Appendix 1: Using Pipetman® Pipettors and Concentrations and Dilutions

A. Pipetting

In the biochemistry lab one often works with very small volumes of solutions that are measured with the use of Pipetman® pipettors. To achieve accurate measurements, pipettes must be used correctly. Incorrect use of pipettes results in inaccurate measurements and can also damage these expensive devices. Please read this appendix to familiarize yourself with Pipetman® pipettors and how to use them correctly in order to get reproducible results.

Use of a Pipetman®

1. We have the following sizes of Pipetman® pipettes in the lab: P-20, P-200, P-1000 (volumes are in µL). Select the proper size Pipetman® by choosing the smallest one that can measure the volume you need. **Never use a Pipetman® to measure less than ten percent of its maximum volume.**

2. Set the desired volume by holding the Pipetman® in one hand and turning the volume adjustment knob until the correct volume shows on the digital indicator.

3. Different size pipettes have different units on their dials, for example:

   P-20 is in microliters _ _ . _ (example 125 = 12.5 µL).
   P-200 is also in microliters _ _ _ (example 045 = 45 µL).
   P-1000 is also in microliters _ . _ _ (example 096 = 960 µL).

   Be careful to use the correct unit when dialing.

4. Attach a new disposable tip (be sure to use the proper size tip) to the shaft of the pipette. Press on firmly to insure a positive, airtight seal.

5. Depress the plunger to the FIRST POSITIVE STOP. This part of the stroke is the calibrated volume displayed on the digital micrometer.

6. Holding the Pipetman® vertically, immerse the disposable tip into the solution. Avoid touching any part of the Pipetman® except the tip to the solution container.

7. Allow the pushbutton to return SLOWLY to the up position. Never permit it to SNAP up. Wait 1 second to ensure that full volume of solution is drawn into the tip. Withdraw the tip from the solution. Check to make sure the solution does not drip out of the tip on its own. If it does, expel the solution in the tip, discard the tip, and try again. If this does not help, let the instructor know. Under no circumstances should you use a pipettor that drips on its own.

8. To DISPENSE SAMPLE, place the tip end against side wall of the receiving vessel and depress the plunger slowly to the FIRST STOP. WAIT 1-2 seconds, then depress plunger to SECOND STOP (bottom of stroke), expelling any residual liquid in the tip.

9. With the plunger fully depressed, withdraw the Pipetman® from vessel carefully.

10. Allow the plunger to return to the top position without allowing it to snap up.

B. Concentrations and dilutions

Absolute amounts are given in moles, mmoles, µmoles, nmoles, etc. One does not write 10^{-3} µmol because this is 1 nmol (or 10^{-9} mol). When the molecular weight of a substance is unknown (ex. a mixture of proteins), the amount can be given as the mass -- g, mg, µg, ng, etc.

Concentration is always given in M, mM, µM, nM, etc. (symbol "M" means **molarity,** or moles per liter and so "µM" means micromoles per liter). Again, one would not write 10^{-3} µM because this is 1 nM, or 10^{-9} M. For substances of unknown molecular weight (i.e. for a mixture of proteins), the concentration can be given as g/liter, mg/ml, µg/ml, ng/µl, etc.

If you need to calculate an amount from a volume and concentration, use the following formula:

volume x concentration = amount

For example, (60 µM) x (1.0 ml) = 60 nmol or 0.06 µmol.

An easy way to think about this is: 60 µM = 60 µmol/L OR 60 nmol/ml so 1 ml contains 60 nmol.

Molarity (M) is defined as the number of moles of solute per liter of solution. In biochemistry, most concentrations are in the millimolar (mM), micromolar (µM), or nanomolar (nM) range.

Biochemical experiments frequently require the dilution of a **stock solution**. If the concentration of stock solution needed in the experiment is known, the ***dilution factor*** can be calculated. For example, if you want to dilute a 1 M stock solution to 5 mM in your experiment, the dilution factor is .005/1 or 1/200 (also indicated as a 1:200 dilution). So you must dilute 1 part stock solution with 199 parts solvent. Another way of stating this is that 1 part stock solution must be diluted into 200 parts total solution - commonly described by biochemists as a 1 ***in*** 200 (or two hundred-fold) dilution. Generally, if you want 200 ml of the diluted solution, you would mix 1 ml stock solution with 199 ml solvent. T

You can determine the concentration of a diluted solution (C_{final}) or calculate what volume of stock solution ($V_{initial}$) must be added to a solvent to make a diluted solution of known concentration. ***The following formula is very useful:***

$$\mathbf{C_{initial}\ V_{initial} = C_{final}\ V_{final}}$$

For example, if you have a stock solution of 5 mg/mL protein, and you want to make 250 µL of a 50 µg/mL working solution, how much of the stock solution do need to use?

$$V_{initial} = C_{final}\ V_{final} / C_{initial}$$
$$= 50\ \mu g/mL * 250\ \mu L / 5\ mg/mL = 25\ \mu L$$

Thus, you would add 25 µL of the stock solution to **225 µL of solvent**. This is a **1:100 dilution** (250/25 = 100). It is good practice to add smaller volumes to the larger volume of solvent. In biochemistry most dilutions will be made in a V_{final} of less than 1 mL. This mean that a typical 1:200 dilution will involve adding 1 µL of stock solution to 199 µL of diluent. Unfortunately the technical limitations of the pipettors does not accurately allow the measurement of 1 µL. Therefore a typical 1:200 dilution would most likely be done by adding 5 µL of stock solution to 995 µL of diluent.

Dilution Series

A special type of dilution is the ***serial dilution***. In this method, a stock solution is systematically diluted in fixed steps of the same dilution factor (see Fig. 1). A high degree of accuracy and precision is required, because errors made in an early dilution are carried to all subsequent dilutions.

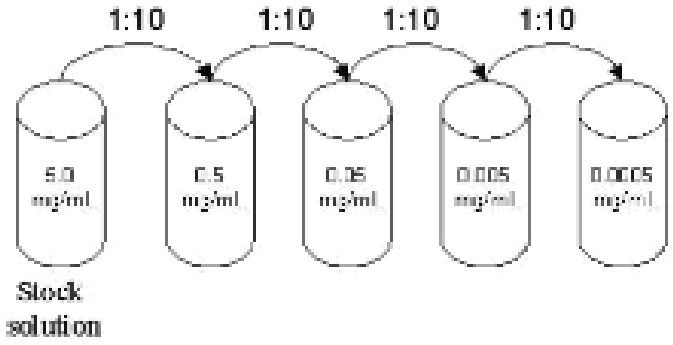

Fig. 1. A serial dilution scheme.

A more accurate method for preparing dilutions is the systematic dilution of a single stock standard. Note: it is not always reasonable to prepare all dilutions from a single standard.

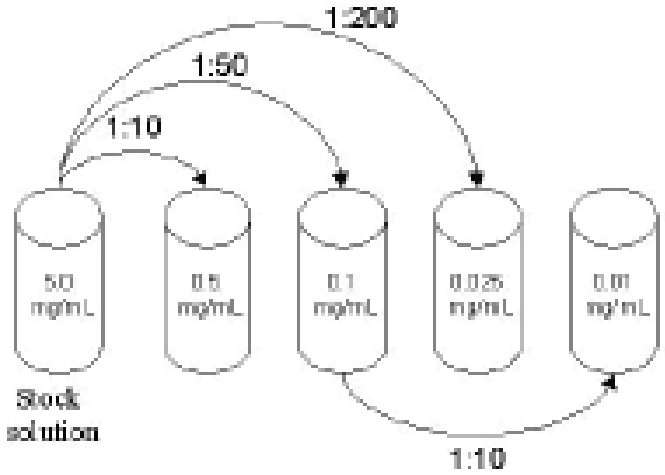

Fig. 2. Systematic dilution scheme