

Biochemistry Laboratory I

CHEM 4401

Pipetting & Scales

Pipettors are one of the most basic, and most used, instruments in the biochemistry laboratory. pipettors are used to transfer small volumes of liquids, usually between 0.2 microliters (ul) and 1 milliliter (1 ml, 1000 ul). They are easy to use, but require care as they are rather expensive (~\$ 250 apiece).

The first thing to learn about pipettors is what liquids are *NOT* to be pipetted. These include strong acids, bases and very non-polar substances, all of which can damage the internal gears, gaskets and pistons.

Pipettable liquids	Non-pipettable liquids
Water	Strong Acids (HCl, H ₂ SO ₄ , HNO ₃ , etc.)
Buffers	Strong Bases (NaOH, KOH, etc.)
Ethanol solutions	Phenol
Salt solutions (MgCl ₂ , NaCl, etc.)	Chloroform
Protein Solutions	Hexane, etc.
DNA-containing solutions	
Most aqueous (water-based) solutions	

If in doubt, ALWAYS ask your instructor if it would be appropriate to use the pipettor for a given substance.

Operation

We will use three different pipettors, which differ in the range of volume they will pick up and dispense. The first one is known as the “P-20”, the 20 indicates that 20 microliters (ul) is the maximum volume it can dispense. The second is the P-200, which handles volumes between 20 - 200 ul and the third is the P-1000, which operates with volumes from 200 - 1000 ul (1 ml). The P-20 and P-200 pipettors use the same tips, which are in the smaller tip boxes and are often either yellow or clear. The P-1000 uses a larger tip, which is typically blue or clear. They are found in their own, larger tip boxes. In order to pick or dispense any volume, the pipettor must have a tip on the end of the shaft. This is done by grasping the pipettor and inserting the shaft into the open end of the appropriate tip, while it is in the box. Slight pressure from your hand on the pipettor will seat the tip.

After deciding if a pipettor is the appropriate instrument for transferring a reagent, the most important thing to know about pipettors is what the values on the different pipettors mean. As indicated, the P-20 pipettor pipets volumes between approximately 1-20 ul. If you look on the dial of the pipettor you will see a column of three values: top, middle and bottom. The top value is gauged in “tens” of microliters. It can have the values of “0” “1” or “2”, which represent ranges of 1-10, 10-20 or 20 ul, respectively. The middle value is gauged for individual microliters and the bottom value for tenths of microliters. To set a volume of, say, 14.5 ul you would turn the plunger knob on the pipettor handle until the top value is “1” (for 10) the middle value is “4” (which added to 10 makes 14) and the bottom value is .5. To set a volume less than 10 ul, you would turn the plunger knob until the top value read “zero” and then dial in the volume desired.

The P-200 and P-1000 operate in the same manner, except for the P-200 the “0”, “1” or “2” values in the top of the column represents ranges of 0-100, 100-200 or 200 ul, respectively. The middle value represents “tens” of microliters and the bottom value individual microliters. You will notice that values on the bottom are hashed off in 0.2 ul increments. The P-1000 can have a reading of either “0” or “1” in the top of the column, representing a 0-1000 ul range. The middle value represents hundreds of microliters and the bottom value is gauged in “tens” of microliters, hashed off into 2 ul increments. Much as for the P-20, setting a pipetting volume on either the P-200 or P-1000 involves dialing the plunger knob to the desired value. Here are some examples

P20 (1 to 20 ul volumes)

17.5 ul would read	2.2 ul would read	0.8 ul would read
1	0	0
7	2	0
5	2	8

P200 (20 to 200 ul volumes)

175 ul would read	24.6 ul would read	102 ul would read
1	0	1
7	2	0
5	4(.6 hash mark)	2

P1000 (200 to 1000 ul)

612 ul would read	1000 ul would read	920 ul would read
0	1	0
6	0	9
1(.2 hash mark)	0	2

To review pipettor operation :

1. The volume desired is “dialed in” to the pipettor by rotating the handle plunger knob (top).
2. An appropriate pipettor tip is seated onto the shaft using slight pressure on the pipettor.
3. The handle plunger knob is depressed by the thumb to the first stopping (resistance) point.
4. The “tip” of the pipettor tip is inserted into the liquid you wish to transfer. Slow release of pressure by your thumb draws liquid into the pipet tip.
5. The pipettor is transferred to the container where the liquid is to be dispensed. Gently insert the liquid-containing tip into the solution where it is to be dispensed. If the tube, container, etc. is empty, touch the pipet tip to the wall of the container near the bottom.

6. Depress the plunger to the very end to completely dispense the liquid. Do not release pressure on the plunger while the tip is still in the container containing the dispensed solution. This may cause solution to be drawn back into the tip, or splash out.
7. Remove the pipettor from the solution. At this point it is OK to gently release pressure on the plunger (NO Snap Releases!).
8. Eject the used tip into the waste container by depressing the button below the plunger on the top of the pipettor

Procedure

Practice pipetting technique by following the directions on the next page. Turn in your sheet to your Laboratory instructor when you are finished

Biochemistry Laboratory I
CHEM 4401
Pipettors & Pipetting (10 pts)

Use ordinary tap water for all pipetting operations

9. Which pipettor would you use to transfer the following volumes? (0.5 pt each.)

Volume	pipettor
650 ul	
0.18 ml	
68 ul	
0.95 ul	
110 ul	
0.4 ml	
7.5 ul	

10. Pipet the above volumes of water onto a weigh boat on one of the analytical balances (tare between each measurement to record accurate values). Record the mass of each pipetted amount below. (0.5 pt each)

Volume	mass
220 ul	
0.85 ml	
68 ul	
195 ul	
19.5 ul	
0.075 ml	
350ul	

11. Read each column below. The top cell indicates pipettor. The next three cells show the volume reading on the pipettor. Indicate in the bottom cell the value of this volume reading, in microliters (ul). (0.5 pt each.)

P-20	P-1000	P-200	P-1000	P-200	P-20
1	0	0	0	1	0
9	9	5	8	8	9
5	3	7	0	2	1