

Protocol for Expt 2-1 to 2-4.

Read Chapters 1 and 2.

Do the concentration problems 1-1 to 1-5 on p 35 and the pH problems 1-6, 1-8, 1-10 on p 43-44.

Write out an experimental protocol to hand in to the TA at the start of the laboratory session. Include answers to questions 1-1 to 1-5 on p 35 and 1-6, 1-8, 1-10 on p 43-44 and the calculations described for Expt 2-4 on p 70.

Changes to the published experiment.

1. We will calibrate pipettors to
  - a. Make sure both that they are working properly
  - b. Make sure that you are using them properly
  - c. Determine the inherent errors involved in pipetting

A 1 ml pipet (Rainin P-1000) will be used to dispense 1ml of distilled water into a weigh boat on an analytical balance, according to the pipetting instructions given in the pipettor handout. The balance will be tared just before each addition and the weight recorded just after each addition. After several additions you should begin to get the feel for the pipette. When you are satisfied that you can deliver consistent masses of water, record 4 more weights and calculate the mean and standard deviation of the measurements. Repeat the procedure with the pipette set on the 100 micro liter setting.

2. All volumes to be measured in the spectrophotometer will be reduced to 1 ml. For example in part 2-1 although the book says to add 1ml of PNP to 3 ml of buffer, we will be adding 0.25 mls of PNP to 0.75 mls of buffer directly in methacrylate cuvettes (also called "cells").
3. We will do parts 2-1 and 2-2 together. Make up 10 samples. The first 8 will contain 250  $\mu$ l of the 100  $\mu$ M stock of PNP plus 750  $\mu$ l of pH 5, 6, 7, 7.5, 8, 8.5, 9, 10 buffers supplied. The remaining two cuvettes should contain only water. A Cary 100 spectrophotometer will be used. This instrument has dual or single beam operations modes, a 12 position thermostated cell holder and is controlled by a computer. Individual programs that utilize different functions of the spectrophotometer can be accessed either through the folder "Cary WinUV" or through saved methods. All of the methods needed for this laboratory are in the folder:

C:\Varian\CaryWinUV\BiochemLab2002\Methods

(Also a shortcut on the desktop labeled **Biochem Lab Methods**)

the method for this part is called **Expt2\_Scans**.

- a. Open the method file (double click).
- b. When you are prompted to allow for a Dual single beam calibration, open the top of the spectrophotometer and check to make sure that cell positions 1 and 7 are empty, close the top then click OK.
- c. Insert blank cuvettes in positions 1 and 7, make sure that the long axis of the lower portion of the cell (1 cm) is parallel to the light path and close the lid.
- d. Click the baseline button to perform a baseline scan. This corrects for the inherent efficiency of the light sources and the absorbance properties of the cuvettes.
- e. Click the Start (greenlight) icon to begin the experiment. A loading guide will appear to show you how to position your cells.

12 unused	11 pH 10
10 pH 9	9 pH 8.5
8 pH 8	7 Blank
6 unused	5 pH 7.5
4 pH 7	3 pH 6
2 pH 5	1 Blank

- f. After the cells are in place close the lid and click OK.
  - g. When prompted, save your data onto a floppy disk in drive A, two files will be saved one specific to the Cary software and a Comma delimited file that can be opened in a spreadsheet program like Excel.
4. Expt 2-3 will use the method called **Expt2\_Conc**. Again make up you samples to a final volume of 1ml..
  5. Expt 2-4 make up the pH buffer samples to 10 mls as indicated in the lab Manual, but use only 750  $\mu$ l of buffer plus 250  $\mu$ l of PNP as before. Use **Simple Reads** to collect the data.