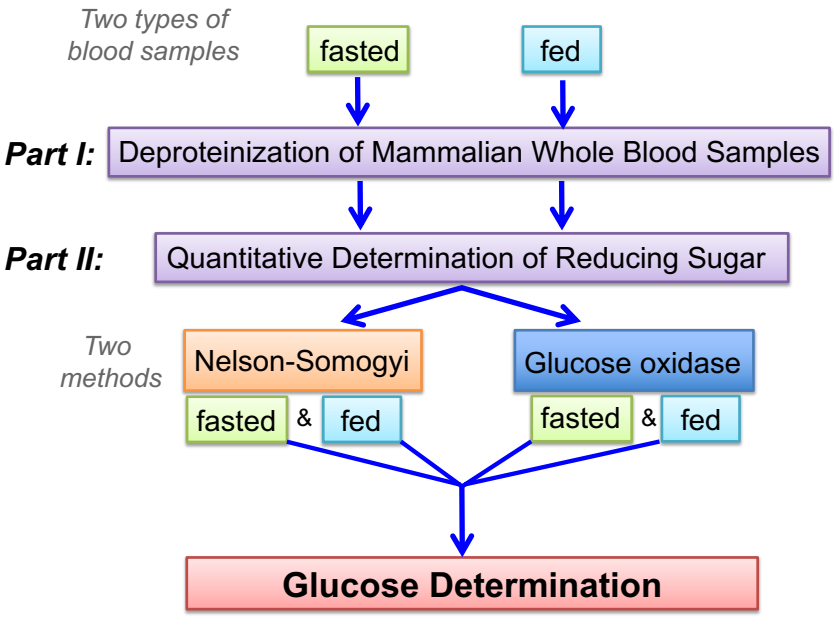
Chapter 7: Quantitative Determination of Sugar in Blood

Objectives

- Perform blood deproteinization procedure
- Perform two sugar concentration determination methods
- Estimate concentration of free sugar in original blood sample

Procedures

- Use the **Nelson-Somogyi method** to ***estimate free reducing sugars*** in solution
- Use **Glucose Oxidase method** to ***estimate glucose*** in solution



Why measure sugars?

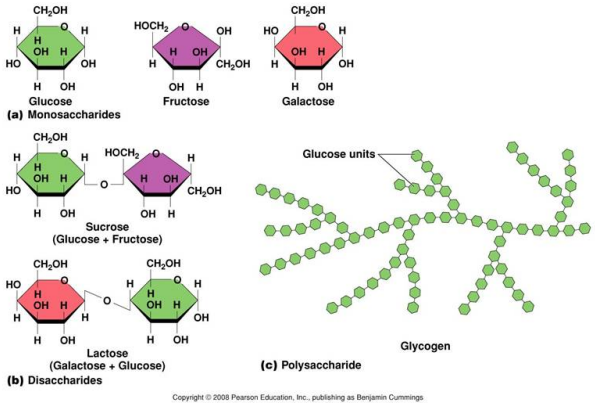
- Glucose levels heavily influence mammalian homeostasis

- Reference range for normal levels of glucose in blood
  - Normal levels during fasting = 60 ~ 100 mg/dL
  - Hypoglycemia – low glucose levels
  - Hyperglycemia – high glucose levels

- Levels used to diagnosis of disease
  - Diabetes: Hyperglycemic during periods of fasting

- Hemoglobin A1c marker, is a measure of glycosylated Hb, and is used for measuring chronic glucose levels over a 3-month period

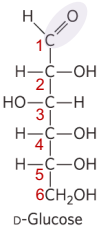
Carbohydrates play a central role in energy metabolism

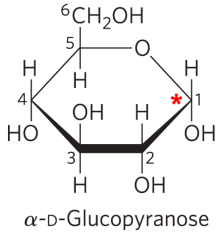


- Key roles in cell structure & communication
- Most abundant of all biomolecules
- Glucose → most significant molecule
- Can exist as linear chains or complex branched structures

Monosaccharides: Glucose (Glc) & fructose (Fru)

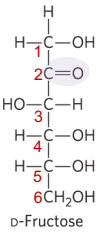
Glucose (Reducing sugar)

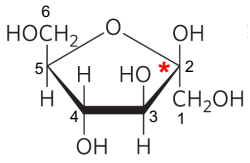




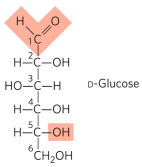
- Hexose
- Aldose
- Chiral

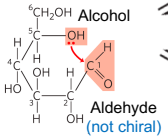
Fructose (Reducing sugar)



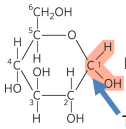


- Hexose
- Ketose
- Chiral



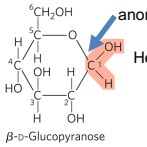


Attack sp2 carbon from "top" or "bottom"



Hemiacetal (chiral)

The carbon (with 2 bonds to oxygen) is now chiral; it is called the anomeric carbon.



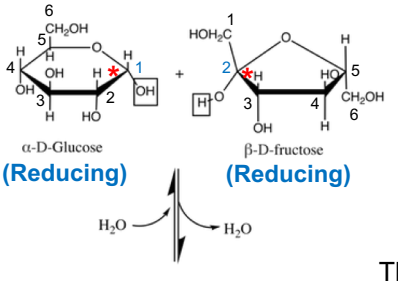
Hemiacetal (chiral)

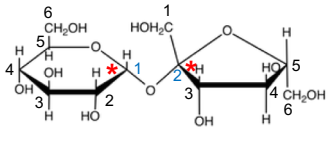
* = anomeric carbon

Announce | Concepts | Procedure | Hazards | Tips | Clarification | End

Disaccharides like sucrose form from a condensation reaction between 2 sugars

Sucrose (Glu + Fru) (Non-reducing sugar)





(Non-reducing)

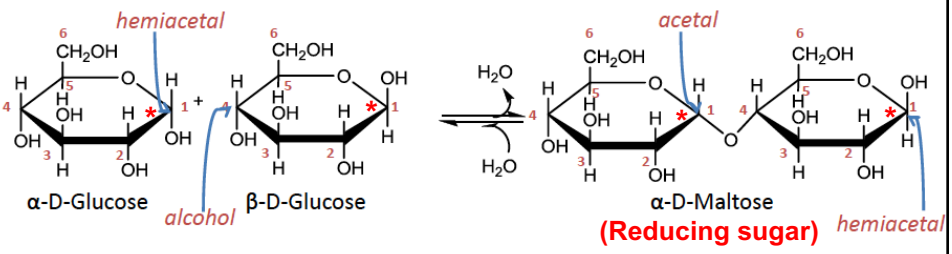
The anomeric carbon involved in the glycosidic linkage is fixed in its chirality and is therefore non-reducing.

* = anomeric carbon

Announce | Concepts | Procedure | Hazards | Tips | Clarification | End

Disaccharides, like maltose can also be reducing sugars

Anomeric Carbon = Hemiacetal



* = anomeric carbon

Announce | Concepts | Procedure | Hazards | Tips | Clarification | End

Nelson-Somogyi method to determine total reducing sugar

- Based on the reduction of cupric ions (Cu^{2+}) to cuprous (Cu^{+}) ions by reducing sugars.
  - *Reducing end (anomeric carbon) of sugars is reactive to dilute base*
- Cu^{+} reduce arsenomolybdate complex which produce blue color that is measured spectrophotometrically.

Alkaline cupric sulfate (Cu^{2+}) + reducing sugars → Cuprous ion (Cu^{+})

Cuprous ion (Cu^{+}) + Nelson arsenomolybdate reagent → Molybdenum Blue (Mo_2O_7)

Measure with benchtop spectrophotometers

Cons:

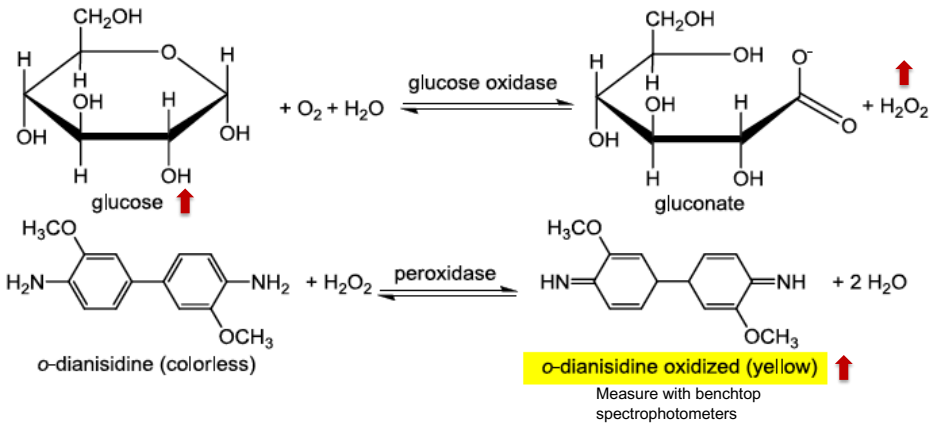
- Very dependent on temperature, pH, oxidant concentration and time.
- Different standard curves are required for different sugars

Not specific for any one sugar!

Announce | Concepts | Procedure | Hazards | Tips | Clarification | End

Glucose Oxidase method for specific detection of glucose concentration

- Enzyme will only oxidize glucose (and rarely 2-deoxy-D-glucose)
- Primary method used in clinical laboratories (aside from A1c test)
- Far less affected by small variations in time or temperature



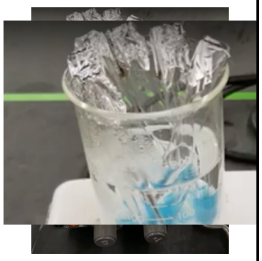
Announce | Concepts | Procedure | Hazards | Tips | Clarification | End

Lab Part I: Deproteinization of Blood Samples

- Use 15-mL conical tubes for samples
  - **Take 0.4 mL of blood for each sample**
- Dilute with dH_2O **(3.6 mL)** , mix, let stand (5 min)
- Add $Ba(OH)_2$, mix, wait (5 min), add $ZnSO_4$, mix, wait (5 min)
- Adjust pH to 6-8 if needed
  - pH strips located near TF bench
  - Use barium hydroxide if pH is too low, zinc sulfate if pH is too high
- Centrifuge at **4,000 *g*** for 5 min (TF's will assist)
  - Balance tubes before centrifuging!
- Collect supernatant fraction for next steps
  - Do not forget to **measure volume** of supernatant fractions. Very important for back calculations!

Announce | Concepts | Procedure | Hazards | Tips | Clarification | End

Lab Part II-A: Nelson-Somogyi Method

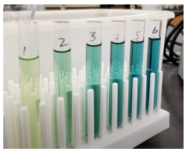


- Boil water in a 250 mL beaker
  - ➢ Cheesecloth at bottom to soften glass tube impact
- Add tubes prepared according to table on pg. 218
  - ➢ Everything **EXCEPT** arsenomolybdate reagent
- AVOID TAPE LABELS!! (will fall off in boiling water)
- Cap tubes with foil; label BOTH tube & foil
- Heat reactions for 20 minutes; cool in ***warm*** tap water [DO NOT SHAKE NOR DISTURB SOLUTION]
- Add arsenomolybdate reagent (toxic!), mix and wait 5 min
- Add 5 mL water, mix again, and let stand at RT for >30 min
- Read absorbance of samples with Tube #1 as BLANK at $A_{750\ nm}$

Table 1. Preparation of Test tubes 1-12 for Nelson-Somogyi assay

Tube #	1	2	3	4	5	6	7	8	9	10	11	12
Addition	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)
Glucose, 0.5 mM	–	0.10	0.20	0.30	0.40	0.50	–	–	–	–	–	–
Fructose, 0.5 mM	–	–	–	–	–	–	0.50	–	–	–	–	–
Sucrose, 0.5 mM	–	–	–	–	–	–	–	0.50	–	–	–	–
Deproteinized blood, fed	–	–	–	–	–	–	–	–	0.20	0.40	–	–
Deproteinized blood, fasted	–	–	–	–	–	–	–	–	–	–	0.20	0.40
DI-H_2O	1.00	0.90	0.80	0.70	0.60	0.50	0.50	0.50	0.80	0.60	0.80	0.60
Copper Reagent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Arsenomolybdate Reagent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Positive control
Negative control
Add only after heating 20 minutes and cooling
Varying [glucose] to make standard curve

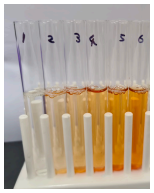


Announce > Concepts > Procedure > Hazards > Tips > Clarification > End

Lab Part II-B: Glucose Oxidase Method

- Set up tubes similar to Nelson-Somogyi Method
  Your sugar and sample volumes will be doubled, and adjust to 1 mL
- Add glucose oxidase reagent
  - ➢ **Be careful**: *o*-dianisidine is a potential carcinogen!
- Incubate in 37 °C water bath for 30 min
- Add HCl and mix to stop reaction
- Wait 5 min, read absorbance at $A_{540\ nm}$ using Tube #1 as BLANK

Measure $A_{540\ nm}$ on benchtop spec
Use tubes with varying [glucose] to make standard curve



Announce > Concepts > Procedure > Hazards > Tips > Clarification > End

Hazardous wastes

• Nelson arsenomolybdate reagent (***poison***) and Nelson-Somogyi assays will go into a special waste container

• Glucose-Oxidase reagent (containing o-dianisidine; potential ***carcinogen***) and assays will go into a special waste container

Chapter 7 Lab Tips

- During the Nelson-Somogyi heating\incubation period, begin the Glucose-Oxidase experiment
- For hot plate and boiling beaker apparatus, you only need a soft boil, nothing too vigorous
  - We have thermometers and tongs available throughout the lab, always ask TFs for assistance

For the first week of lab... And before every lab each week:

- Please make sure to submit your Pre-Lab to Gradescope *before* your lab section (30 min)
- Go to SCI-162, show up 5-10 min early!
- TFs will begin lab by introducing themselves and taking attendance, pairing you up with a lab partner.
- TFs will go over lab policies, lab safety, and answer questions.
- Please submit your in-lab data collection assignment on Gradescope *by the end of the day after* your lab section (midnight)

Chapter 7 requirements

- TFs will preview Chapter 7 (record any procedural changes in your notebook)
- Complete Chapter 7 experiments

Chapter 7

Before the lab period, you should have:

- ✓ Completed your Pre-lab Write-up and submit on Gradescope
  - ✓ Title, purpose and procedures
  - ✓ Remember to include:
    - ✓ Prompts for recording volumes and pH values
    - ✓ Reagents/data table for Nelson-Somogyi assay
    - ✓ Reagents/data table for Glucose oxidase assay

At the end of lab, you should have:

- ✓ Produced two deproteinized blood samples (*fed & fasted*)
- ✓ Performed Nelson-Somogyi and Glucose oxidase assays
  - ✓ Obtained absorbance readings for glucose standard curves & controls
  - ✓ Collected absorbance data for both types of blood samples
- ✓ Submit in-lab data collection assignment to Gradescope by the end of the day after your lab section

Questions?
In-class activity
&
Discussion Quiz