

Here's the math for the chlorophyll lab:

$$A_{645} = 16.75C_a + 45.60 C_b \quad (1)$$

$$A_{663} = 82.04C_a + 9.27 C_b \quad (2)$$

From equation 1

$$C_a = \frac{A_{645} - 45.60C_b}{16.75}$$

$$C_a = 0.0599 A_{645} - 2.730C_b$$

Substitute into equation 2

$$A_{663} = 82.04 (0.0599A_{645} - 2.730C_b) + 9.27A_{663}$$

Solve for  $C_b$

$$A_{663} = 4.914 A_{645} - 223.969C_b + 9.27C_b$$

$$A_{663} = 4.914 A_{645} - 214.699C_b$$

$$C_b = \frac{A_{663} - 4.914 A_{645}}{-214.699}$$

$$C_b = 0.0229A_{645} - 0.00468 A_{663}$$

Substitute into equation 1

$$A_{645} = 16.75C_a + 45.60 (0.0229 A_{645} - 0.00468 A_{663})$$

Solve for  $C_a$

$$16.75 C_a = A_{645} - 45.60 (0.0229 A_{645} - 0.00468 A_{663})$$

$$16.75 C_a = A_{645} - 1.0442 A_{645} + 0.213 A_{663}$$

$$16.75 C_a = 0.213 A_{663} - 0.0442 A_{645}$$

$$C_a = \frac{0.213 A_{663} - 0.0442 A_{645}}{16.75}$$

$$C_a = 0.0127 A_{663} - 0.00264 A_{645}$$

$$C_a + C_b = C_{\text{total}} = 0.0229A_{645} - 0.00468 A_{663} + 0.0127 A_{663} - 0.00264 A_{645}$$

$$C_{\text{total}} = 0.0203A_{645} + 0.00802 A_{663}$$

These calculations are in grams per liter. Convert to ug/mL:

$$C_{\text{total}} = 20.3A_{645} + 8.02A_{663}$$

### THIS IS THE EQUATION YOU SHOULD USE

#### Notes:

Arnon (1949) shows the solution for  $C_a$  as

$$C_a = 0.0127 A_{663} - 0.00269 A_{645}$$

In several tries we were unable to get 0.00269 (we get 0.00264). Thus the equation from Arnon (1949) may be incorrectly calculated:

$$C_{\text{total}} = 20.2A_{645} + 8.02A_{663}$$

This hardly matters as far greater errors have been reported in the literature in later papers.

Other equations have been derived that minimize the problems with the Arnon equations. Lichtenthaler & Welburn (1983) report the following equations to determine chlorophyll a and chlorophyll b content in 80% acetone extracts:

$$\begin{aligned} C_a (\mu\text{g/ml}) &= 12.21 (A_{663}) - 2.81 (A_{646}) \\ C_b (\mu\text{g/ml}) &= 20.13 (A_{646}) - 5.03 (A_{663}) \\ C_{\text{total}} &= 17.32A_{645} + 7.18A_{663} \end{aligned}$$

Porra (2002) reports the following equations in buffered aqueous 80% acetone:

$$\begin{aligned} C_a (\mu\text{g/ml}) &= 12.25 (A_{663.6}) - 2.55 (A_{646.6}) \\ C_b (\mu\text{g/ml}) &= 20.31 (A_{646.6}) - 4.91 (A_{663.6}) \\ C_{\text{total}} (\mu\text{g/ml}) &= 17.76 (A_{646.6}) + 7.34 (A_{663.6}) \end{aligned}$$

#### Literature Cited

Arnon, D (1949) Copper enzymes in isolated chloroplasts, phytophenoloxidase in *Beta vulgaris*. Plant Physiology 24: 1- 15.

Lichtenthaler, HK and AR Wellburn (1983) Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochemical Society Transactions 11: 591 - 592.

Porra, RJ (2002) The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. Photosynthesis Research 73: 149 - 156.