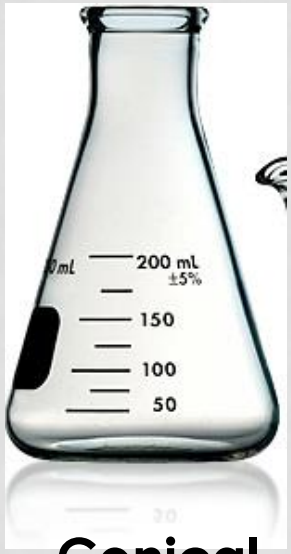
IDENTIFICATION OF GLASSWARE, PH METER, SPECTROPHOTOMETER

AIMS

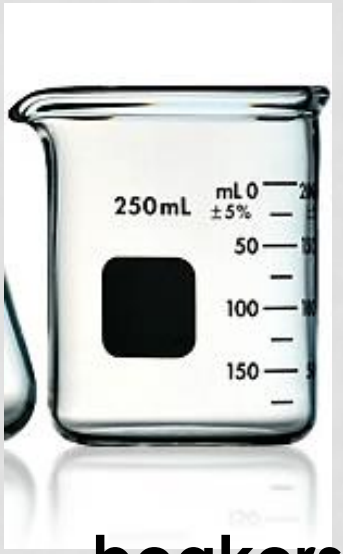
- Identify different glassware and the accuracy of them
- To be familiar with the use of pipetting technique
- To learn how to handle the ph meter and to measure ph values
- To learn how to handle the spectrophotometer

GLASSWARE

- **1-Conical flasks and beakers**:
- They are used for mixing, transporting and reacting,
- but not for accurate volume measurements.



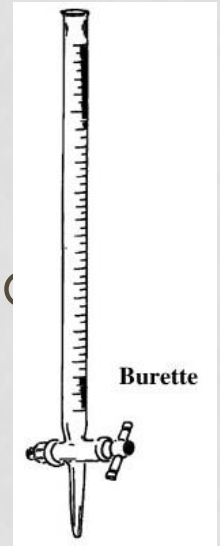
Conical flasks



beakers

- 2-**Graduated cylinders**:
- They are for general purpose use, but not for quantitative analysis.
- If greater accuracy is needed, use a pipette or volumetric flask.

- 3-**Burettes**:
- It is used to deliver solution in precisely-measured variable volumes. Burettes are used primarily for titration



- **4-Volumetric flasks**: It is used to make up a solution of fixed volume very accurately.

- 4) **Pipettes**: A pipette is used to measure small amounts of solution very accurately. Two types of pipettes commonly used are transfer pipettes and measuring pipettes.

PIPETTING TECHNIQUES

Transfer pipettes(volumetric)

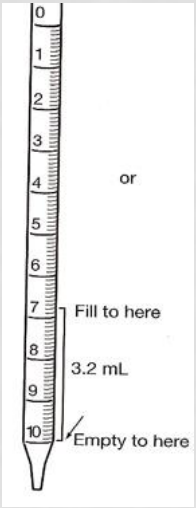
- designed to deliver accurately a fixed volume of liquid
- Not graduated
- consist of a cylindrical bulb joined at both ends to narrowed glass tubing
- More accurate than measuring pipettes
- Non-blown out

Measuring pipettes(graduated)

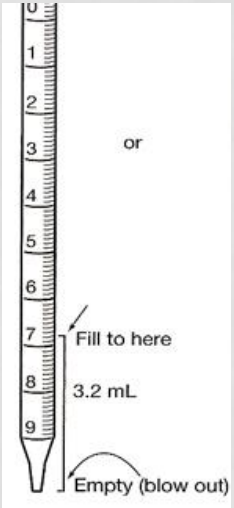
- Graduated
- Some are blown out
- There are two types: Mohr and serological

MEASURING PIPETTES(GRADUATED)

Mohr
Graduated between two marks

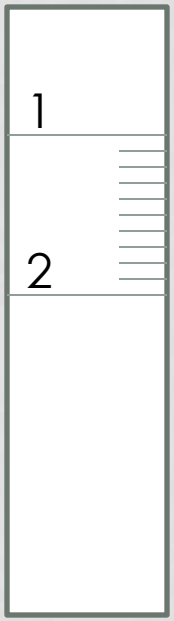


Serological
Pipettes with graduation mark down to the tip

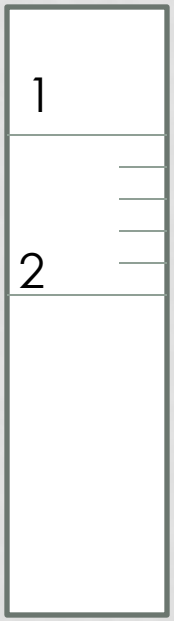


SMALLEST DIVISION OF GRADUATED PIPETTE

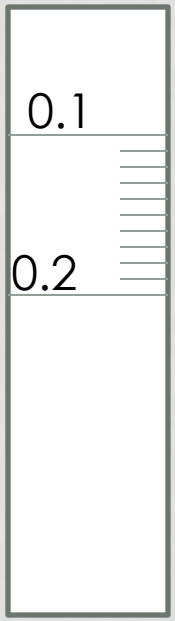
- How to know the smallest division of a pipette:

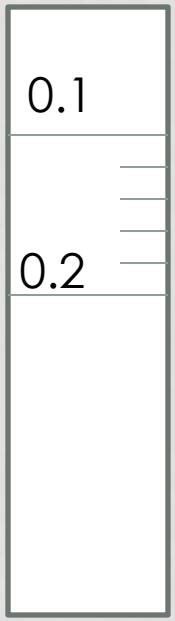


Smallest division: 0.1 ml



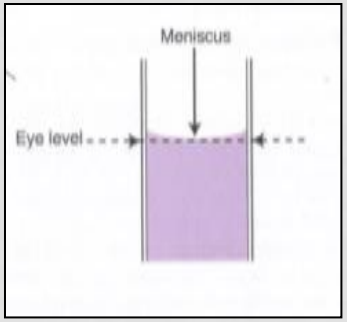
Smallest division: 0.2 ml





HOW TO USE PIPETTES

- The pipette first should be washed with water
- Then rinsed with a little of the solution to be used. Why?
- The bottom of the curved surface is read at eye level and the volume measurement is read to the proper number of significant digits



- The solution is allowed to drain into the appropriate vessel with the jet of the pipette touching the wall of the vessel

PH METER

- Hydrogen ion concentration of many solution is low and difficult to measure accurately.
- So, the term pH introduced as a way of expressing hydrogen ion concentration .
- PH define as the negative logarithm of the hydrogen ion concentration .
- PH = - log10 [H+]

- Since the PH determines many important aspects of the structure and activity of biological macromolecules and thus of the behavior of the cell and organisms .
- Note: PH range value (0 - 14) ,, the higher PH number , the lower the hydrogen ion concentration and vice versa..

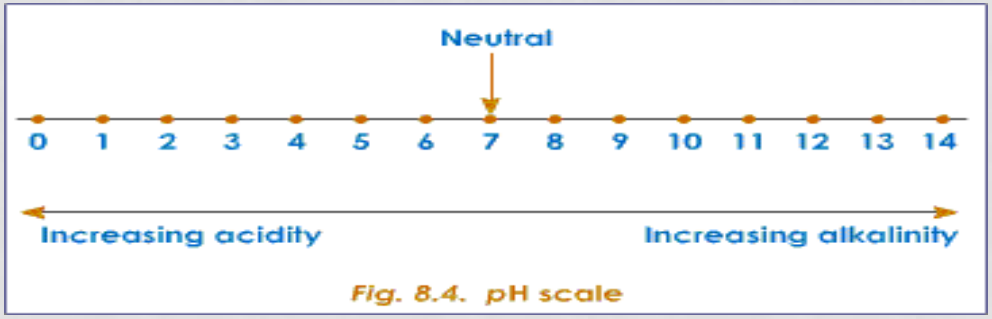
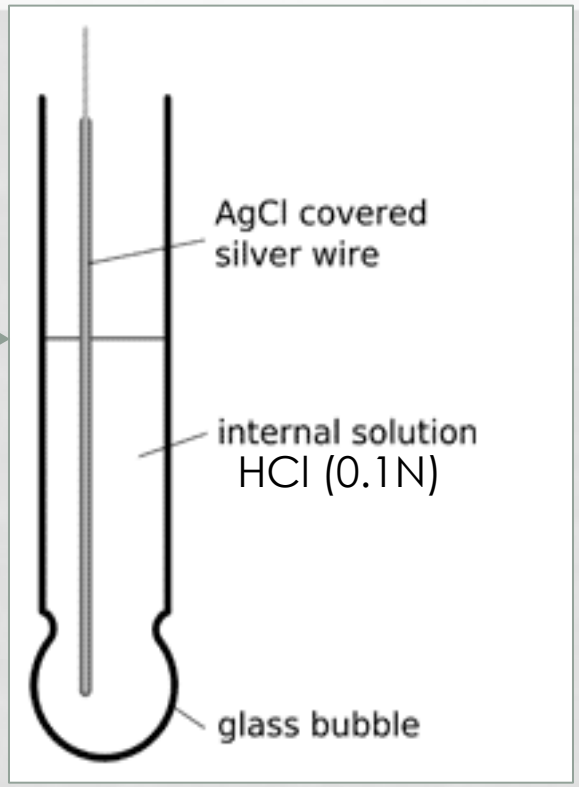


Fig. 8.4. pH scale

- There are many ways in biochemical laboratory to measure PH value such as ; and
- litmus s paper,
- a field kit
- PH meter. The most accurate and reliable method is

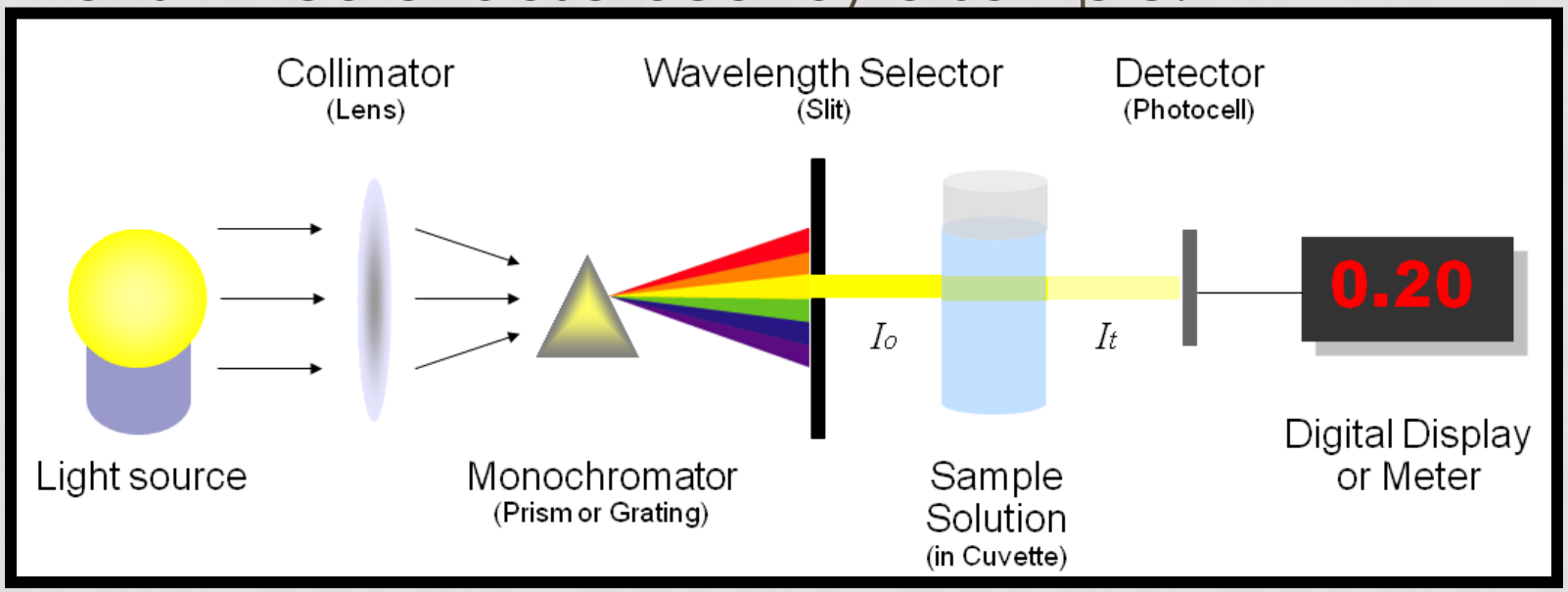


- The glass electrode

Note: before use it needs to be calibrated

SPECTROPHOTOMETER

- spectrophotometer is instrument used to measure the intensity of light at a given wavelength that is transmitted or absorbed by a sample.



*Wavelength in this instrument divided into: -Invisible range(ultraviolet) from 100 to 360 nm [Quartz cuvette are used] -Visible range (400 -700 nm) [Glass or plastic cuvette are used] Blank: contain everything except the compound to be measure.

METHOD

- Four parts:
- 1-Examine the 3 pipettes A,B, and C record their types and smallest division

Pipette	*Type*	*Smallest division*
A		
B		
C		

- Using distilled water, pipette a

	Weight of the beaker	**Weight of beaker +water**	**Weight of water**
1- graduate pipette			
2-measuring cylinder			

- 1-standardize the PH meter by placing the electrode in a solution of known PH(PH 4 , 7 , 9) . 2-Wash the electrode with distilled water and dry by tissue then put it into sample solution A & B , read PH . Note: After use the electrode you should storage it in distilled water and never be allowed to dry out .IF the electrode ge

Solution	*PH Value*
Standard PH 4	
Standard PH 7	
Standard PH 9	
A	
B	

- - Adjust the spectrophotometer to zero using blank solution in the cuvette and read the absorbance of standard solution and the solution of unknown concentration at 280 nm. - Read your result in the table below:

NO.	Solution	Absorbance
1	Standard solution (0.5 gm/100 ml of BSA)	
2	Solution of Unknown concentraton	

- - Calculate the concentration of unknown solution from the following formula: Au x Cs As Where Au= Absorbance of the solution of unknown concentration As= Absorbance of the solution of standard solution Cs= concentration of standard solution
- - Concentration of unknown solution is =