CLS 281

Basic Biochemistry and Biomolecules

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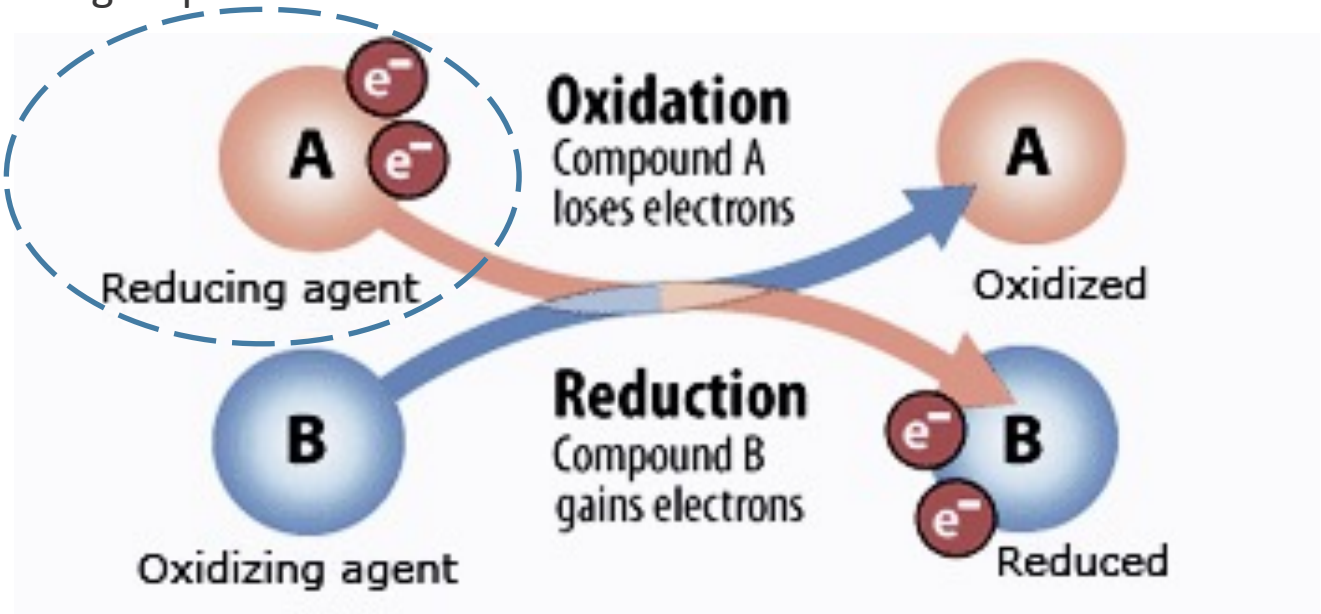
King Saud University
1957

Experiment 7

Determination of Reducing Sugars by Somogyi-Nelson Method

Reducing Sugars

- A reducing sugar is a sugar with a free or potentially free aldehydic or ketonic group.

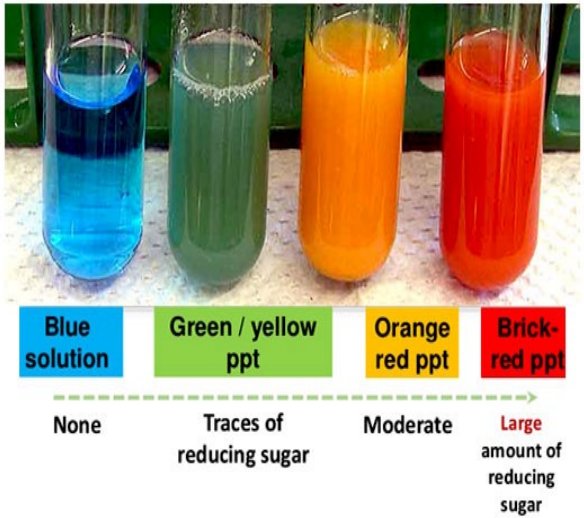


- Oxidation is the loss of electrons
- Reduction is the gain of electrons

Review of Reducing Sugars Test

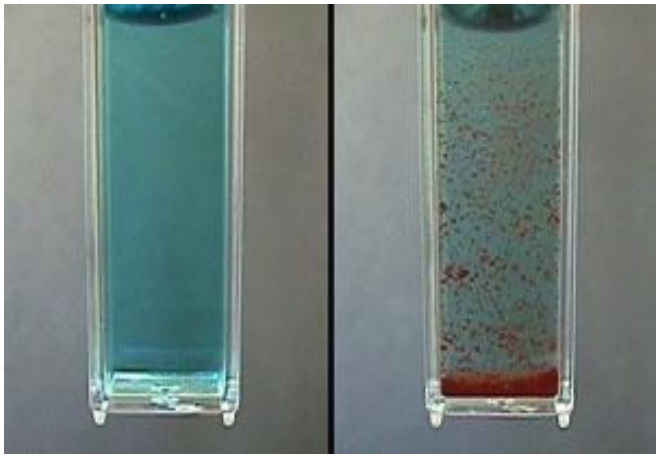
- ***Benedict's Test Result***

Color of the Precipitate	% of Reducing Sugar
Green	0.5%
Yellow	1%
Orange	1.5%
Red	2% or more



Semi-quantitative

Barfoed's Test Result



Blue Solution

Carbohydrates absent

Red Precipitation

Within few minutes - monosaccharides
After 3 minutes- disaccharides

Qualitative

Type of Testing

- **Qualitative** examinations measure the presence or absence of a substance.
- **Semi-quantitative** examinations provide an estimate (e.g. %) of how much of the measured substance is present.
- **Quantitative** examinations are used for determining the amount of an analyte in a sample. The amount is always expressed as a number with appropriate units.

Somogyi-Nelson Method

- The Nelson-Somogyi method is one of the classical and widely used methods for the **quantitative** determination of reducing sugars.

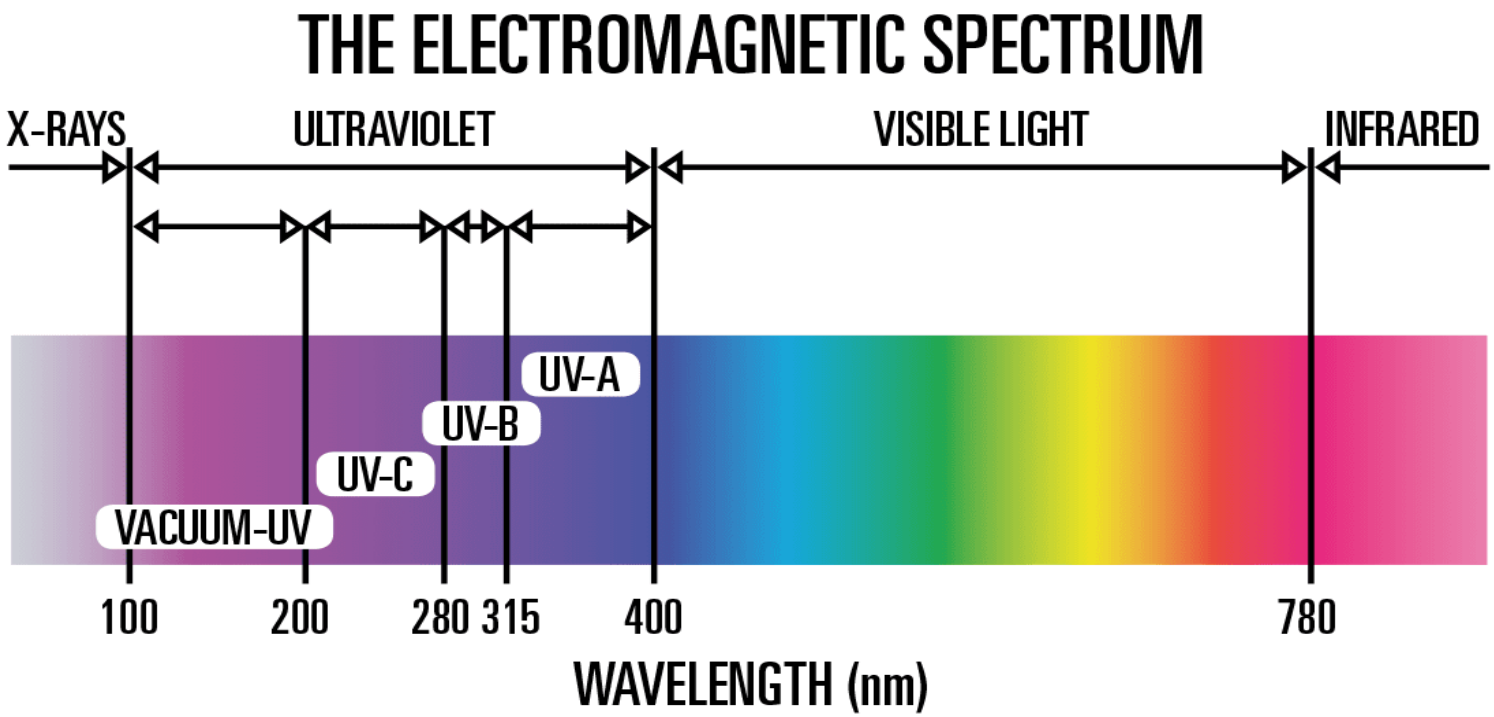
1. Cu^{+2} + glucose $\xrightarrow{\triangle,\ \text{Alkaline pH}}$ Cu^{+1} +oxidation products of glucose
2. Cu^{+1} + Phosphomolybdic acid(MO^{+6}) → MO^{+4} (blue color)

- The reducing sugars, when heated with alkaline copper tartrate, reduce the copper from the cupric to the cuprous state, and thus cuprous oxide is formed.
- When cuprous oxide is treated with Phosphomolybdic acid, the reduction of molybdic acid to molybdenum blue takes place.

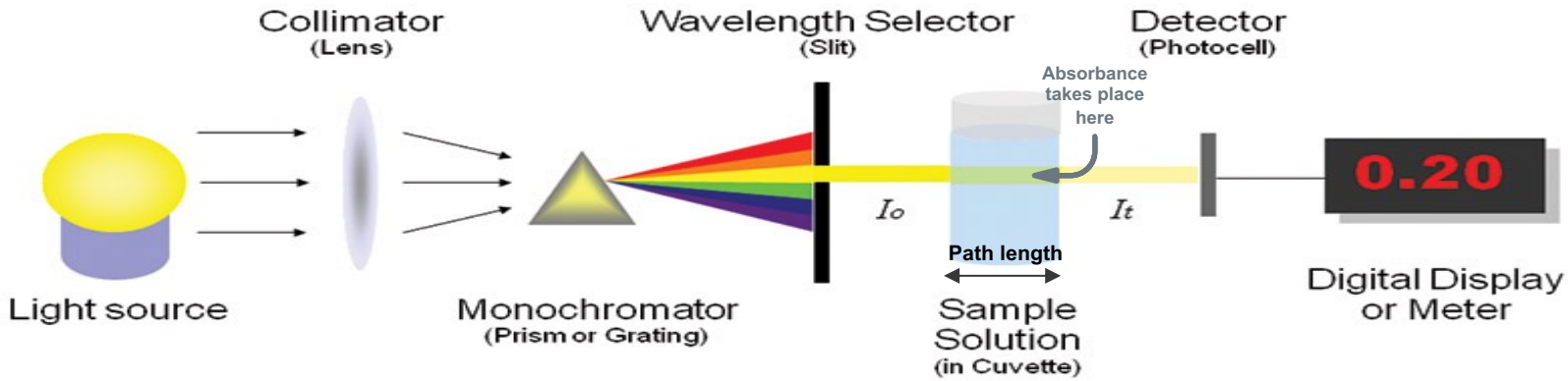
- **How do we analyze this test quantitatively and measure the concentration of glucose?** **Using a spectrophotometer to measure the concentration of MO+4 at 520 nm.**

△ = Heat

The Electromagnetic Spectrum



Spectrophotometer



- If the light is sent through a solution (Io) and only some of the light passes through (I), this suggests that the rest of the light has been absorbed by the molecules in the solution.

Converting light intensity to concentration

- To convert light intensity to concentration the Beer-Lambert Law is usually applied.
- It is commonly applied to determine the concentration of various molecules in solution.

Beer's law

- States that the **absorbance is directly proportional to the concentration** of a solution.
- If you plot absorbance versus concentration, the resulting graph yields **a straight line.**

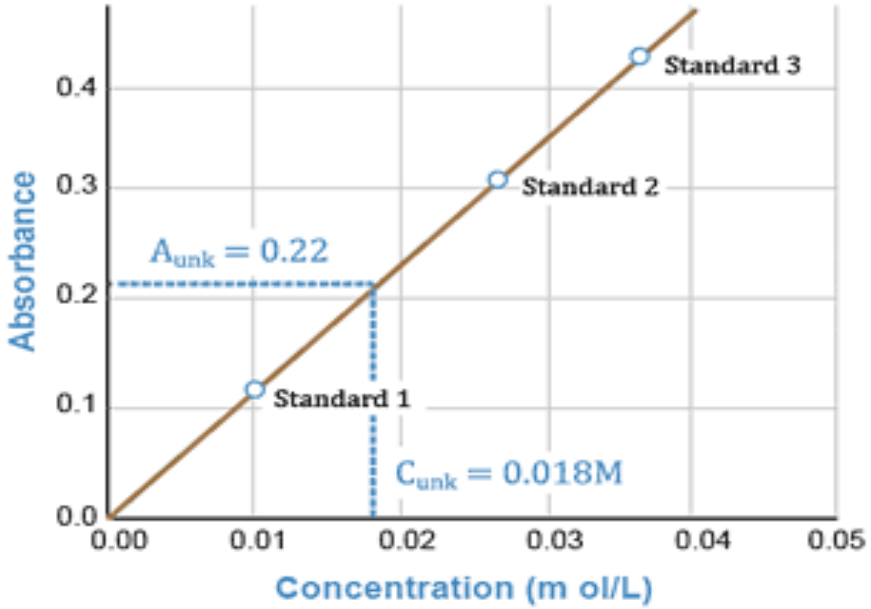
$$A = \varepsilon b C$$

Concentration Mol/L

Absorbance

Path length → cm

Molar absorptivity → L/(mol cm)



C1× V1 = C2 × V2

(Start reading) (final reading)

Procedure steps

Step 1: Construction of glucose standard curve:

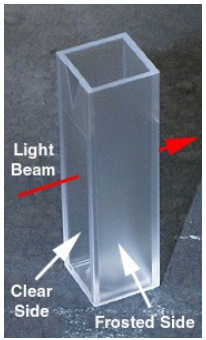
Use beer's law to measure standards of glucose.

Step 2: Measure the unknown glucose sample:

Calculate the concentration using data obtained from the standard curve.

Materials

- Spectrophotometer
- Cuvettes
- micropipette
- Tubes
- Rack
- Water bath



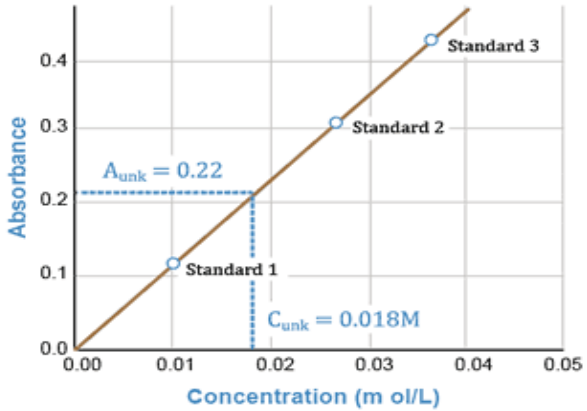
Preparation of a Standard Curve

• A standard curve is generated to be used to determine the concentration of an unknown sample.

How to make a standard curve?

1. Create a series of solutions (4-7 tubes) in known increasing concentrations of your analyte of interest (in this case, glucose of know concentration)
2. Use the formula C1V1= C2V2 to measure the concentration of each standard tube.
3. Measure the absorbance of all your standard at 520 nm (lambda max (λmax) for glucose).
4. Used the values of concentration and absorbance to create a standard curve.

Now you can move to **step 2** and use the standard curve to determine the concentration of glucose in an unknown sample.



Step 1 Construction of Glucose std. Curve

Note: Accurate pipetting is very important in performing your standard curve.

Total Volume (V2) = 1 ml

Steps	Tube No.	Blank	Std.1	Std.2	Std.3	Std.4	Std.5	Unknown sample
1	Volume of Glucose ([C1] 0.2mg/ml) (ml) [V1]	-	0.2	0.4	0.6	0.8	1	-
2	D.W (ml)	1	0.8	0.6	0.4	0.2	-	-
	Unknown sample (ml)	-	-	-	-	-	-	1
3	Copper reagent (ml)	1	1	1	1	1	1	1
4	Mix and incubate in a boiling water bath for 20 mins and cool.							
5	Arsenomolybdate reagent (ml)	1	1	1	1	1	1	1
6	Incubation Room Temperature for 1 minute.							
7	D.W (ml)	10	10	10	10	10	10	10
8	• Add each into a cuvette. • Set the spectrophotometer and read the absorbance of standards against the blank at wavelength 520 nm.							

Step 1 Standard Curve Calculation

1- Calculate the final concentration C2 for each standard using C1× V1 = C2 × V2 :

- C1 = Concentration of standard glucose = 0.2mg/ml
- V2=total volume of glucose + D.W dilution = 1ml in all tubes
- V1 = volume of std. Glucose was added to each tube.
- C2 = ? → The final concentration of standards. Glucose after dilution

2- Create The Result Table.

Read Abs. at 520 nm.

Tube No.	Blank	Std.1	Std.2	Std.3	Std. 4	Std. 5
Glucose (0.2mg/ml) V1	-	0.2	0.4	0.6	0.8	1
D.W	1	0.8	0.6	0.4	0.2	-

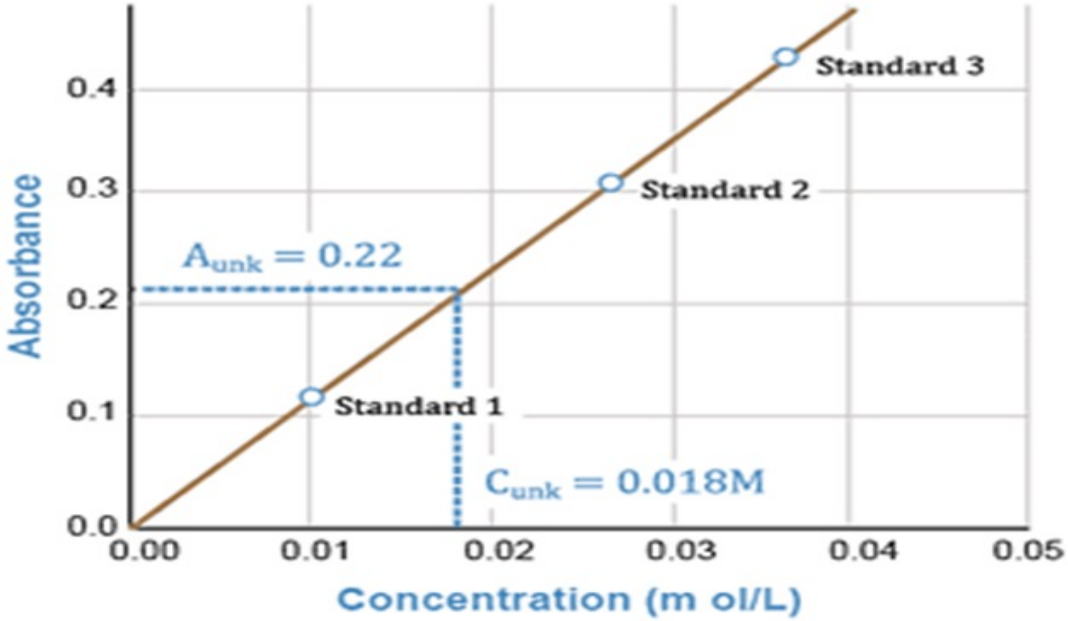
Record your result here	Abs	C2
Blank		
Std.1		
Std.2		
Std.3		
Std.4		
Std.5		
Unknown		

Step 1

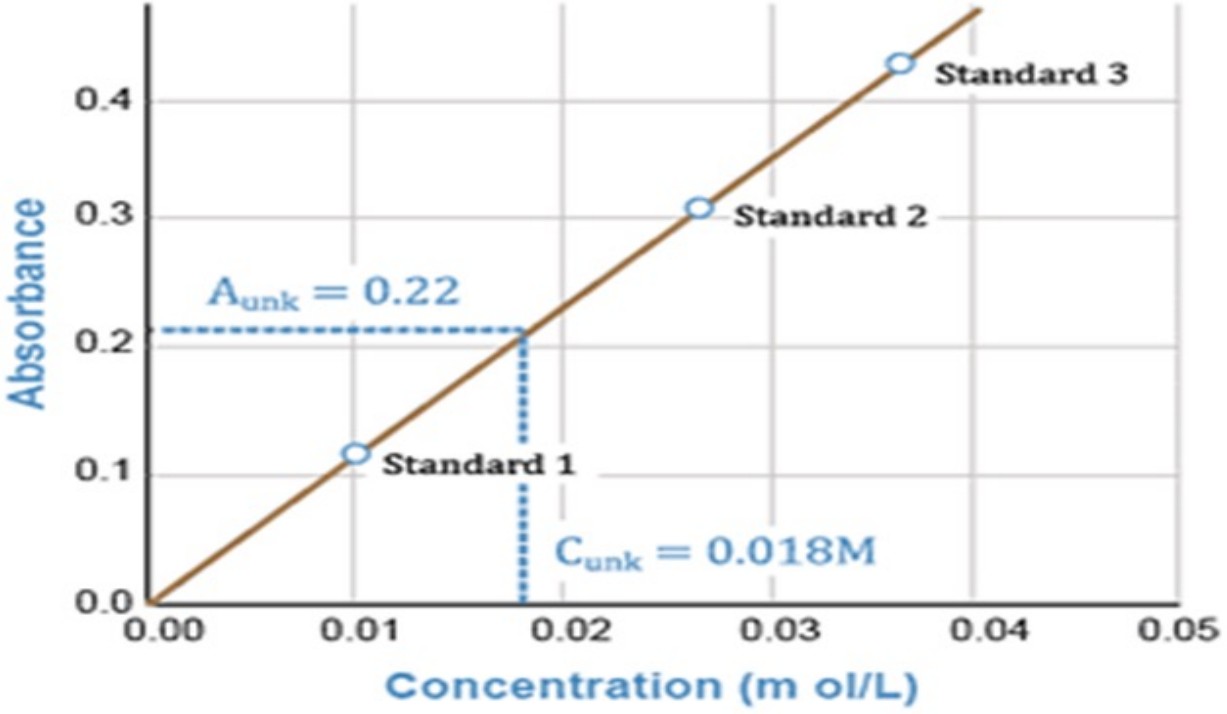
Standard Curve plot

3- Draw The Curve of absorbance against concentration

- **Standard curves** are graphs of light absorbance versus solution concentration which can be used to figure out the solute concentration in unknown samples.



Step 2 *Finding the concentration of unknown*



Definitions

- **Standards:** a series of tubes containing increasing concentrations of the substance to be assayed (glucose) are treated with the reagent.

- **Blank:** a blank is prepared to contain all reagents except the compound to be measured (glucose), and then Absorbance is measured for each of the standard tubes against the blank.

- **Unknown sample:** the sample to be measured of unknown concentration of the analyte under investigation.

FYI

Using Excel to plot standard curve

- https://www.youtube.com/watch?v=KeTFzJUG_rA

Note

- Reoxidation of cuprous ions by oxygen from the air is prevented by adding Sodium Sulphate in the reagent to decrease the solubility of oxygen.

- The Somogyi-Nelson method has been replaced recently using rapid colorimetric procedures like O-toluidine or enzymes (such as glucose oxidase and peroxidase).

Report Criteria **Total: 5 marks**

- Course # (CLS 281), Experiment title, Date of the experiment, Student's names and university ID#, Section #, Experiment title.
- The **aim** of the experiment (objective, or what the test detects specifically) (1 mark).
- **Principle** (chemical reaction) (1 mark).

- **Result (2 mark):**
  - Table of standard concentration and absorbance value, write detailed calculation with units.
  - Standard curve – do not forget the units.
  - The absorbance of the unknown sample.
  - The concentration of the unknown sample + explains how you obtained the concentration value.

- **Interpretation or Comment (1 mark)** - Describe the observed relationship between the absorbance and the concentration.

Deadline: Next lab
Submission: Handout