BCH 312

Biochemical Calculations [Practical]

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Mark Distribution

- Report [5 Marks]
- Quiz [7 Marks]
- Final [13 Marks, 8 for practical and 5 for theoretical]

- Final exam date:
  - Monday 30/2/1435H - 22/12/2014

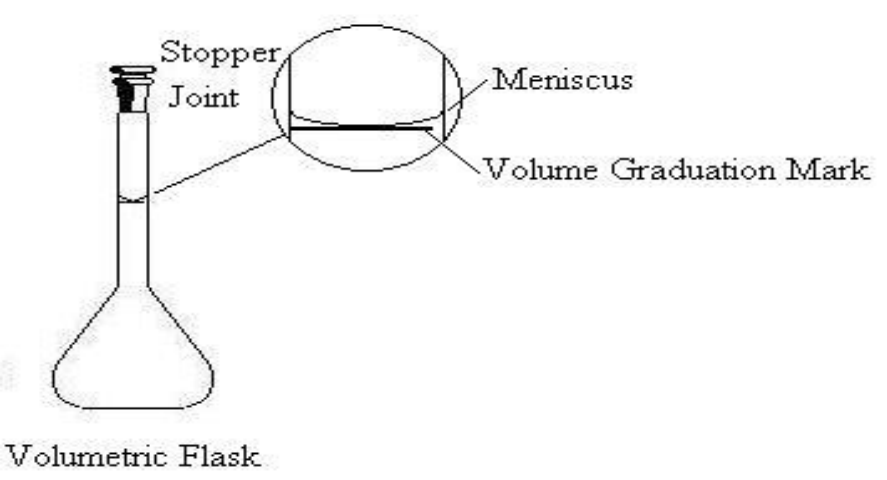
Identification of Glassware, pH meter, and Spectrophotometer

Objectives

- 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

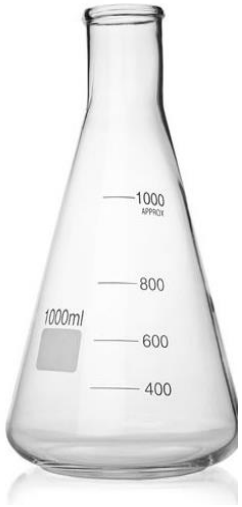
- **Volumetric flask**
  - It is used for preparing large amounts of standard solutions and reagents that require highly accurate concentrations.
  - The most common volumes are 250, 500 ,1000 , and 2000 ml. However smaller volumes are available such as 100, 50 , 25 , and even 1,2, and 5 ml.



Volumetric Flask

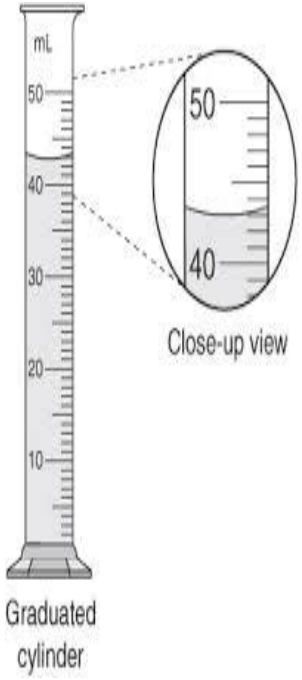
Ehrlenmeyer flasks

- This type of flask is a conical container with multiple volume markings that sever as estimate of volume and is available in a variety of sizes, such as 250, 500 and 1000 ml.
- It is used in solution preparation but are less accurate than volumetric flasks.
- The purpose of an Ehrlenmeyer flask is to help dissolve a solid solute into solution before transfer to a volumetric flask for final volume adjustment



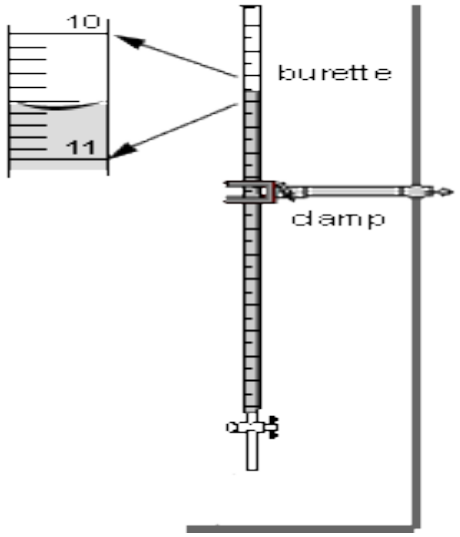
Graduated cylinder

- It is a tall flask with multiple volume gradation.
- The purpose of this flask is to aliquot volumes of a fluid in making up a reagent dilution or to determine the volume of an unknown fluid.



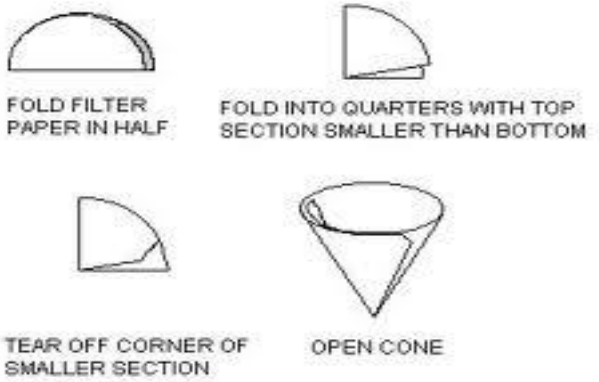
Beakers

- They are used to hold stock solutions or diluents for short-term storage or during laboratory procedures.
- They are available in a variety of total volume sizes, but have the least accuracy of the three types of flasks.



Burette and its stand

Glass rod



Filter paper

Funnel

Pipettes

- **There are two main type of pipettes are used in biochemical laboratory:**

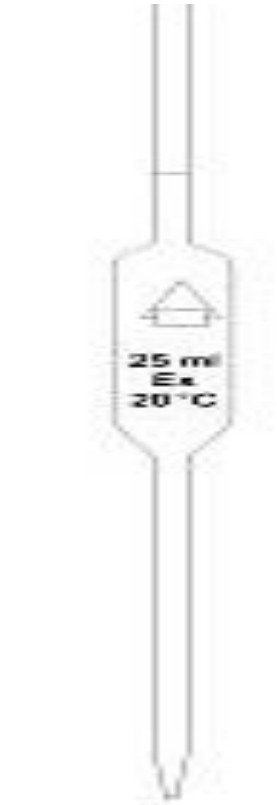
a) **Volumetric or transfer pipettes**

- They are designed to deliver a fixed volume of liquid
- It is consists of a cyclindrical bulb joined at both ends to narrowed glass tubing.
- Non-blown out
- More accurate than measuring pipettes

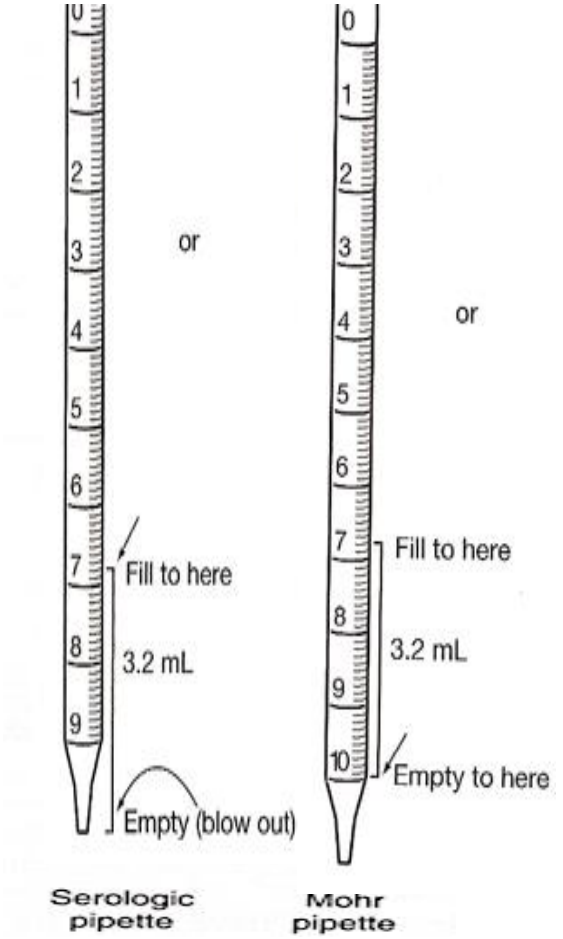
b) **Graduated or measuring pipettes**

- These consists of a plain narrow tube drawn out to a tip and graduated uniformly along its length.
- Some are blown out
- There are two types of graduated pipettes are available:
  1-Mohr, graduated between two marks
  2-Serological pipettes with graduation marks down to the tip.

Pipette Types



Volumetric pipette

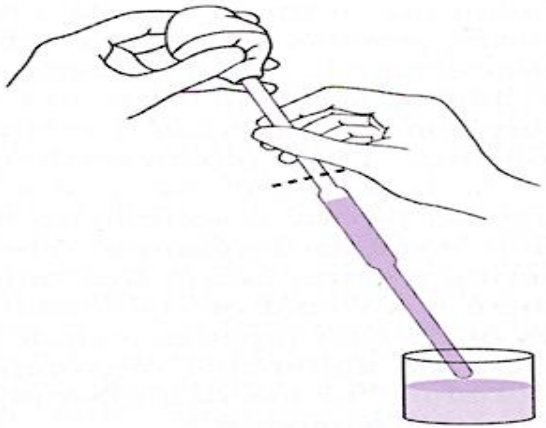


Graduated pipettes

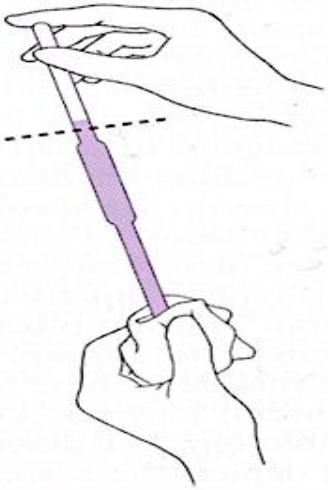
How to use pipettes

- The pipette is first washed with water ,then rinsed several times with a little of the solution to be used and finally filled to just above the mark , the liquid is allowed to fall to the mark .
- The solution is allowed to drain into the appropriate vessel with the jet of the pipette touching the wall of the vessel .
- After the flow of the liquid has stopped, the jet is held against the wall for some times and then removed .

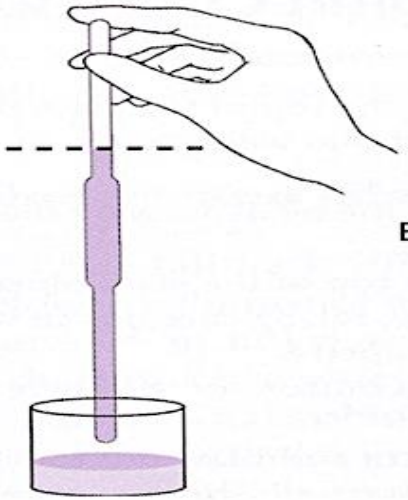
1. Use mechanical suction

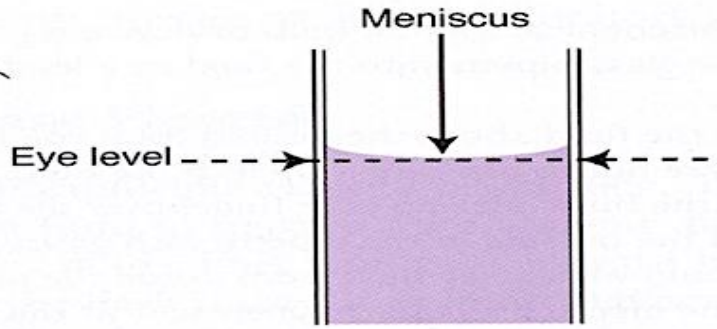


2. Wipe off outside of pipette with gauze



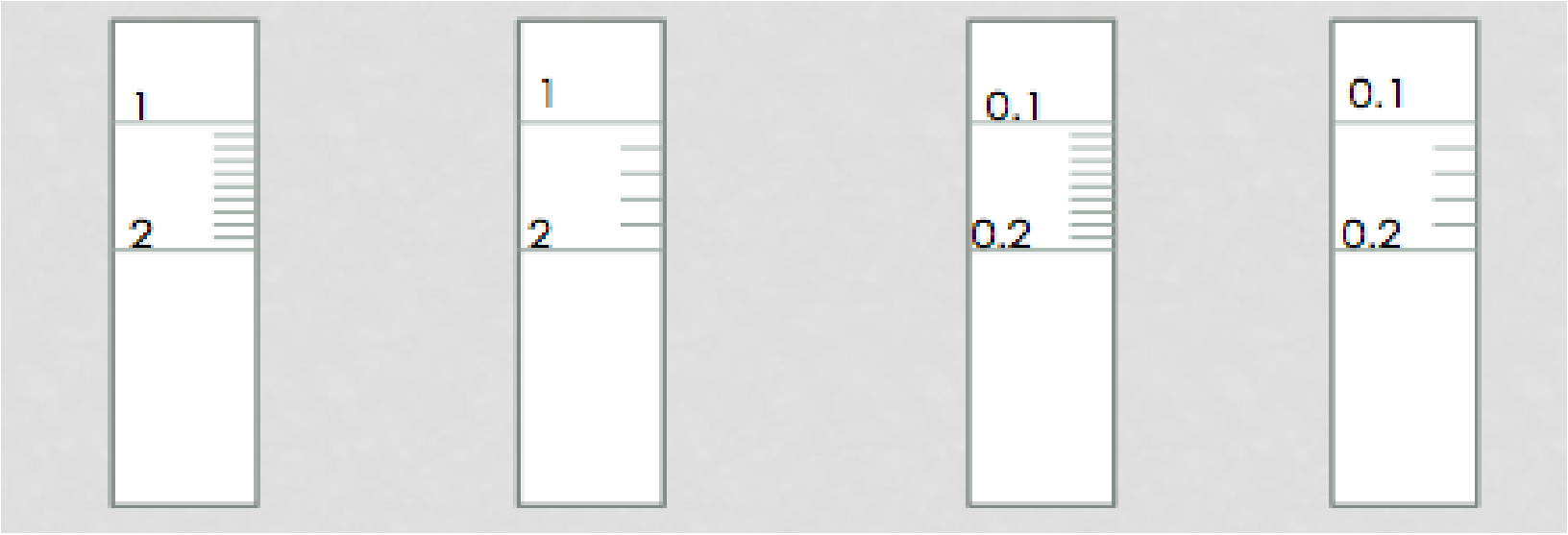
3. Adjust the meniscus; read meniscus at the bottom of the curved liquid





Smallest division of graduated pipette

- How to know the smallest division of a pipette:



Smallest division: 0.1 ml

Smallest division: 0.2 ml

Smallest division: 0.02 ml

Smallest division: 0.02 ml

Method

Part 1

- Examine the 3 pipette placed on your laboratory bench .
- Record their types and the volume of their smallest division .

Pipette	Type	Smallest division
A		
B		
C		

Part 2

- By using distilled water , pipette into weighted beaker
  - with 5ml graduate pipette(Mohr) =5ml water
  - with 5ml measuring cylinders =5ml water

	Weight of the beaker	Weight of beaker +water	Weight of water
graduate pipette			
measuring cylinder			

Laboratory Equipment

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+]**

- pH range value (0 – 14), the higher pH number, the lower the hydrogen ion concentration and vice versa

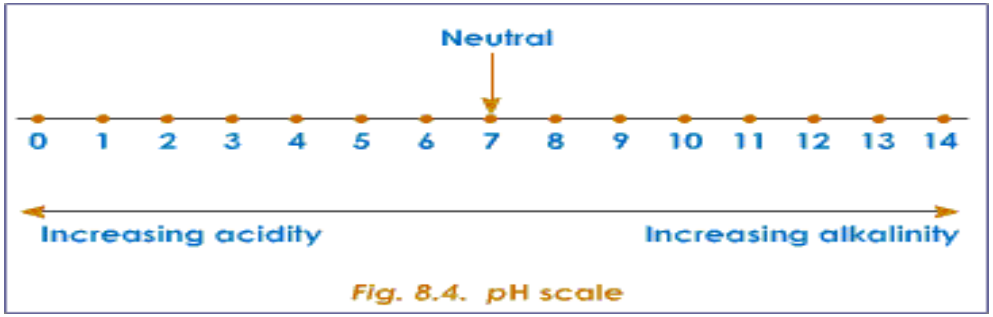
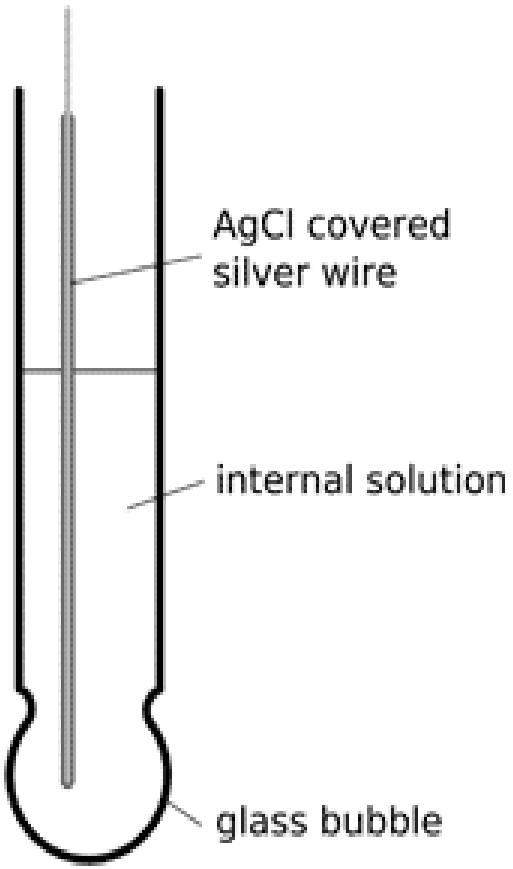


Fig. 8.4. pH scale

Glass electrode

- The glass electrode consist of a very thin bulb ,blown onto a hard glass tube.
- The bulb is made of high conductivity glass which is sensitive to pH.
- The bulb contains a solution of hydrochloric acid (0.1N) and is connected to a platinum lead via silver - silver chloride electrode which is reversible with respect to hydrogen ions .
- The glass electrode is very sensitive and readily responds to changes in hydrogen ion concentration .



Method

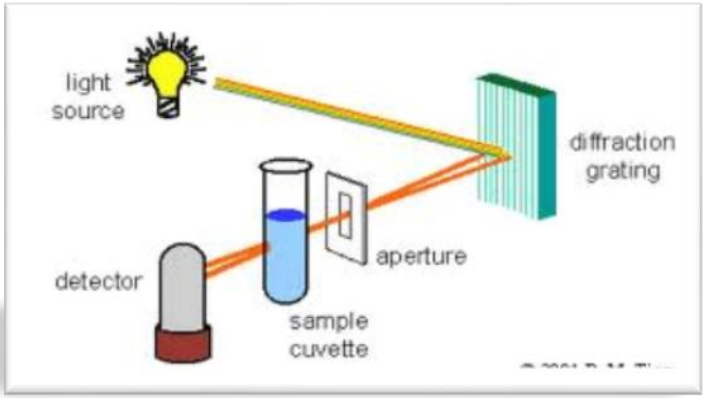
- Standarize the PH meter by placing the electrode in a solution of known PH(PH 4 , 7 , 9) .
- Wash the electrode with distilled water and dry with a tissue then put it into sample solution A & B , read PH .
- **Note:** After use the electrode you should storage it in distilled water and never be allowed to dry out . If the electrode get dry it will required reactivation.

Solution	pH Value
Standard 4	
Standard 7	
Standard 9	
A	
B	

Spectrophotometer

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

- It consist of two parts:
  - **Spectrometer** is designed to emit the light at different wavelength
  - **Photometer** contains photoelectric cell and the potentials are recorded on a scale which read out as absorbance or transmittance.



Wavelength in this instrument is 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: It contains everything except the compound to be measured.

Method

- 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 concentration	

- **Calculate the concentration of unknown solution from the following formula:**

$$Cu = (Au / As) \times Cs$$

Where:

Au= Absorbance of the solution of unknown concentration

As= Absorbance of standard solution

Cs= Concentration of standard solution

Cu= Concentration of the solution of unknown concentration