Here’s the math for the chlorophyll lab:

\[ A_{645} = 16.75C_a + 45.60C_b \]  \hspace{1cm} (1)

\[ A_{663} = 82.04C_a + 9.27C_b \]  \hspace{1cm} (2)

From equation 1

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

\[ C_a = 0.0599A_{645} - 2.730C_b \]

Substitute into equation 2

\[ A_{663} = 82.04(0.0599A_{645} - 2.730C_b) + 9.27A_{663} \]

Solve for \( C_b \)

\[ A_{663} = 4.914A_{645} - 223.969C_b + 9.27C_b \]

\[ A_{663} = 4.914A_{645} - 214.699C_b \]

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

\[ C_b = 0.0229A_{645} - 0.00468A_{663} \]

Substitute into equation 1

\[ A_{645} = 16.75C_a + 45.60(0.0229A_{645} - 0.00468A_{663}) \]

Solve for \( C_a \)

\[ 16.75C_a = A_{645} - 45.60(0.0229A_{645} - 0.00468A_{663}) \]

\[ 16.75C_a = A_{645} - 1.0442A_{645} + 0.213A_{663} \]

\[ 16.75C_a = 0.213A_{663} - 0.0442A_{645} \]

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

\[ C_a = 0.0127A_{663} - 0.00264A_{645} \]

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

\[ C_{total} = 0.0203A_{645} + 0.00802A_{663} \]
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:

\[
\begin{align*}
C_a \text{ (µg/ml)} & = 12.21 \ (A_{663}) - 2.81 \ (A_{646}) \\
C_b \text{ (µg/ml)} & = 20.13 \ (A_{646}) - 5.03 \ (A_{663}) \\
C_{\text{total}} & = 17.32A_{645} + 7.18A_{663}
\end{align*}
\]

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

\[
\begin{align*}
C_a \text{ (µg/ml)} & = 12.25 \ (A_{663.6}) - 2.55 \ (A_{646.6}) \\
C_b \text{ (µg/ml)} & = 20.31 \ (A_{646.6}) - 4.91 \ (A_{663.6}) \\
C_{\text{total}} \text{ (µg/ml)} & = 17.76 \ (A_{646.6}) + 7.34 \ (A_{663.6})
\end{align*}
\]

Literature Cited


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.