

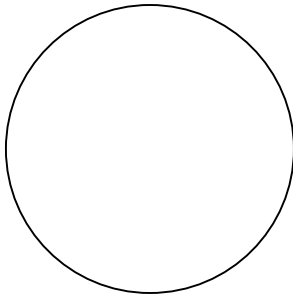
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\_\_\_\_\_  
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## Datum Sheet for Laboratory 3 Spectrophotometric Analysis of Membrane Stability in Beet Root Cells

### B. Microscopic Observation of Beet Root Cells

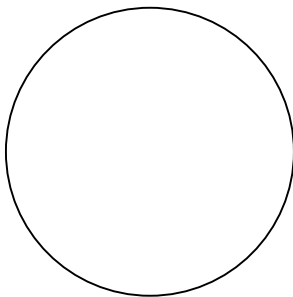
- Using the following circles to represent the microscopic fields, draw the cells in the beet root sections as seen with the 40X objective with bright field illumination in distilled water and in 2.0 M NaCl. Indicate the total magnification in each case (1 point each, 2 points total). Include descriptions next to the drawings.

Distilled Water:



Total Magnification \_\_\_\_\_ X

2.0 M NaCl:



Total Magnification \_\_\_\_\_ X

- What is the significance of these observations? (0.5 points)

D. Determination of the Absorption Spectrum of Betacyanin

1. Enter your raw data for the absorption spectrum of betacyanin (2 points).

Wavelength(nm)	Absorbance (A)
440	
460	
480	
500	
520	
540	
560	
580	
600	
620	

2. Attach to this datum sheet a **graph** of the absorption spectrum of betacyanin (3 points).
3. What wavelength did you use for the rest of the experiments? (0.5 points)

\_\_\_\_\_ nm

E. Measurement of Betacyanin Released from Beet Root Segments

1. Enter your raw data for the absorbance measurements of the betacyanin solutions obtained by the various treatments include the mean and subtract the control from each for the net absorbance (4 points).

**Absorbance of Betacyanin Solutions at \_\_\_\_\_ nm after Various Treatments**

Treatment	1	2	3	Mean	Net absorbance
25°C					0
F/T					
75°C					
70°C					
65°C					
60°C					
55°C					
50°C					
Ethanol					
Isopropanol					
SDS					
TX100					

2. If you diluted any of the samples, indicate which were diluted and give the calculated absorbance value for the original solution.

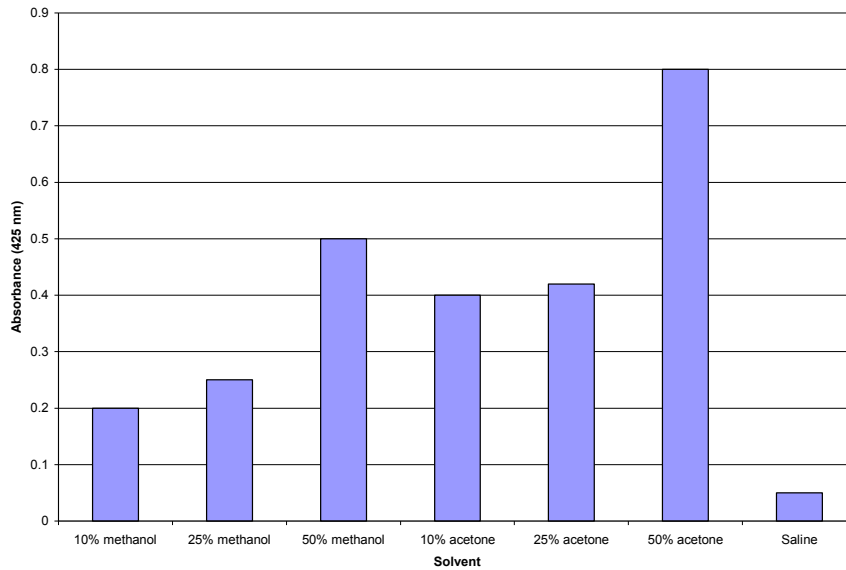
## F. Preparation of Tables and Graphs

1. Attach to this datum sheet a **table** summarizing the results of the freeze/thaw, solvent, and detergent treatments (2 points).
2. Attach to this datum sheet a **graph** summarizing the results of the freeze/thaw, solvent, and detergent treatments (3 points).
3. Attach to this datum sheet a **graph** of the absorbance in the solution as a function of the temperatures treatments (3 points).
4. What type of graph did you use for each datum set? Why is this the best type of graph for the data? (2pts)

## G. Additional Questions (2 points each, 8 points total)

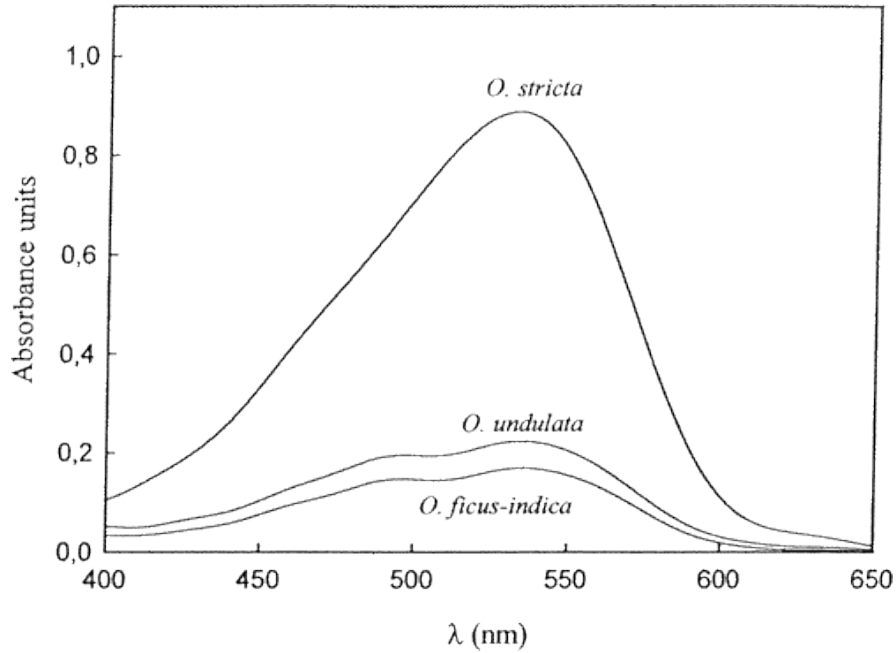
1. The molar extinction coefficient ( $E$ ) at 538 nm for a betacyanin from *Portulaca oleracea* seedlings was estimated to be  $65 \times 10^6 \text{ L cm}^{-1} \text{ mol}^{-1}$  [S. J. Schwartz and J. H. von Elbe, Quantitative determination of individual betacyanin pigments by high-performance liquid chromatography, *J. Agric. Food Chem.* **28** (1980), pp. 540–543]. If a solution betacyanin from the organism has an absorbance of 0.459, what is its concentration? Assume the measurement was made in a 1 cm light path.

2. A Google search for other experiments involving beet root betacyanin turned up the following datum set. In this case, beet root segments were exposed to different concentrations of methanol or acetone for 20 minutes and the amount of pigment released determined by measuring the absorbance of the solutions. Here are the results:



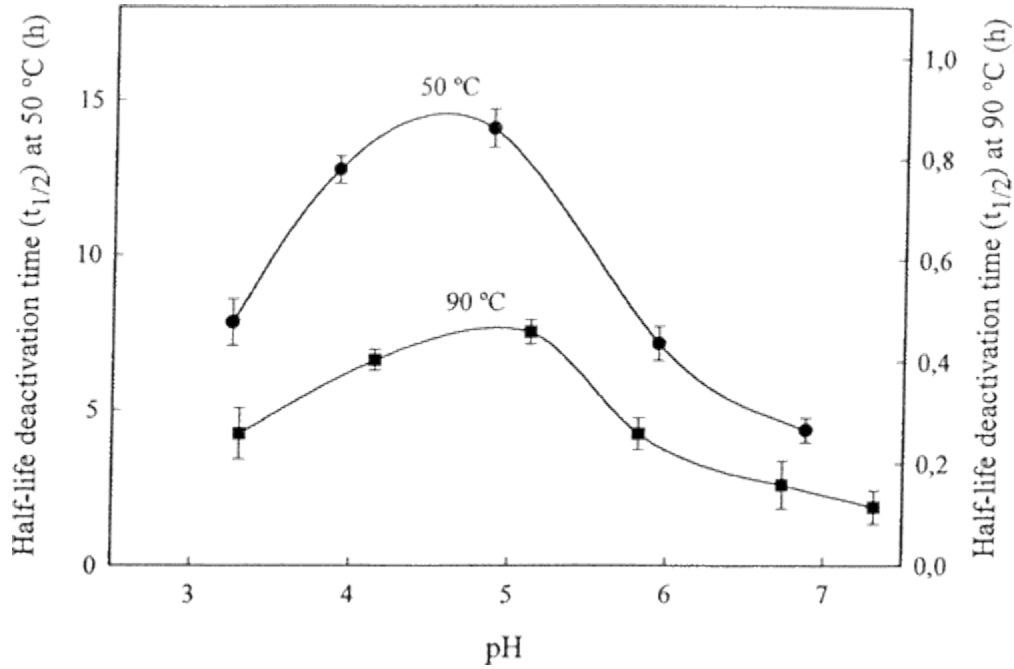
Describe what is shown in this graph.

3. In another article entitled “Color Properties and Stability of Betacyanins from *Opuntia* Fruits” by Rosario Castellar, José M. Obón, Mercedes Alacid, and José A. Fernández-López published in the *J. Agric. Food Chem.*, 2003, 51 (9), pp 2772–2776, the authors investigated the color properties of betacyanins from fruits of various species of *Opuntia* (prickly pear cactus). The results are shown in the next figure.



What conclusions can be drawn from these data?

4. In this same article, the authors measured the effect of pH on the thermal stability of the betacyanins from *O. stricta*. The results are shown in the following figure.



Describe what is shown in this graph (NOTE THE DIFFERENT SCALES FOR THE MEASUREMENTS AT 50°C and 90°C).