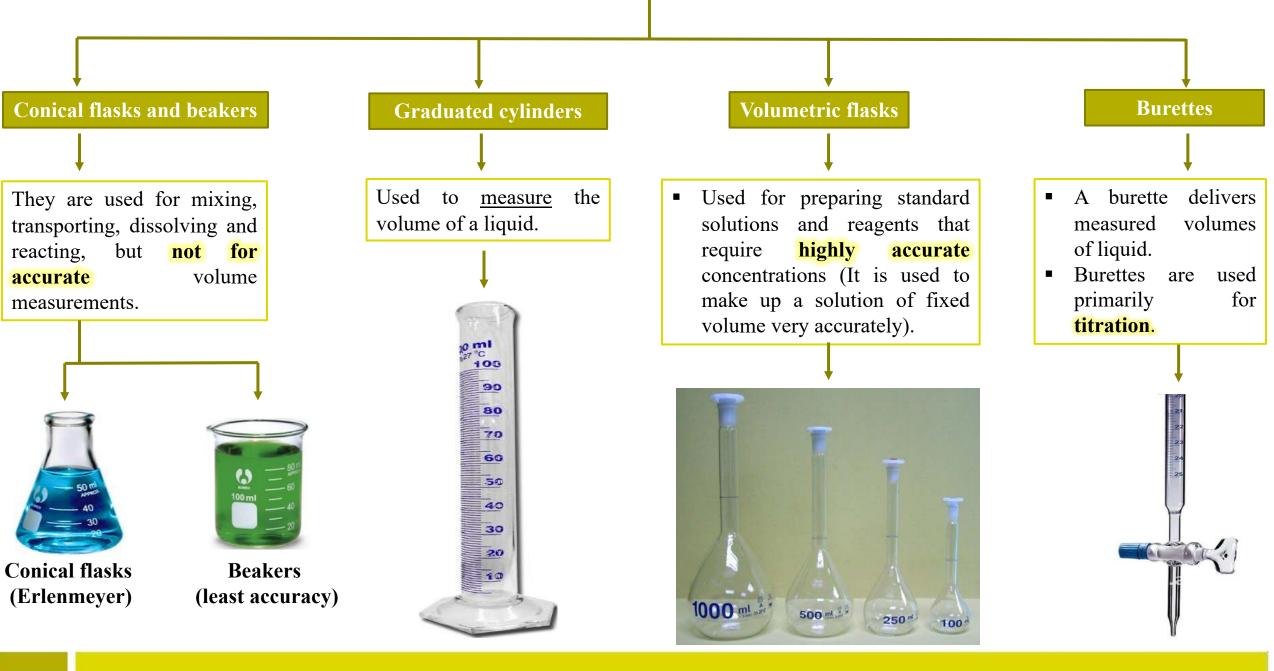
BCH 312 [Practical]

Identification of the common laboratory glassware, pipettes and equipment

(1) Identification of the common laboratory Glassware:

- a. Conical flasks and beakers
- b. Graduated cylinders [*measuring cylinder*]
- c. Volumetric flasks
- d. Burettes
- e. Pipettes

Glassware



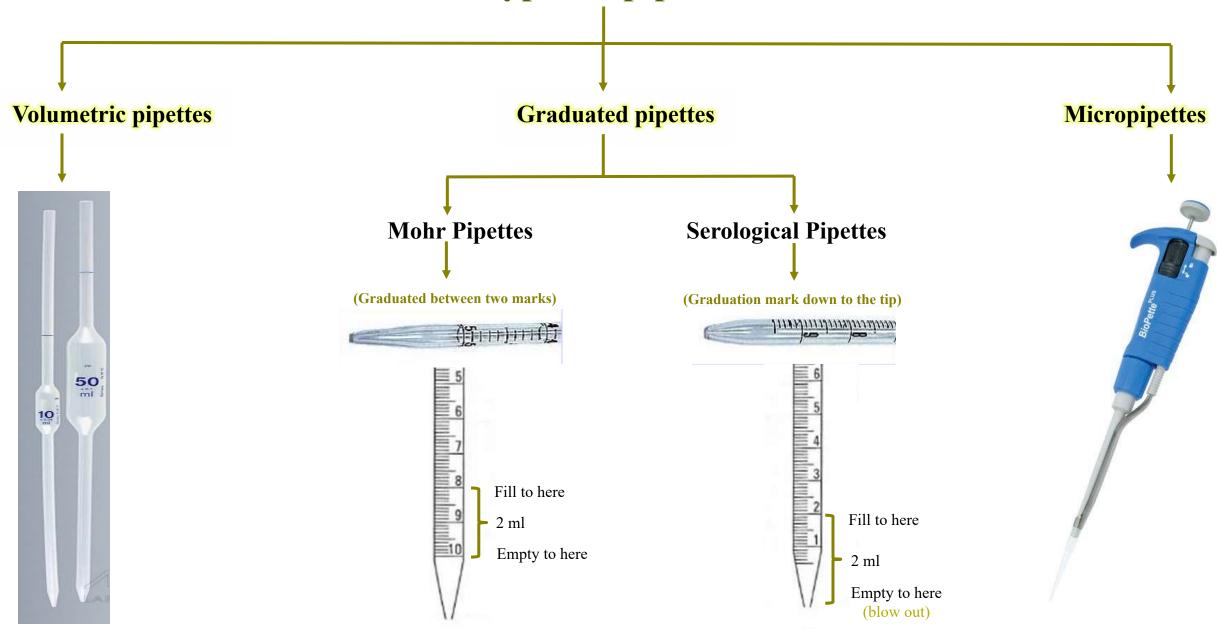
(2) Identification of the common laboratory **pipettes**:

- Sometimes spelled <u>pipet.</u>
- Commonly used to transport a measured volume of liquid.
- Pipettes come in several designs for various purposes with <u>differing levels of accuracy</u>.

There are three types of pipettes are used in biochemical laboratory:

 (a) Volumetric or transfer pipettes
 (b) Graduated or measuring pipettes (Mohr and Serological Pipettes)
 (c) Micropipettes

Types of pipettes



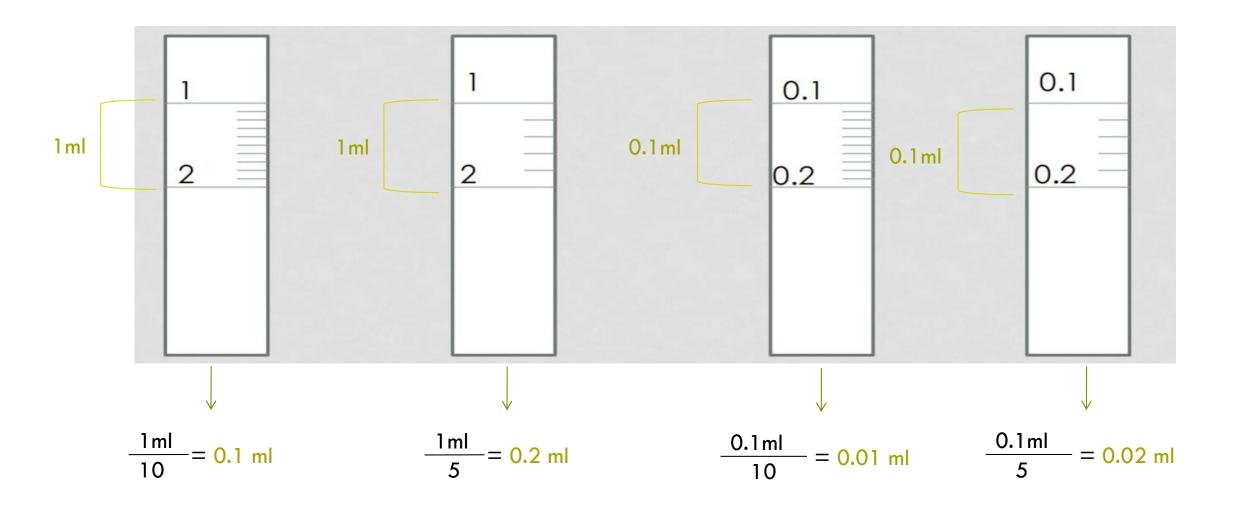
Comparison between types of pipettes

Volumetric pipettes	Graduated pipettes
Transfer (designed to deliver accurately fixed volume of liquid)	Measuring a range of volumes
Not graduated	Graduated
More accurate	Less accurate
Non-blown out	Some are blown out
Consists of a cylindrical bulb joined at both ends to narrowed glass tubing	Don't contain a cylindrical bulb

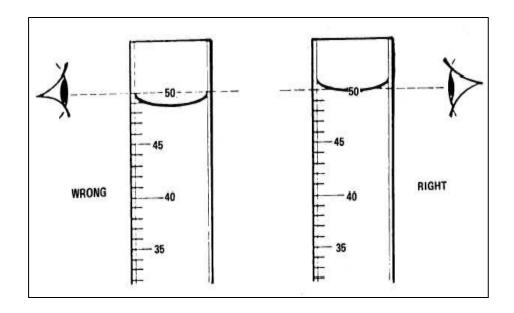
Card

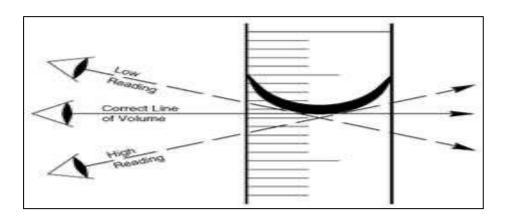
25ml 20°c

Smallest division of graduated pipette

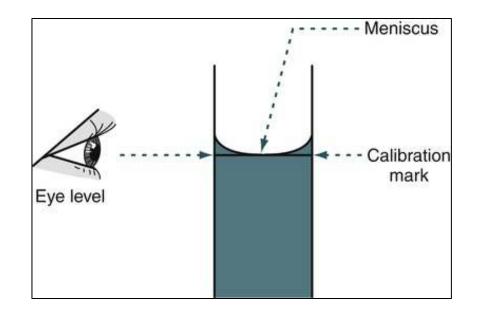


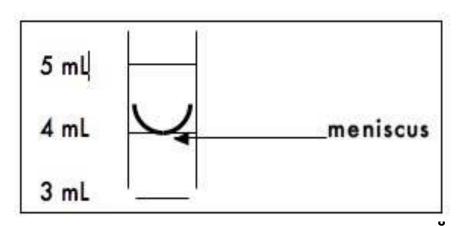
Reading the meniscus:











Steps of the use of the pipettes:

- 1. Choose appropriately sized pipette i.e if you need to measure $8 \text{ ml} \rightarrow \text{choose } 10 \text{ ml}$ pipette
- 2. Press the pipette into the pump with a slight twisting motion.
- 3. The pipette is first washed with water, then rinsed several times with a little of the solution.
- 4. The pipette then filled to just above the mark, the liquid is allowed to fall to the mark .
- 5. The solution is allowed to drain into the appropriate vessel with the jet of the pipette touching the wall of the vessel.
- 6. After the flow of the liquid has stopped, the jet is held against the wall for some times and then removed.

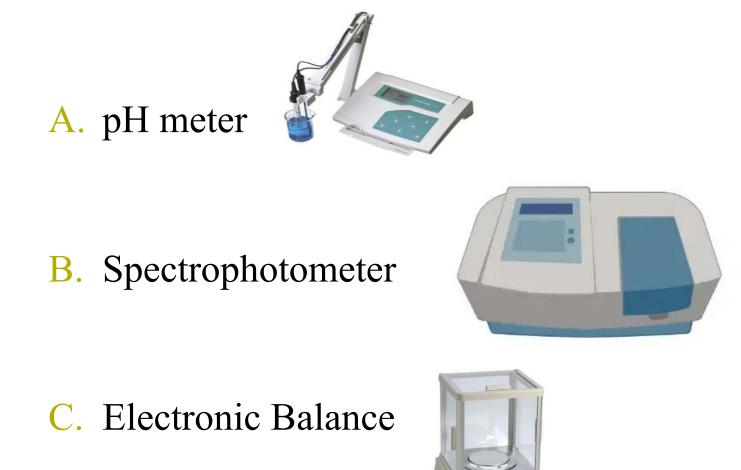
Note:

- For **serological pipette**, some are of the <u>blown out type</u>; the last drop being blown out against the vessel wall.
- For volumetric pipette and Mohr a certain amount of liquid will remain at the tip and this must <u>not be "blown</u> <u>out".</u>

Accuracy:

- 1. Volumetric flasks and volumetric pipettes \rightarrow most accurate
- 2. Burettes and graduated pipets
- 3. Graduated cylinders
- 4. Beakers and conical flasks → least accuracy used only when a rough estimation of volume is required -

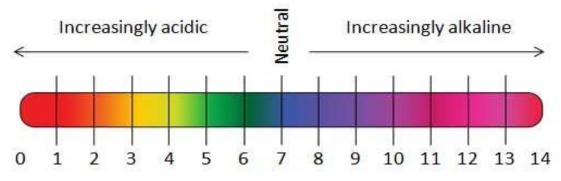
(3) Identification of the common laboratory equipment:



pH and solution acidity:

• **<u>pH define as:</u>** the negative logarithm of the hydrogen ion concentration.

- So the term pH introduced as a way of <u>expressing hydrogen ion concentration</u> (acidity or alkalinity of a solution).
- pH range value (0 14) → the higher pH number, the lower the hydrogen ion concentration and vice versa [inverse relationship].



pH cont.:

- pH determines many important aspects of the structure and activity of biological macromolecules and thus of the behaviour of the cell and organisms.
- There are many ways in biochemical laboratory to measure pH value such as :

3. pH meter **>** The most accurate and reliable method (quantitative)

1. Litmus paper (*qualitative*)



2. Test strips (*semi-quantitative*)

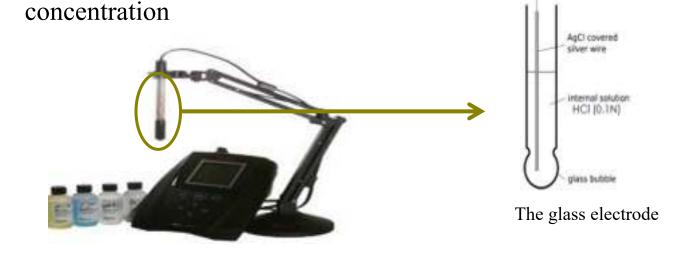




• A nice video show you how to use the pH meter: <u>https://www.youtube.com/watch?v=vwY-xWMam7o</u>

(1) pH meter:

- A pH meter is an electronic device used for <u>measuring the pH</u> (acidity or alkalinity) of a liquid
- Before use it needs to be calibrated
- pH meter contain <u>glass electrode</u> which is very sensitive and readily responds to changes in hydrogen ion





(2) Spectrophotometer:

• **Spectrophotometer** is instrument used to measure the intensity of light that is transmitted

or absorbed by a sample at a given wavelength

- Wavelength in this instrument divided into:
- a. Invisible range-ultraviolet- (from 100 to 360 nm) \rightarrow [*Quartz cuvette are used*]

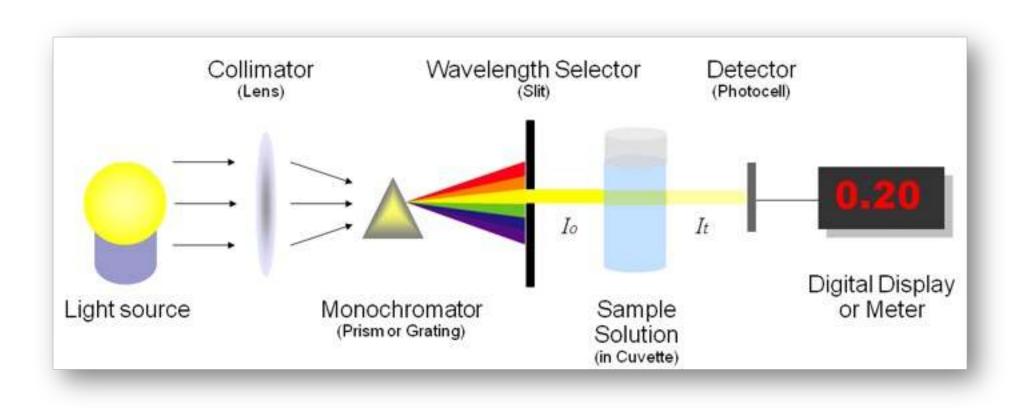
- b. Visible range (above 360 nm -700 nm) → [*Glass or plastic cuvette are used*]
- **Blank**: contain everything <u>except</u> the compound to be measure







Spectrophotometer Principle

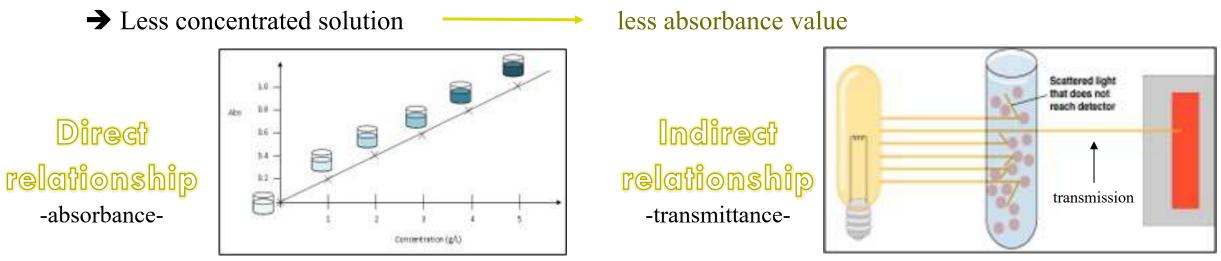


A nice video show you how dose spectrophotometer work:

http://www.youtube.com/watch?v=pxC6F7bK8CU

(2) Spectrophotometer cont':

- By using the spectrophotometer, we can quantitatively measure absorbance, and this information can be used to determine the concentration of the absorbing molecule [concentration of unknown sample].
- More concentrated solution will absorb more light and transmits less:
 - \rightarrow So, the more concentrated solution \longrightarrow high absorbance value



(3) Electronic Balance:

- Electronic Balance is a device used to find accurate measurements of <u>weight</u>
- It provide the results <u>digitally</u>, making them an easy tool for use
- The weight can be displayed by <u>different units</u>
- Before waiting any substance, you should (Zero) the balance.
- → What does mean zeroing of the electronic balance?

(mass of paper + substance) - (mass of paper) = (mass of substance)



• A nice video show you how to use the electronic balance: <u>https://www.youtube.com/watch?v=0UymyTJATLc</u>

Practical Part

Objective:

- To be familiar with most common biochemistry lab tools and equipment.
- To compare the accuracy of different glassware.

A. Identification of the common laboratory glassware:

Glassware number	Type of glassware	Final volume (capacity)
1		
2		
3		
4		
5		

B- Identification of the common laboratory pipettes:

- 1. Examine the three pipettes placed on your laboratory bench
- 2. Record their types and the volume of their smallest division

	Type of pipette	Smallest division
Α		
В		
С		

3- Comparing between glassware accuracy:

- 1. Place a beaker in the electronic balance, and read the weight.
- 2. Remove the beaker from the balance, and add 5ml of water using a graduated pipette (Mohr).
- 3. Record the weight.
- 4. Repeat the procedure again by using measuring cylinder this time.
- 5. Record the weight.

Type of glassware	Weight of beaker (g)	Weight of beaker + water (g)	Weight of water (g)	
Graduated pipette				
Measuring cylinder				
Which one is more accurate?				

4- Identification of the common laboratory equipment: A. pH meter:

- 1. Standardize the PH meter by placing the electrode in a solution of known pH (pH 4, 7, 9) \rightarrow Calibration.
- 2. Wash the electrode with distilled water and dry by tissue then put it into sample solution A then wash it again and place it in solution $B \rightarrow Read pH$.

Note: After use the electrode, you should storage it in distilled water and never be allowed to dry out. If the electrode get dry it will required reactivation.

Solution	pH value	Neutral, acidic or basic
Standard 4		
Standard 7		
Standard 9		
Sample A		
Sample B		

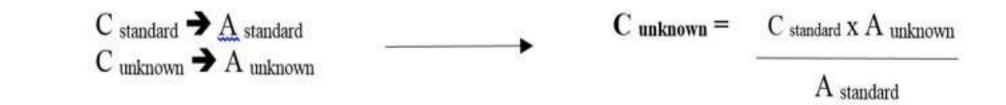
4- Identification of the common laboratory equipment:

B. Spectrophotometer:

- 1. Adjust the spectrophotometer to zero using water as blank solution in the cuvette
- 2. Read the absorbance of standard solution and the solution of unknown concentration at 280 nm
- 3. Read your result

Solution	Absorbance at 280nm
BSA standard solution (0.5 g/100 ml)	
Solution of Unknown concentration	

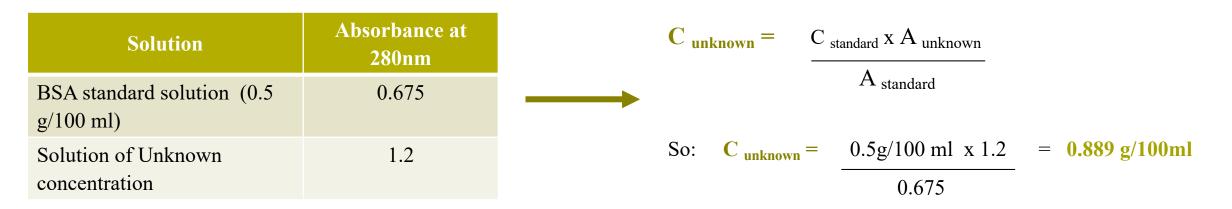
Calculation:



Where:

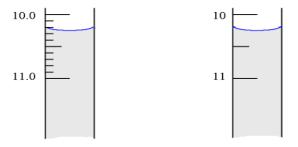
 $C_{standard}$ = concentration of standard solution, $C_{unknown}$ = concentration of unknown solution, $A_{standard}$ = Absorbance of standard solution, $A_{unknown}$ = Absorbance of unknown solution.

Example:



Homework:

1. What is the smallest division for the following:



- 2. Measuring cylinder cannot be a substitute for the pipette or a burette, why?
- 3. What is the meaning of Calibration?
- 4. There are three different solution have pH values 3, 7 and 10:
 - solution 1 is basic (T or F)
 - solution 2 is neutral (T or F)
 - solution 3 is acidic (T or F)
- 5. Why in the invisible range wavelength quartz cuvette is used?