

Report Guidelines for Isolation of Chlorophyll and Carotenoid Pigments from Spinach

Pre-lab report MUST be written inside your lab notebook.

Note: The experimental procedure for this experiment is provided via email together with these pre-lab and post-lab guidelines.
You will start this experiment on Tuesday, May 10 or Wednesday, May 11, 2016.
The project is scheduled to be completed in TWO lab periods.

(I) On-line Technique Videos for this experiment

Click on the title below to download the video (require [Real Player](#))

If you have trouble downloading the videos, go to the following Web site and click on the appropriate title to download the video.

<http://www.oid.ucla.edu/edtech/impcastvideos> (Watch Column Chromatography and TLC)

[Guides for Writing Lab Reports](#)**(II) Pre-lab Report Guidelines**

In preparing for lab, set up your notebook with the following information:

- Title of the experiment
- A reference identifying the procedure handout
- A short introduction summarizing the goals of the experiment and the techniques that you will use in the experiment
- A flow chart summary of the ENTIRE experimental procedures
- **SDS:** The following chemical will require you to use the SDS database on the Web

Methanol

(II) Pre-lab Report Guidelines (Continued)

You should record the following SDS information in your notebook for the chemical listed above. **(Printouts directly from the Web pages will NOT be accepted!)**

- (a) Product Name
- (b) Chemical Formula
- (c) Formula Weight
- (d) Melting Point; Boiling Point and Density
- (e) Health Hazard Data (**summarize in your own words**)
- (f) Spill and Disposal procedures (**summarize in your own words**)

IMPORTANT: Make sure that you always follow the proper laboratory safety protocol (refer to the course syllabus) BEFORE going to the lab.

(III) Pre-lab Questions

1. Why is it important that the organic layer is dried over anhydrous sodium sulfate before applying the solution to the column?
2. Arrange the solvents used in the experiment according to increasing eluting power. Explain briefly.
3. The TLC plate should not leave the lab. Explain briefly. How is the TLC reported?
4. Why is it important to use a small spotter to apply the sample to the TLC plate?

DATA TABLES & OBSERVATIONS

USE A NEW PAGE TO SET UP THE FOLLOWING BLANK TABLES.

- Set up a blank table for recording the column chromatography data. Include a column for the number of the fraction being collected, the time over which the fraction is collected, the concentration of the solvent on the column, the appearance of colors on the column, and the color of the solution in the fraction being collected.
- Set up a blank data table for recording the thin-layer chromatography data. The data should include the components of the mobile phase; the identity of the initial spots on the TLC plate; the distance travel by each spot on the TLC plate at the end of the analysis; the distance traveled by the solvent front at the end of the TLC analysis. Keep the original TLC plate in your lab notebook for future reference.

Report Guidelines for Isolation of Chlorophyll and Carotenoid Pigments from Spinach

Post-lab report **MUST** be written inside your lab notebook

This is an INDIVIDUAL REPORT (due May 19 or May 20, 2016)

1. ABSTRACT – Summarize the goal(s) of the experiment plus a summary of your experimental results (i.e., identities of components and their R_f -values etc.)

2. OBSERVATIONS**Isolation**

Rewrite all your observations during the extraction of the pigment and the chromatographic separation in an organized format. In tabular format, record the number of the fraction being collected, the time over which the fraction is collected, the composition of the solvent used in the column (i.e., the mobile phase used), the appearance of colors on the column, and the color of the solution in the fraction being collected.

Characterization

Attach a picture of the ORIGINAL TLC plate in your report. In tabular format record the color of each component separated on the TLC plate, and its corresponding R_f -value.

3. DATA ANALYSIS

Identify the absorption signal(s) in the UV spectra that give rise to the colors of the chlorophyll and β -carotene solutions (*refer to the table listed below for colors of various components*)

Based on the absorption signals you identified, explain whether the locations of the absorptions make physical sense.

Calculate the R_f -values for all the components on the TLC plate. *Show all your work.*

Using the guide below, identify as many spots on the TLC plate as you can. Determine which pigments were present in the yellow band and which were present in the green band from your column.

Pigments in spinach in order of decreasing R_f -values:

Carotenes (1 spot) (yellow-orange)

Pheophytin **a** (gray, may be nearly as intense as chlorophyll **b**)

Pheophytin **b** (gray, may not be visible)

Chlorophyll **a** (blue-green, more intense than chlorophyll **b**)

Chlorophyll **b** (green)

Xanthophylls (possibly three spots: yellow)

4. CONCLUSIONS

In your conclusion comment on the quality of the separation of the various pigments based on the following results.

- (i) Compare the separation of components on the TLC plate.
- (ii) Based on the locations of the absorption bands on the UV-Vis spectra

*Attach the a colored picture ORIGINAL EXPERIMENTAL TLC plate with this post-lab report and turn in the completed report to **your TA on the due date listed on the lab schedule***