Experiment- 5

Sugar identification using Polarimetry

- Approximately 25% of all drugs are marketed as either racemates (mixtures of two enantiomers) or mixtures of diasteromers.
- The drug Thalidomide, was prescribed to millions of women to suppress morning sickness associated with pregnancy during the late 50's and early 60's.
- Horrible birth defects, including missing limbs, resulted. The cause of these birth defects was assigned to the S-enantiomer, which did not undergo clinical trials.
- Chiral synthesis and purification is a crucial aspect of all successful drug manufacture.

- **Chirality**

Chiral molecules have an asymmetrical center which respond to light as a lens and rotate the light. The ability to rotate light is termed **optical activity**.

Enantiomeric compounds rotate light by exactly the same amount but in the opposite direction. The degree to which a substance rotates light may be used to determine:

a) the identity of the substance

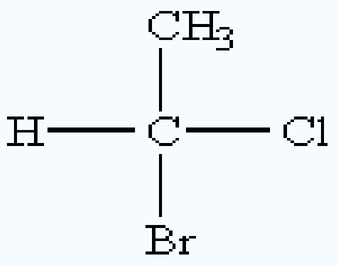
b) the enantiomeric purity of the substance

c) the concentration of a known substance in a solution.

An object is considered to be **chiral** if it is *nonsuperimposable* on its mirror image.
An object is considered **achiral** if it is superimposable on its mirror image.
All objects can be classified as chiral or achiral.

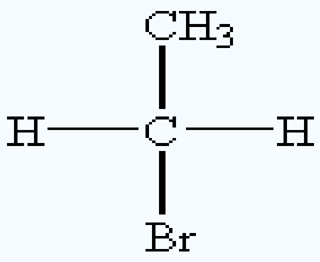
(b) Achiral objects

A molecule is considered chiral if there exists another molecule that is of identical composition but which is arranged in a non-superposable mirror image. Also the presence of an asymmetric carbon atom is often the feature that causes chirality in molecules.



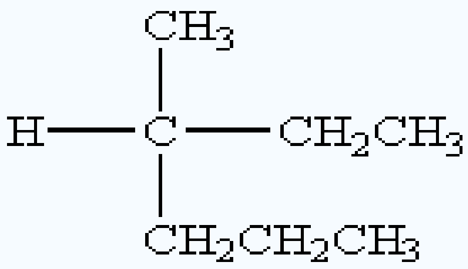
chiral

Has 4 differnt atoms bonded to the carbon



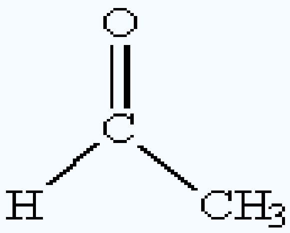
achiral

Does not have 4 different atoms or groups bonded to the carbon (2 hydrogens)



chiral

Has 4 differnt groups bonded to the carbon



achiral

Only has 3 atoms bonded to the carbon

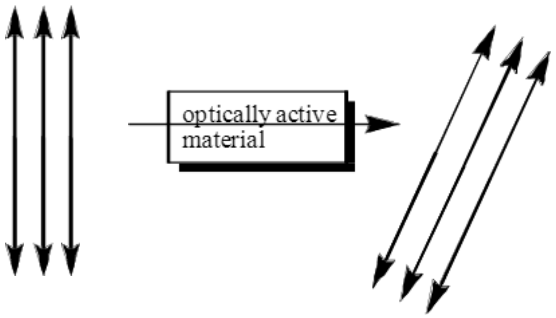
Two mirror images of a chiral molecule are called enantiomers or optical isomers. Pairs of enantiomers are often designated as "right-" and "left-handed".

- Polarimetry is used in many ways like dealing with process control in the pharmaceutical industry, flavor, fragrance and essential oil industry, food industry, or chemical industry.
- optical purity of the product: measuring the specific rotation of compounds like amino acids, antibiotics, or steroids and comparing them with reference values.

The purpose of this lab is to become familiar with use of a polarimeter and to use optical rotation as a method of determining the identity of unknown sugars.

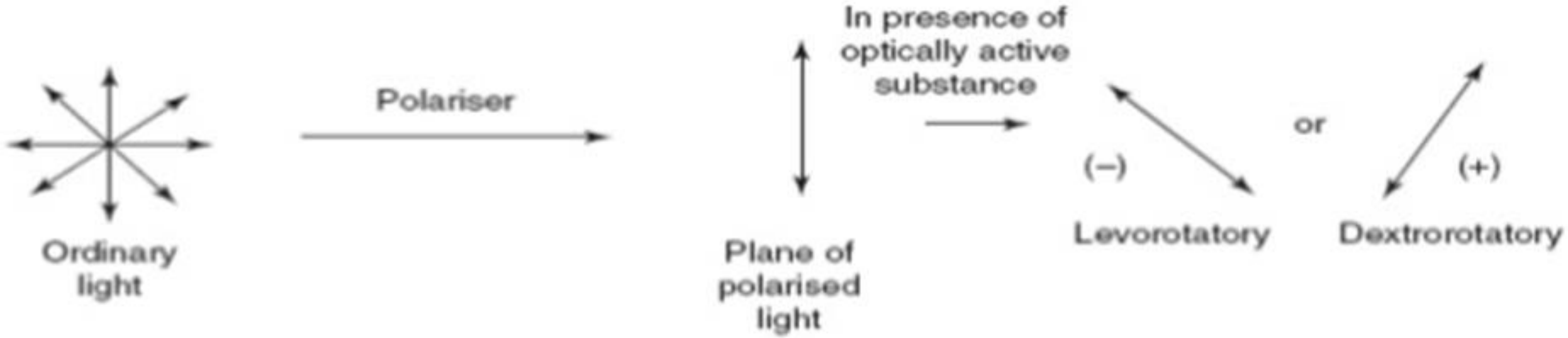
When a beam of plane polarized light passes through a solution of optically active material the light will rotate.

Each pure chiral material has a set specific rotation [c‹] which is a fixed physical characteristic for that material. The specific rotation describes how far a material will rotate light. A [c‹] of **+87.6** means that pure material will rotate light in the clockwise manner by 87.6 degrees.
The enantiomer will rotate the plane of polarized light by exactly the same amount but in the opposite direction. **An [c‹] of -87.6** would result in a rotation in the counterclockwise direction by 87.6 degrees.



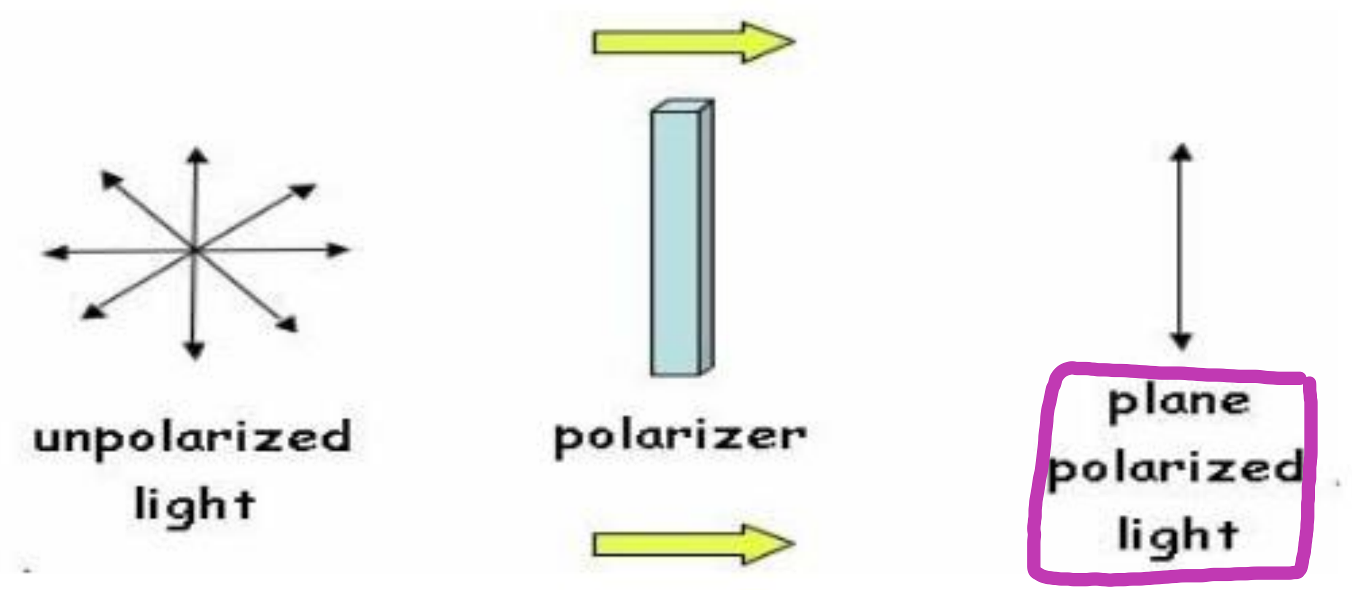
INTRODUCTION

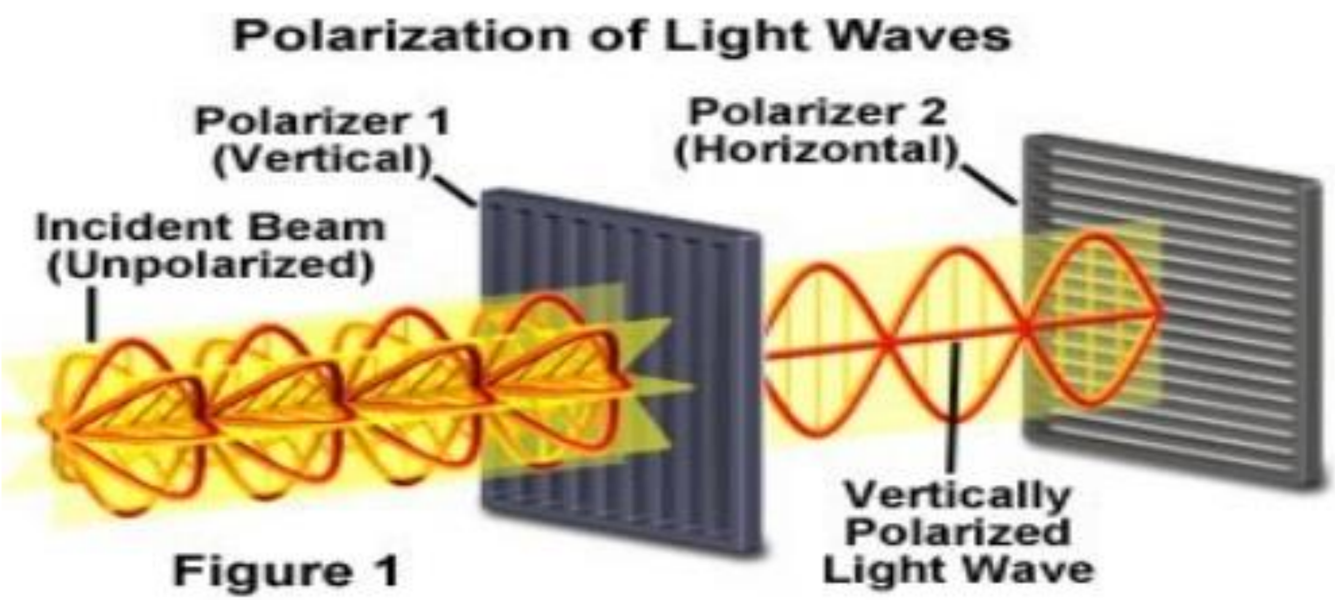
Polarimetry is one of the important instrumental methods employed in analysis. This measures the rotation of the polarized light as it passes through an optically active compound. **This technique involves the measurement of change in the direction of vibration of polarized light when interact with an optically active compound**. A substance is said to be optically active if it rotates the plane of the polarized light.

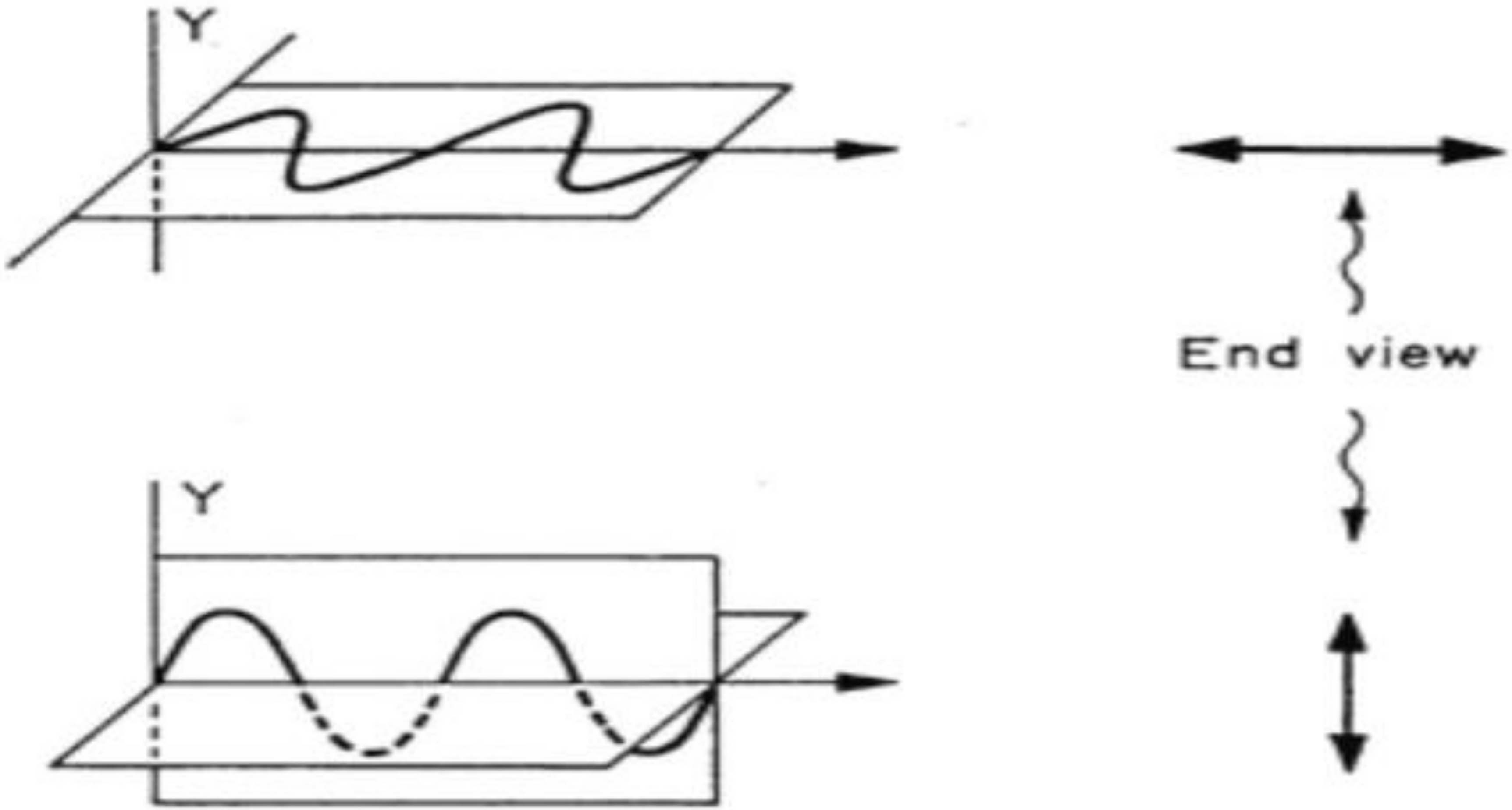


PLANE POLARISED LIGHT

According to wave theory of light, an ordinary ray light is considered to be vibrating in all planes at right angle to the direction of propagation. If this ordinary ray of light is passed through a Nicol prism, the emergent ray has its vibration only in one plane. This light having wave motion in only one plane is known as Plane Polarised Light.

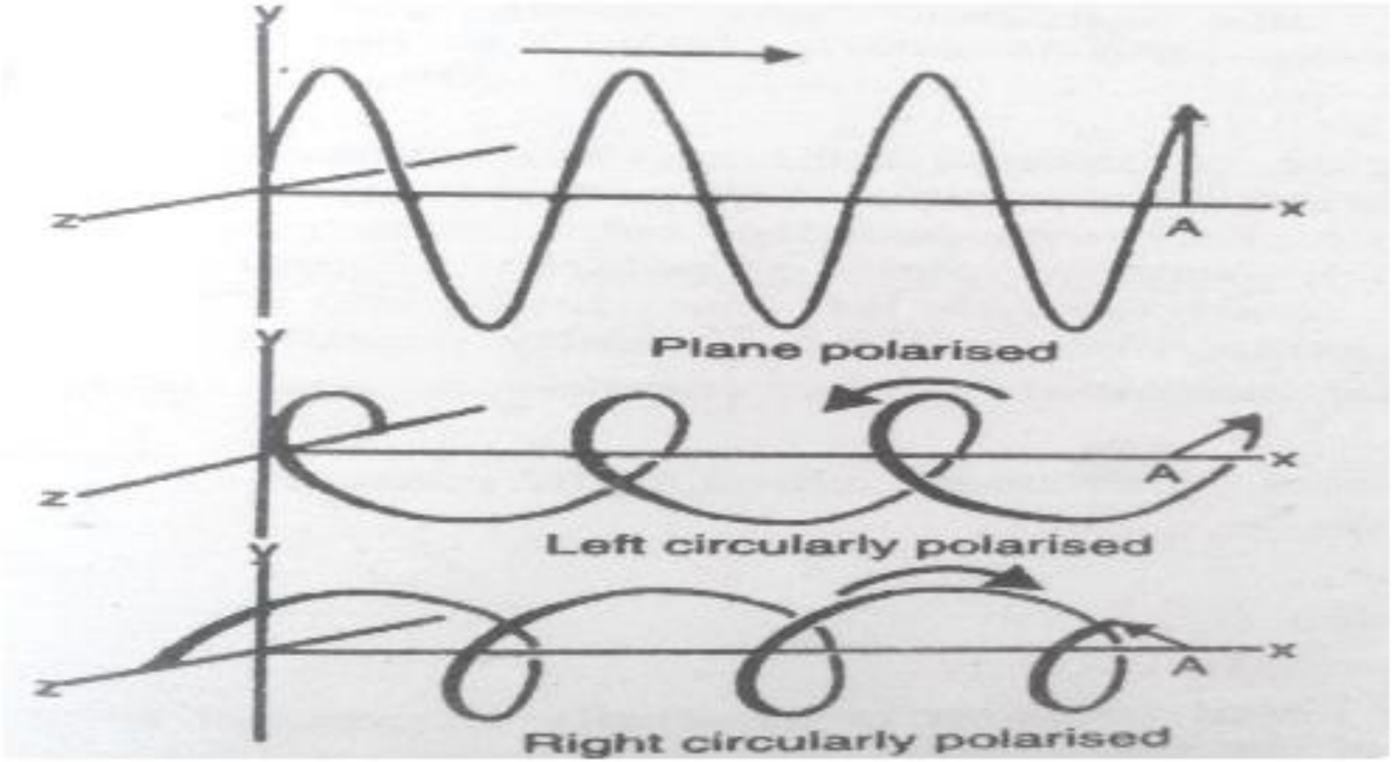

unpolarized
light
polarizer
plane
polarized
light


Polarization of Light Waves
Polarizer 1
(Vertical)
Polarizer 2
(Horizontal)
Incident Beam
(Unpolarized)
Vertically
Polarized
Light Wave
Figure 1



Linearly polarized light

- A plane polarized light which consists of two components of fixed magnitude rotating in opposite directions to one another;
- The right circulatory polarized light
- The left circulatory polarized light
- Plane polarized light is the vector sum of these two components



OPTICAL ACTIVITY

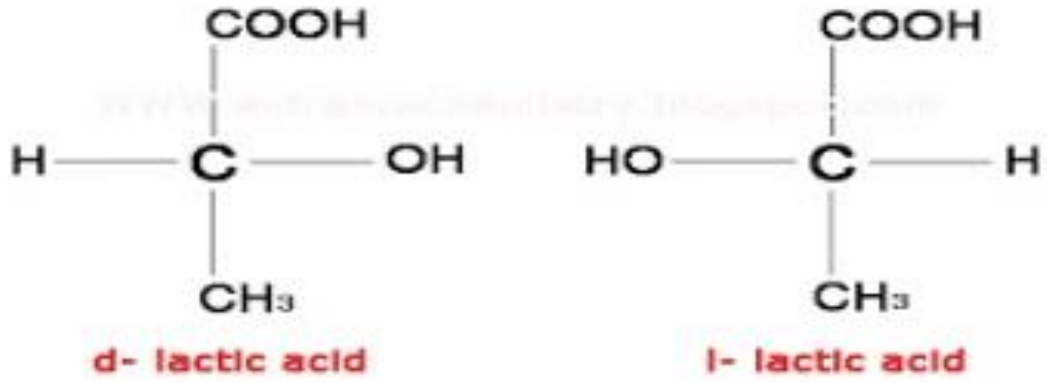
- **Definition**
- It Has find that magnitude of rotation depend upon the following factors:

1. Nature of Substance
2. Length of liquid column (l) through which light passes.
3. Concentration of the solution.
4. Nature of the solvent.
5. Temperature of the solution (t)
6. Wavelength of the light used

Theory of Optical Activity

- An optically active substance is one that rotates the plane of polarized light

  Example: (1) Lactic acid



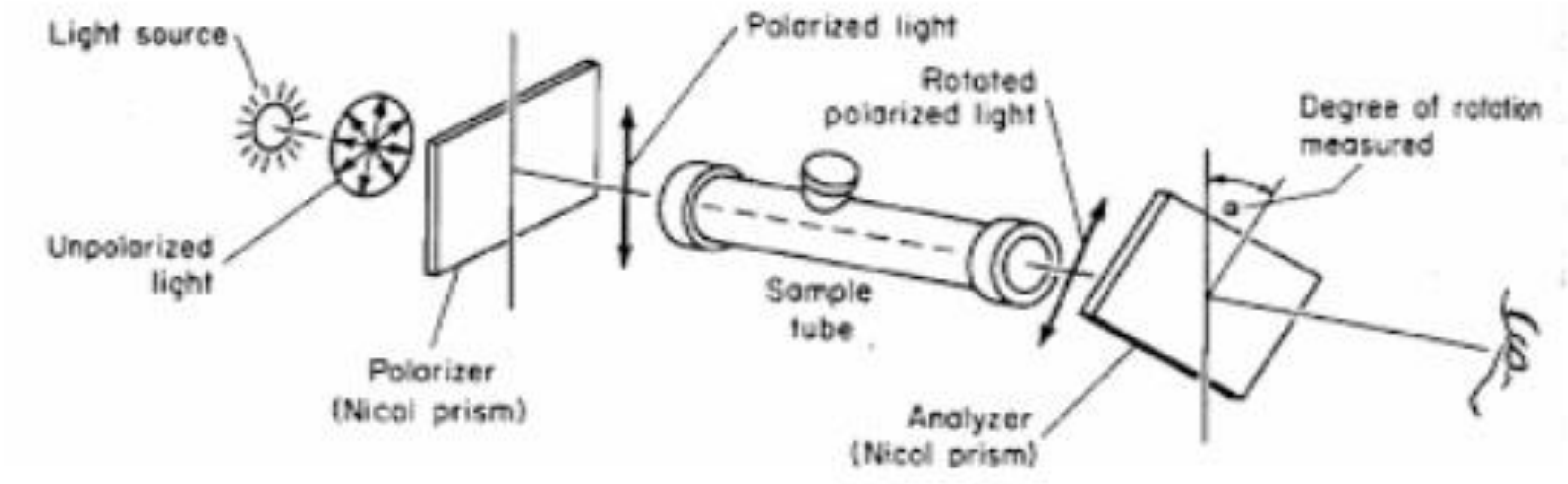
  Dextro= right designated by 'd', (+), clockwise

  Levo= left designated by 'l', (-), counterclockwise

- **<u>Configuration:</u>**

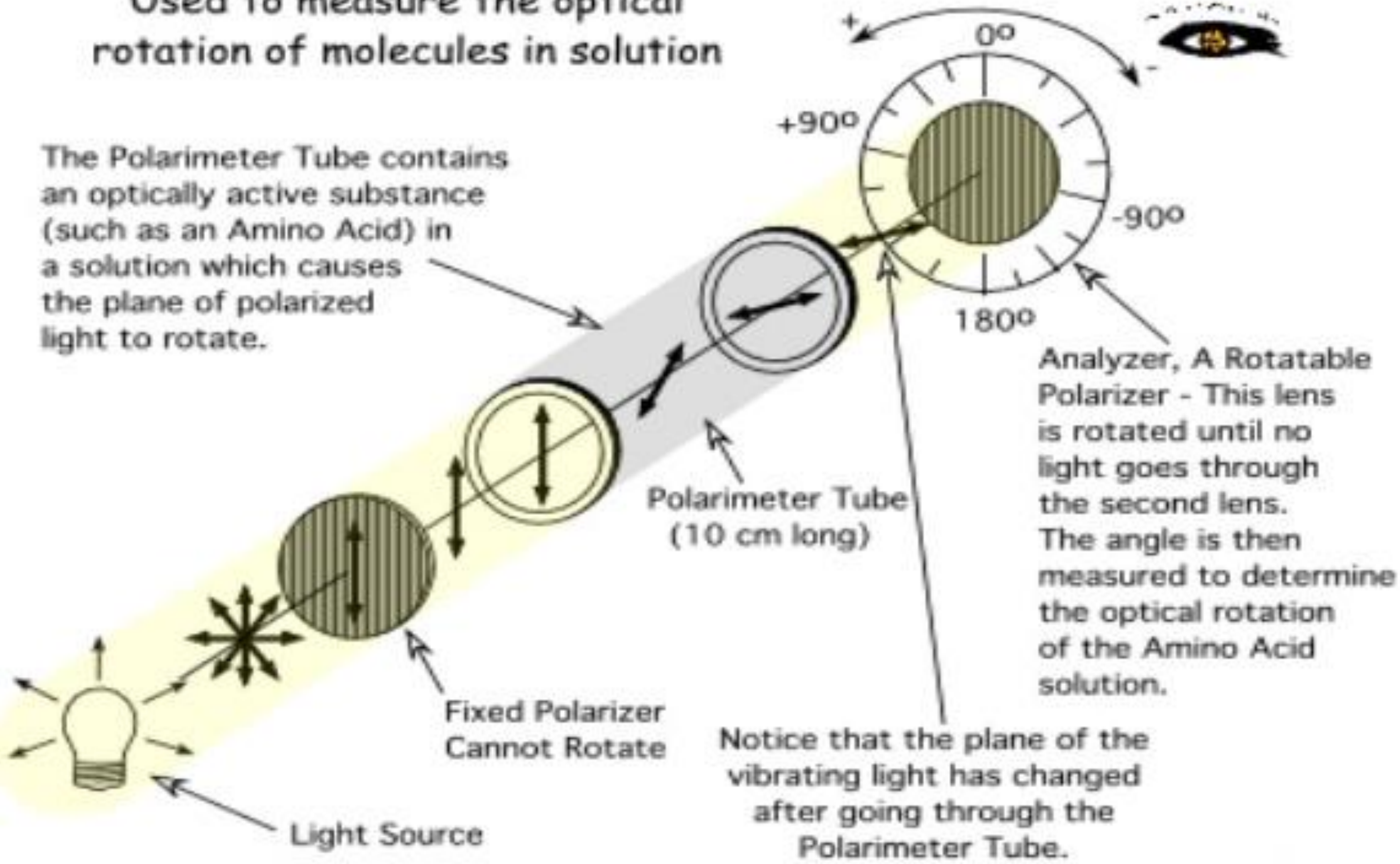
INSTRUMENTATION

- Optical activity may be detected & measured by an instrument → Polarimeter

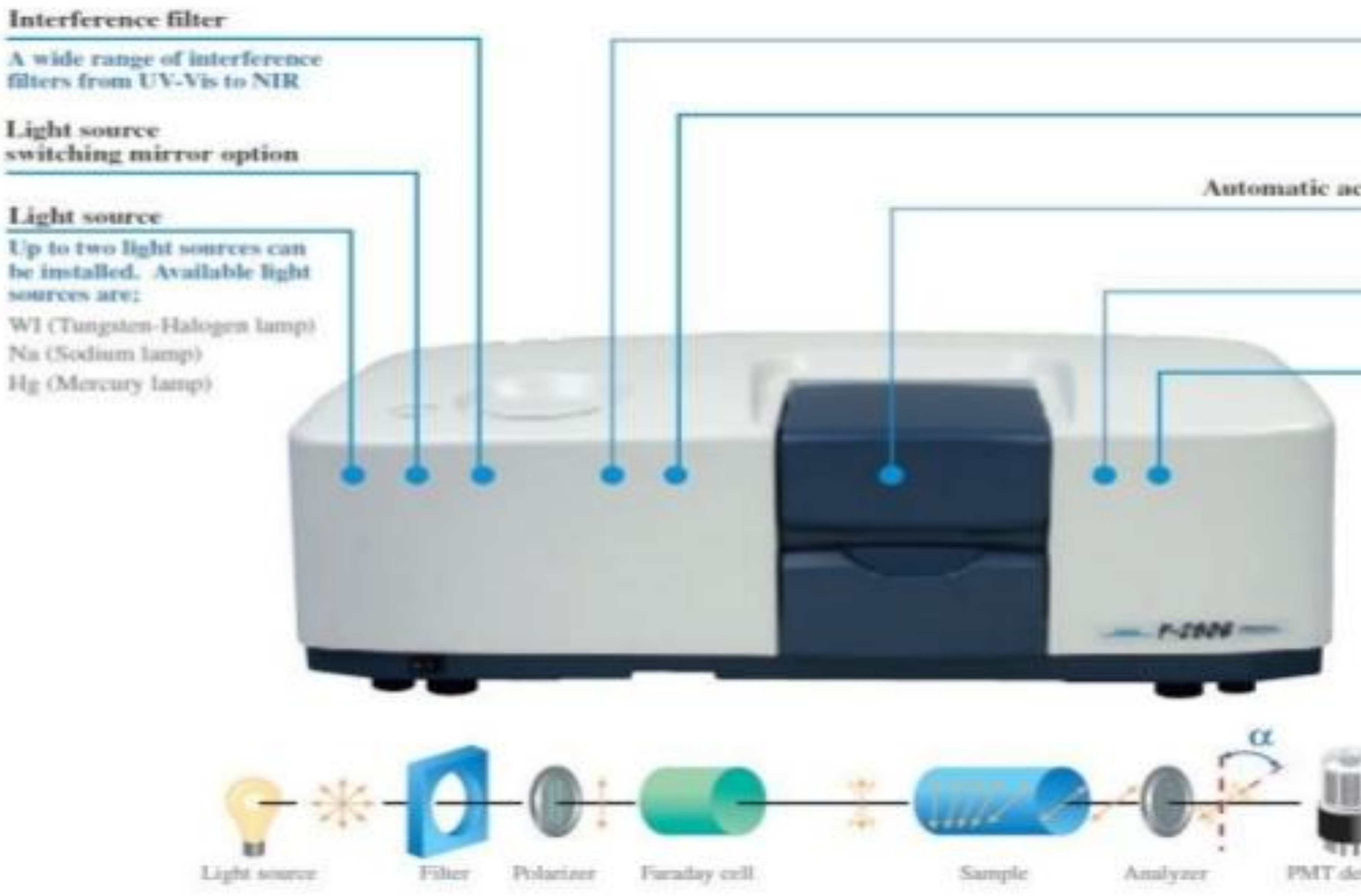


Polarimeter

Used to measure the optical rotation of molecules in solution

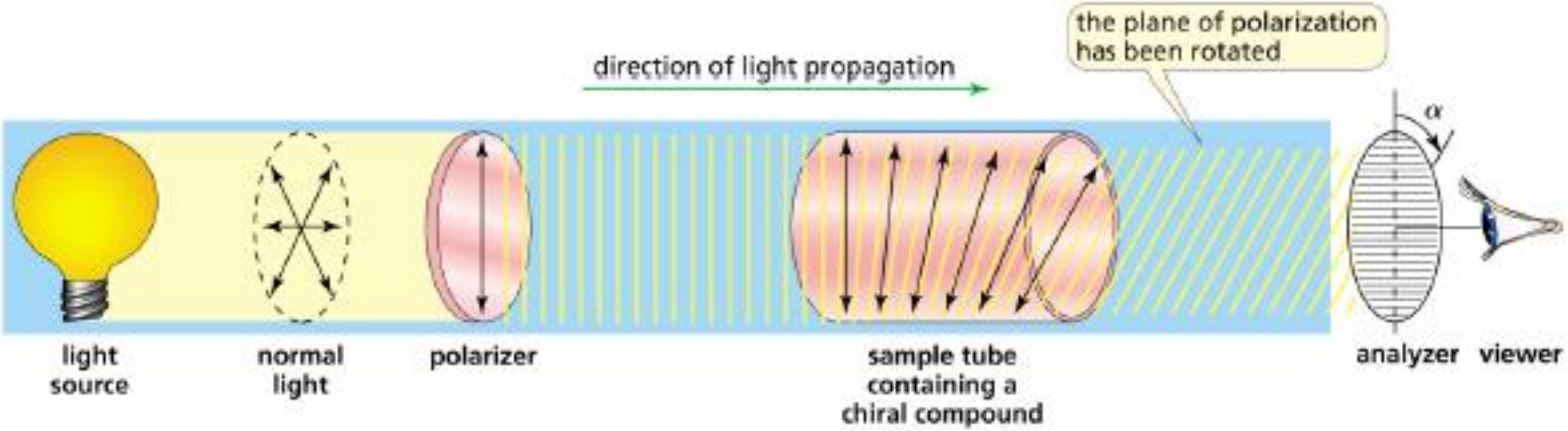


- <u>Light source</u> – Sodium vapor lamp
- <u>Analyzer</u>– another Nicol prism aligned to intercept the linearly polarized ray as it emerges from the sample solution
- A polarimeter consists of a polarized Light source – Sodium vapor lamp, Polarizer –quartz prism material and Analyzer – Nicol prism aligned to intercept the linearly polarized ray as it emerges from the sample solution, a graduated circle- to measure the rotation angle, and sample tubes.



digital-polarimeter

A Polarimeter



$$[\alpha]_{\lambda}^{T} = \frac{\alpha}{l \times c}$$

$$[\alpha] = \alpha_{obs} / (c \ * \ \ell)$$

$[\alpha]$= specific rotation of the compound

α_{obs} = observed rotation of light in degrees

c = concentration in **grams per milliliter** (g/ml)

ℓ= cell length in decimeters (dm)

The specific rotation ([α]) of a compound is a fixed physical property of that compound (as is its boiling point or melting point or density). The observed rotation (α obs) depends on the concentration of the sample in solution (c) and the length of the cell (ℓ) as well as the specific optical rotation of the compound [α].

Doubling the concentration of a material in a solution will double the observed rotation. Cutting the cell length in half will half the observed rotation. The specific rotation [α] remains the same regardless of the concentration or cell length.

$$[\alpha]=\alpha_{obs} / (c \ * \ell)$$

[α]: specific rotation of the compound
(α obs): observed rotation of light in degrees
C: concentration in grams per milliliter (g/ml).
ℓ: cell length in decimeters (dm)

What is the specific rotation of a compound 'X' when the concentration is 0.5% w/v, angle of rotation is 1.3 and tube length is 25 cm?

- ○ 10.4°
- ○ 52.0°
- ● 104.0°
- ○ 5.2°

Angle of rotation (α) = 1.3° , Tube length = 25 cm = 2.5 dm

Concentration = 0.5% w/v

Formula

$$[\alpha]_{\lambda}^{T} = \frac{100 \times \alpha}{l \times c}$$

$$[\alpha]_{\lambda}^{T} = \frac{100 \times 1.3}{2.5 \times 0.5}$$

$$[\alpha]_{\lambda}^{T} = 104.0^{\circ}$$

Enantiomeric Excess

Enantiomeric excess tells us how much of an excess of one enantiomer is in a mixture.

$$\text{enantiomeric excess} = \frac{\text{observed specific rotation}}{\text{specific rotation of the pure enantiomer}} \times 100\%$$

For example, if the sample of 2-bromobutane has an observed specific rotation of +9.2, then the enantiomeric excess is 40%. In other words, the excess of one of the enantiomers comprises 40% of the mixture.

$$\text{enantiomeric excess} = \frac{+9.2}{+23.1} \times 100\% = 40\%$$

optical purity = % enantiomeric excess
= % of one enantiomer - % of other enantiomer

2. Calculate the % ee for the following:

100% 0%

a) a solution containing a single enantiomer

$$\% ee = 100 - 0 = 100\%$$

b) a solution containing equal amounts of both enantiomers (racemic mixture)

$$\% ee = 50\% - 50\% = 0$$

c) a solution containing 75% of one enantiomer and 25% of the other enantiomer

$$\% ee = 75 - 25 = 50\%$$

So we have 50% excess of this enantiomer,

$$\% \text{ ee} = \frac{\text{observed } [\alpha]}{[\alpha] \text{ of pure enantiomer}} \times 100$$

% ee = 93% − 7% = 86%

3. A mixture of natural cholesterol and its enantiomer has a specific rotation (at 20°C) of -27. Calculate the % ee of this mixture. What % of the mixture is natural cholesterol?

$\boxed{93\%}$

$$\% \text{ee} = \frac{-27}{-31.5} \times 100 = 86\%$$ nat cholesterol

14%

7% nat chol 7% enantiomer

So that's just a nice
little check to make sure

Example 1: If a mixture of this R and S has an observed rotation (α_{obs}) of +3.97, then the percent composition is calculated as follows.

- The overall rotation is positive so there is an excess of the S enationmer.
- We know from the above equation that ee =α_{obs} /[c‹] x 100%,
- therefore ee =3.97/16.7 * 100 = 23.8%.
- This means that the rest of the material (100.0 — 23.8 = 76.2%) is equally divided between the R and S (100 % - % e.e. = % of balanced material).
- Therefore, there is 76.2/2 or 38.1% R material,
- and 38.1% +23.8 % = 61.9% S material.
- To check, 61.9% + 38.1% = 100 %,
- the difference between the two is 23.8%.

- **Example 2:** If a pure R material has a specific rotation of -16.70
- a mixture of 40% R and 60% S will result in a 20% enantiomeric excess (The 40%R cancels 40% of the S, and leaves 20% excess S).
- The 20%e.e. is then divided by 100 =0.20,
- and multiplied by the absolute value of [u] of |-16.70| or 16.70 to show this mixture will rotate light by =|3.35|.
- Knowing that the R material rotates in the negative direction,
- and knowing that this mixture has an excess of the S enantiomer, means that this material will rotate light in the positive or clockwise direction by 3.35 degrees.

e.e. = |R-S| / (R+S) * 100%= |60-40|/(60 +40) *100% = 20%

20% of |-16.70| = |3.35|, and an excess of S means +3.35.

Practicallities

If the two enantiomers are not present in equal amounts, the amount of this enatiomeric excess (ee) may be calculated using the observed rotation (α_{obs}) compared to the specific rotation [α].

$$e.e. = |R-S| / (R+L) \;\; * \; 100\% = |\alpha_{obs}|/|[\alpha]| \; * \; 100\%$$

e.e. = |R-S| / (R+S) * 100%= |60-40|/(60 +40) *100% = 20%

20% of |-16.70| = |3.35|, and an excess of S means +3.35.

Procedure and Observations:

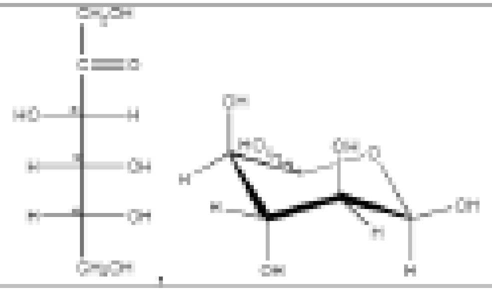
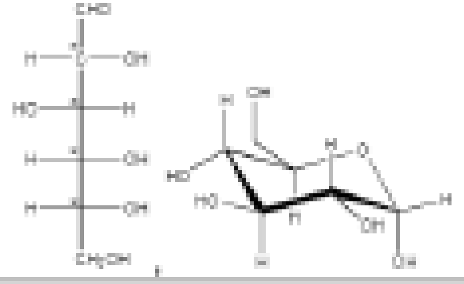
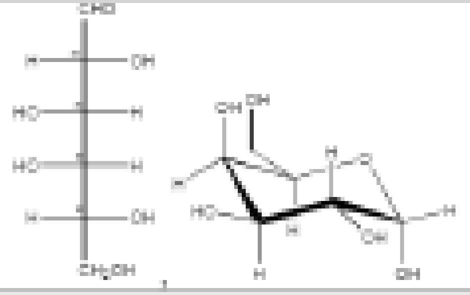
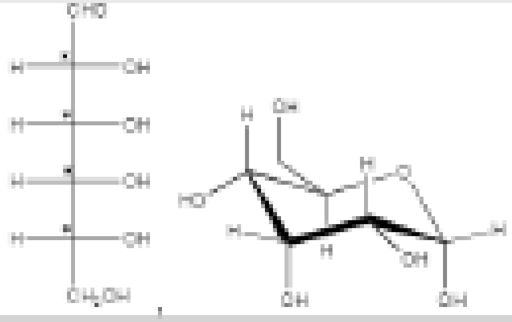
- In this experiment,
- to become familiar with using a polarimeter and how to use optical rotation as a method of determining the identity of unknown sugars.
- The ability to rotate light is optical activity. The degree that a substance rotates light can be used to determine the identity of the substance, the enantiomeric purity of the substance, and the concentration of a known substance in a solution.

- **Part 1:**
- In this part sugar solutions of known concentration (c) will be prepared (note the units of concentration used). The observed rotation (α obs) will be obtained by using a polarimeter. This data will be used to calculate the specific rotation [α] and the identity of the sugars will be hypothesized.
- Weigh out approximately 1 g of unknown sugars. Record all the digits of this mass in your notebook.
- In 50ml beaker, dissolve your material in approximately 15ml of deionized water. Swirl the contents until all of the solid has dissolved.

1. Carefully transfer this solution to a 50 ml volumetric flask.
2. Rinse beaker with approximately 2 ml of deionized water. Transfer this solution to volumetric flask. Repeat.
3. Carefully drop wise add deionized water to volumetric flask until bottom of meniscus is exactly on line. This is your solution.
4. In notebook, calculate the concentration in g/ml.

1. Obtain the (α obs) by analyzing your solution in the polarimeter using the instructions mounted adjacent to machine and reviewed by your instructor.
2. calculate the specific rotation [c‹].
3. Select the identity of your material from the list provided.

Chemicals:

Name (other names)	Structure (Fisher, Haworth)	Specific Rotation [α]
D- Fructose (D-Levulose)		-86
D-Glucose		+98
D- Galactose		+ 82
D-Allose		+15
Sucrose	glucose-fructose	+64.5
Maltose	glucose-glucose	+118

Part 2:

In this part a sugar solution of unknown concentration (c) but known identity will be given.

The observed rotation (α obs) will be obtained by using a polarimeter. This data in addition to [α] from part 1 will be used to calculate the concentration of the unknown sample.

1. Obtain the (α obs) by analyzing your unknown solution in the
2. Using the above equation and the previously calculate specific rotation [c‹] in part 1 calculate the concentration of your unknown.