Name:_____

Biology 3515/Chemistry 3515
Biological Chemistry Laboratory
Spring Semester 2017
Quiz 1 - 2 February 2017

Please write your name on each page.
Be sure to show your work and include correct units in all of your answers!
25 points total.

1. In preparation for doing an experiment, you need to prepare a buffer solution with a pH of 7.8. A friend in your lab says that she has just the recipe for you, using *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), a sulfonic acid with a pK_a of 7.5. Since you know that the buffer pK_a should be close to the desired pH, this sounds good, and you make the solution according to the following recipe, which is intended to make 600 mL of a 0.5 M solution:

   Dissolve 0.2 moles of HEPES in 400 mL of water.
   Add 0.1 moles of Na-HEPES (the sodium salt of the conjugate base) and dissolve.
   Add water to a total volume of 600 mL.

   (a) (4 pts.) Before adding water to the final volume, you decide to check the pH. It's a good thing you did, because it looks like your friend gave you the wrong recipe! Calculate the expected pH for the solution, made according to the recipe.

   (b) (3 pts.) Now, you want to change the pH to the desired 7.8, but you don't want to add any more HEPES or Na-HEPES. You have on hand stock solutions of 1 M HCl and 1 M NaOH. Which of these solutions should you add to change the pH to 7.8? Explain what will happen to the different components of the solution when you add the HCl or NaOH.

(c) (5 pts) How many mL of the 1 M HCl or NaOH solution should you add?

2. From biochemistry class, you should be familiar with nicotinamide adenine dinucleotide (NAD), which can exist in an oxidized form (designated NAD^+) and a reduced form (NADH). NAD participates as a cofactor in many metabolic reactions and serves as an intermediate in electron transfer processes, such as oxidative phosphorylation. Rather conveniently, NADH has an absorbance peak at 339 nm, with an extinction coefficient of $6,220\,\mathrm{cm^{-1}M^{-1}}$, but NAD^+ does not absorb significantly at this wavelength. This difference is widely used as the basis for kinetic measurements for reactions that involve the conversion of NAD^+ to NADH, or vice versa.

   Someone gives you a sample and tells you that it contains 0.4 mM NAD, but not the specific concentrations of the oxidized and reduced form. You decide to determine the concentrations of the two forms by measuring A_{339}, using a 1- cm path-length cuvette.

   (a) (3 pts) What additional information will you need in order to do this measurement? How will you use this information?

(b) (5 pts) You measure A_{339}, and obtain a value of 0.89. Calculate the concentrations of NAD^+ and NADH in the sample.

(c) (5 pts) What fraction of the light entering the cuvette containing the sample is absorbed by the NADH in the sample?