# *Chlorophyll A determination using the Cintra 6*

## Introduction

Chlorophyll is the green molecule in plant cells that carries out the bulk of energy fixation in the process of photosynthesis.
It is most commonly analysed to estimate the algal biomass in marine and freshwater environments.

Chlorophyll itself is not a single molecule but a family of related molecules, designated Chlorophyll a, b, c and d. Chlorophyll d is only found in marine red algae.
In this application note, Chlorophyll A is extracted using a 90% ethanol solution. The solution is measured at various wavelengths then the trichromatic equation of Jeffery and Humphrey[1] was used to calculate the amount of Chlorophyll A in solution.
The GBC Cintra 6 and UV-Lite software were used to analyse and automate the measurement of Chlorophyll A.

## Procedure

The samples were filtered as soon as possible after collection as chlorophyll pigments react with light and oxygen. A known volume of sample water was vacuum filtered through a glass fibre filter.
Once wrapped in aluminium foil to keep the light out, samples can be stored in this state at -20$^{o}$C for approximately 3 to 4 weeks if the analysis cannot proceed immediately.

The filter was broken up to facilitate extraction and placed into a 15 ml centrifuge tube. 90% ethanol solution was added and shaken thoroughly. The tube was wrapped in foil to keep out any light and refrigerated at 4$^{o}$C for at least 2 hours but less than 24 hours.
The tube was shaken three times during this period.

After it had been left to stand, the contents of the tube was centrifuged for 10 minutes at which point a clear solution remained. This time can be as low as 5 minutes depending on the speed of the centrifuge.

The optical density of the supernatant should ideally be less than 0.05 abs at 750 nm for a 1 cm cuvette.

## Chlorophyll A Determination

In subdued light the supernatant was poured into a 1cm cuvette and measured immediately. The instrument was zeroed on a 90% ethanol solution and the sample was measured at wavelengths 750 nm, 664 nm, 647 nm and 630 nm.

Corrections for turbidity were made by subtracting the absorbance value at 750 nm from the absorbance values at 664 nm, 647 nm and 630 nm.

The amount of Chlorophyll A was calculated by inserting the 750 nm corrected absorbances into the following equation:

**Chlorophyll A = 11.85 (Abs 664) – 1.54 (Abs 647) – 0.08 (Abs 630)**

The amount of Chlorophyll A pigment in the water sample was determined using the following equation:

$$\textbf{Chlorophyll A (ug/L)} = \frac{\textbf{(Chl A) * v (ml)}}{\textbf{V (L) * cell length (cm)}}.$$

Where:
V= volume of sample filtered in Litres
v = volume of extract solvent in mL

## UV Lite software

The equations were automatically calculated performed by the UV-Lite software.

As can be seen in figure 1, in the method form, in the measurement page there is a parameter called “Relation” in which you can enter the equation to find the amount of Chlorophyll A in the sample. The equation was entered and the Test button was clicked to confirm there were no errors in the equation.

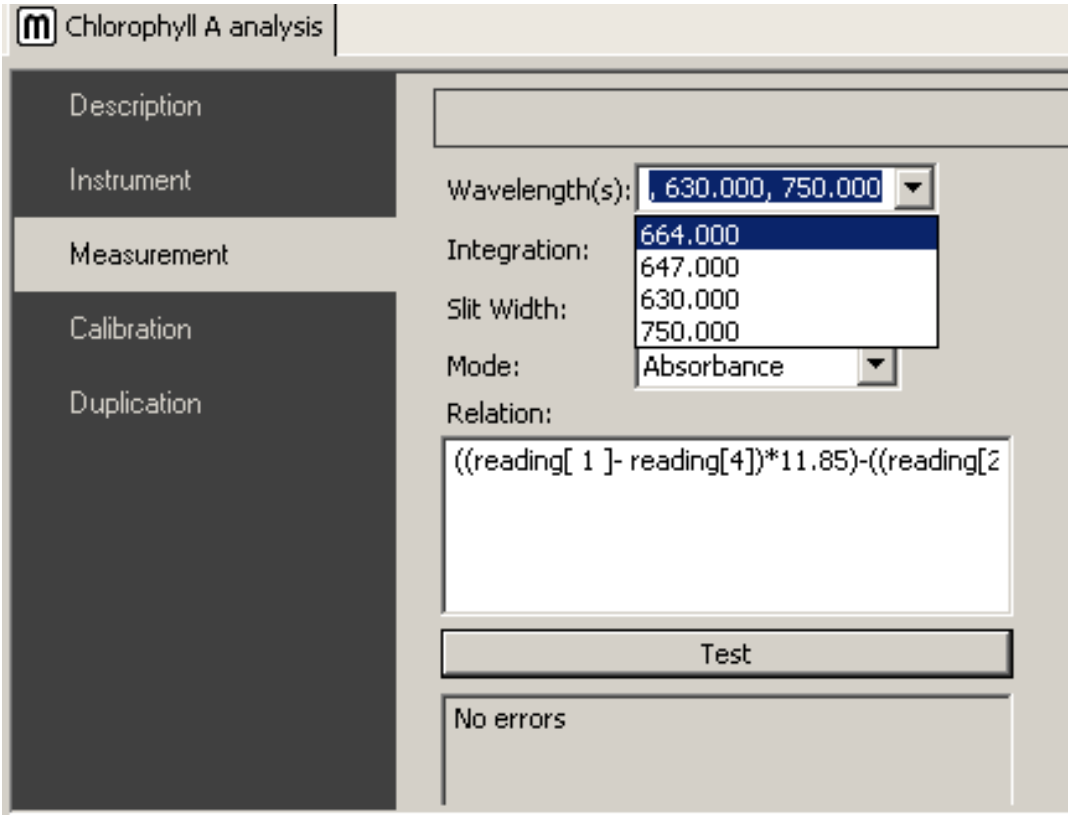


***Figure 1: UV-Lite Measurement Page.***

## Results

The results output of the UV-Lite software for the analysis of Chlorophyll A are shown in figure 2.

Method:

| | |
|---|---|
| Name: | Chlorophyll A analysis |
| Filename: | method1.method |

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Description:
Calculation in the Relation are to determine the concentration in mg\L of Chlorophyll A.

Instrument Parameters:

| | |
|---|---|
| Instrument Type: | Cintra 6 |
| D2 Lamp: | Automatically changed at 350.00nm |
| Beam Mode: | double |

Measurement:

| | |
|---|---|
| Wavelength(s): | 664.00nm, 647.00nm, 630.00nm, 750.00nm |
| Integration Time: | 0.5s |
| Slit Width: | 1.5nm |
| Photometric Unit: | absorbance |
| Relation: | ((reading[ 1 ]- |

Calibration Information:

| Sample | Sample Reading | Conc | Replicate %RSD | Replicate Reading |
|---|---|---|---|---|
| Pond water sample | 0.9119 | - | 3.0571 | 0.9439 |
| | | | | 0.8986 |
| | | | | 0.8931 |

***Figure 2: Chlorophyll A results*.**

## Conclusion

There are many methods available for the preparation samples for the analysis of Chlorophyll A however the measurement at the four different wavelengths is the quickest and most robust of these techniques. This analysis can easily and accurately be performed using the Cintra 6.
While only Chlorophyll A was measured in this application, Chlorophyl B, C and Total Chlorophyll as well as acidification to remove interference of Chlorophyllide can also easily be performed using the Cintra 6.
The use of the relation to calculate results within the software removes the need to perform tedious calculations for every sample, making it a fast and effective tool in the measurement of Chlorophyll.

## References


- Jeffrey, S.W. and G.R. Humphrey. 1975 New spectrophotometric equations for determining chlorophylls a and $c_2$ in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanzen Bd. 167: 191 – 194.
- C. Eijckelhoff & J. Dekker, A routine method to determine the chlorophyll a, pheophytin a and b-carotene contents of isolated Photosystem II reaction center complexes; Kluwer Academic Publishers, 1997
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- E. Arar, Method 446.0 In vitro determination of chlorophylls a, b, $c_1$ + $c_2$ and pheopigments in marine and freshwater algae by visible spectrophotometry; US EPA, Ohio USA, 1997



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