The drinkers of moonshine also became less discriminating, and often ended up dead or blind. The root cause of this blindness and/or death was the presence of methanol, or ‘wood alcohol’ present in the moonshine.

Methanol is a byproduct of alcohol distillation, but only forms in tiny, non-toxic amounts during distillation. It has a lower boiling point and is found in the first few ounces of alcohol that drip from the condenser. Any distiller worth his salt will discard these at the start, eliminating methanol (which also leads to nasty hangovers in small doses, by the way) from the equation. But not all moonshiners would throw these first few ounces out during prohibition. Even worse, some unscrupulous moonshiners would actually add methanol to their product to up the potency. Methanol can cause blindness in very small amounts (~10 milliliters) or can be lethal in larger amounts (a few ounces).

The methanol itself is not toxic but its metabolic byproducts are. The enzyme **Alcohol Dehydrogenase** catalyzes the reaction converting methanol to formaldehyde. The enzyme aldehyde dehydrogenase then converts the formaldehyde to formic acid. The formaldehyde is the main toxin although formic acid is also toxic.

Thus, if conversion to these products can be avoided, the methanol can be excreted by the body unchanged and thus save the drinker from blindness and/or death. Giving the patients a sufficient quantity of an inhibitor of the enzyme Alcohol Dehydrogenase could potentially be life-saving.

The following kinetic data were obtained in the lab for the enzyme, **Alcohol Dehydrogense**, **by itself (A)**, and in the presence of three different inhibitors **B (2, 2, 2, trifluoroethanol), C (cefamandole), & D (pyrazole)**. The velocity for each reaction at various concentrations of substrate with enzyme alone (A) and enzyme in the presence of each inhibitor(B, C, & D) is given below. Graph each set of data in a Lineweaver-Burke plot. Using excel, determine the equation of the line in each plot and include this on the graph.

[S]	**V (A)**	**V(B)**	**V(C)**	**V (D)**
1	12.00	4.29	5.49	9.01
2	20.87	8.00	9.01	12.35
4	27.78	14.08	12.99	15.15
8	34.48	20.83	16.00	17.54
12	40.00	26.32	17.86	18.52

In class assignment:

For your inhibitor, determine the V_{max} for the enzyme alone and in the presence of the inhibitor. Are they the same or different?

Determine the K_m for the enzyme alone and in the presence of the inhibitor. Are they the same or different?

Based on the answers above, what kind of inhibitor is this? How does this type of inhibitor work to inhibit the enzyme?

Suppose someone with methanol poisoning is given one of the above inhibitors as a treatment (either inhibitor B, C, or D). At the same time, they have not learned their lesson and ingest much more of the methanol containing liquid. Will the additional methanol cause any of the inhibitors to lose their effectiveness? If so, which one(s)? Explain how you came to this answer.

Part 2: Exam question

You are trying to find a chemical that inhibits the reaction of Enzyme A. After trying a variety of chemicals, you find one causes the maximal rate of the enzymatic reaction to decrease, and you also find that in the presence of this chemical, you need to add a higher concentration of substrate to get to half the maximal reaction rate. When you test what the inhibitor is binding to, you find that it binds to the enzyme when it is already bound to the substrate.

a) What kind of inhibitor have you found?

b) Is the K_m changed?

c) Is the V_{max} changed?

d) Draw a line on the graph below representing the reaction of the enzyme in the presence of this inhibitor.