

Isolation and Characterization of Protein and Sugar from Milk

1. This experiment is written for data collection with the Vernier Go Direct Polarimeter and one of the following:
 - Instrumental Analysis app (version 1.2 or newer) running on a computer, Chromebook, or mobile device
 - LabQuest 3 (app version 3.0.3 or newer)
 - LabQuest 2 (app version 2.8.7 or newer)

Visit www.vernier.com/downloads for information about how to download the most recent version for your device.

2. There are several ways to locate the angle at which the maximum illumination occurs:

Using Instrumental Analysis

- a. **Statistics:** To simply get the angle with the highest illumination, highlight the peak of interest in Instrumental Analysis, as shown in Figure 1. Click or tap Graph Tools, , and select View Statistics. Record the angle value where the illumination is at a maximum, as presented in the box. This method is the fastest and will result in reproducibility of the angle of rotation measurement of $\pm 2.0^\circ$.
- b. **Examine:** Click or tap the Illumination vs. Angle graph to find the data point associated with the peak that is closest to 0° . This method also lacks accuracy but is useful from a pedagogical standpoint as it connects the feature on the graph in a way that telling students to perform a curve fit and use the parameters does not.
- c. **Cosine Squared:** To incorporate all of the data into the fit, students can fit the data to its true waveform, a cosine squared, in Instrumental Analysis. Choose Curve Fit from the Graph Tools, . From the list of available General Equations, select Cosine Squared. The fit will run automatically. In this fit, the x-value corresponding to the maximum y-value is obtained from the negative of the phase shift parameter, $-C$. This is a nonlinear fit which undergoes numerous iterations and has the possibility of no convergence, which will result in an unreasonable answer. With all nonlinear fits, it is important to make sure the resulting value is reasonable based on the data presented in the graph. This method is the most time consuming; however, it will result in reproducibility of the angle of rotation measurement of $\pm 0.1^\circ$.

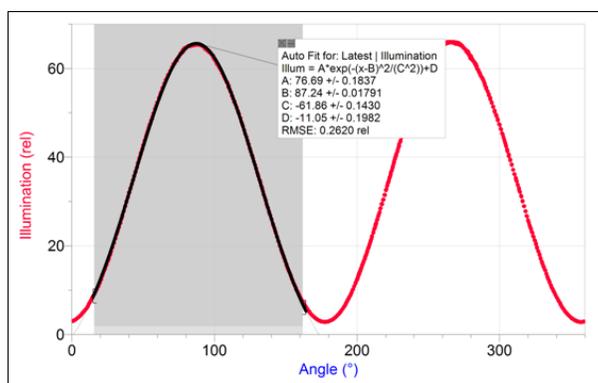


Figure 1 Selection for Statistics and Gaussian fits (applies to all supported software)

Using LabQuest 2 or 3

- a. Statistics: To simply get the angle with the highest illumination, highlight the peak of interest in LabQuest App, as shown in Figure 1. Choose Statistics from the Analyze menu. Record the angle value where the illumination is at a maximum, as presented in the box. This method is the fastest and will result in reproducibility of the angle of rotation measurement of $\pm 2.0^\circ$.
 - b. Gaussian: To improve your accuracy with a better fit, highlight the peak of interest using LabQuest App, as shown in Figure 1. Then, choose Curve Fit from the Analyze menu. From the list of available Equations, select Gaussian. The fit will run automatically. The B coefficient presented represents the angle at maximum illumination. This method will result in reproducibility of the angle of rotation measurement of $\pm 0.3^\circ$. The data are not a true Gaussian, but the ease and accuracy of this methodology make it a good option. For best results, be consistent in the way you select your peaks.
 - c. Cosine Squared: To incorporate all of your data into the fit, you can fit the data to its true waveform, a cosine squared, in LabQuest App. Choose Curve Fit from the Analyze menu. From the list of available General Equations, select Cosine Squared. The fit will run automatically. In this fit, the x-value corresponding to the maximum y-value is obtained from the negative of the phase shift parameter, $-C$. This is a nonlinear fit which undergoes numerous iterations and has the possibility of no convergence, which will result in an unreasonable answer. With all nonlinear fits, it is important to make sure the resulting value is reasonable based on the data presented in the graph. This method is the most time consuming; however, it will result in reproducibility of the angle of rotation measurement of $\pm 0.1^\circ$.
3. Due to the nature of light transmission, it is important that the sample be transparent and homogeneous. The sample can have a moderate amount of color but make sure it is not too dark to ensure that light will still pass through it onto the detector.
 4. The illumination value for Go Direct Polarimeter should not be used for quantitative purposes. If you choose to use the value quantitatively, note that it is very sensitive to change. These slight variations can be brought on by several different variables in the sample including inhomogeneity (such as particulates and bubbles), height of the sample in the cell, concentration of the sample, and aberrations in the glass cell or path length.
 5. α -Lactose interconverts to β -lactose in aqueous solution over time causing the time-dependent change in optical rotation of the synthesized sample. In order for students to see this change,

the α -lactose cannot be sitting in water for too long prior to data collection. To have students observe the kinetics of this reaction, see Go Direct Polarimeter Experiment 2 “Observing the Reaction Kinetics of Sucrose with Polarimetry” available at www.vernier.com/gdx-pol

HAZARD ALERTS

The chemical safety signal words used in this experiment (DANGER and WARNING) are part of the Globally Harmonized System of Classification and labeling of Chemicals (GHS). Refer to the Safety Data Sheet (SDS) that came with the chemical for proper handling, storage, and disposal information. These can also be found online from the manufacturer.

Acetic acid, 1.7 M, CH_3OOH : **WARNING:** Causes skin and eye irritation.

Calcium carbonate: This chemical is considered nonhazardous according to GHS classifications. Treat all laboratory chemicals with caution. Prudent laboratory practices should be observed.

Ethanol: **DANGER:** Highly flammable liquid and vapor. Keep away from heat, sparks, open flames, and hot surfaces. Do not eat or drink when using this product—harmful if swallowed. Causes skin and serious eye irritation. May cause respiratory irritation. Avoid breathing mist, vapors, or spray. Causes damage to organs. Addition of denaturant makes the product poisonous. Cannot be made nonpoisonous.

COMPOUND INFORMATION

Compound	Molar mass (g/mol)	Specific rotation ($^\circ$)
α -lactose	342.3	+92.6
β -lactose	342.3	+34.0

SAMPLE RESULTS

Part I Isolation and purification of casein

Weight of powdered nonfat milk (g)	4.13
Weight of watch glass alone (g)	48.39
Weight of watch glass with dry casein (g)	50.55
Weight of dry casein (g)	2.16

Experiment 3

Part II Isolation of the lactose and albumin

Weight of synthesized albumin (g)	2.38
Weight of filter paper (g)	0.40
Weight of filter paper with synthesized α -lactose (g)	1.82
Weight of synthesized α -lactose (g)	1.42

Part III Measuring the optical activity of lactose

Volume of water added to dissolve α -lactose (mL)	5.0
α -lactose sample concentration (g/mL)	0.28
Height of α -lactose solution (dm)	0.21
Angle of rotation, α ($^{\circ}$)	5.1

ANSWERS TO DATA ANALYSIS QUESTIONS

Part I Isolation and purification of casein

1. $\frac{2.16}{4.13} \times 100 = 52.3\%$

Part II Isolation of the lactose and albumin

2. $\frac{1.42}{4.13} \times 100 = 34.4\%$

3. $\frac{2.38}{4.13} \times 100 = 57.6\%$

Part III Measuring the optical activity of lactose

4.

$$\alpha = [\alpha]lc$$

$$[\alpha]_{abs} = \frac{5.1^{\circ}}{(0.21 \text{ dm})(0.28 \text{ g/mL})} = 86.7^{\circ}$$

$$\frac{86.7^{\circ}}{92.6^{\circ}} \times 100 = 93.6\%$$

5.

$$\frac{86.7^{\circ}}{92.6^{\circ}} \times 100 = 93.6\%$$

Therefore, only 93.6% of the sample is α -lactose. The impurity is contamination, most likely from the β -lactose enantiomer.

SAMPLE GRAPH

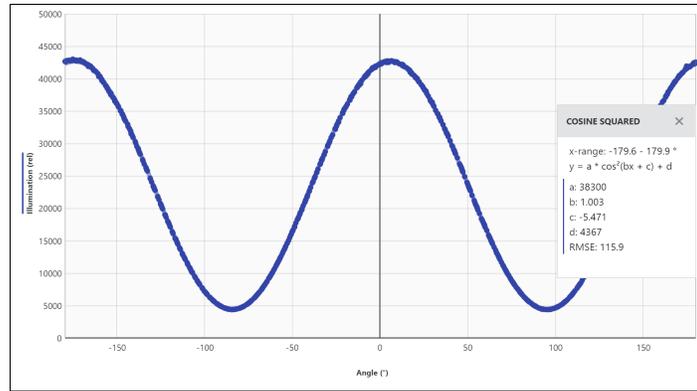


Figure 2 Optical rotation data for lactose