Quantify chlorophyll *a* and chlorophyll *b* with a custom method

Using the NanoDrop One Spectrophotometer

Abstract

Scientists can accurately quantify chlorophyll *a* and chlorophyll *b* on the Thermo Scientific™ NanoDrop™ One/One^{C} Microvolume UV-Vis Spectrophotometer using a user-defined custom method.

Introduction

Chlorophyll *a* is the principal pigment that converts light energy to chemical energy, and chlorophyll *b* is the accessory photosynthetic pigment that transfers light it absorbs to chlorophyll *a*. Chlorophyll *a* is found in all plants, green algae, and cyanobacteria, and chlorophyll *b* is found in plants and green algae. Chlorophyll quantitation is valuable in a vast array of disciplines including but not limited to plant biology, environmental science, ecotoxicology, disease prevention, and medical drug discovery.

Spectrophotometry is a common method used to measure the absorbance of light by the chlorophyll molecules. The NanoDrop One/One^{C} UV-Vis Spectrophotometer can be used to measure the absorbance of chlorophyll. Chlorophyll *a* and chlorophyll *b* absorb light at slightly different wavelengths. Chlorophyll *a* absorbs light at 433 nm and 666 nm and chlorophyll *b* absorbs light at 462 nm and 650 nm. The NanoDrop One/One^{C} UV-Vis application can be used to observe the spectrum of each chlorophyll *a* and chlorophyll *b* and identify major absorbance peaks (Figure 1). With this information, a user-defined custom method including user-defined formulas can be created to measure the absorbance and determine the concentration of chlorophyll.

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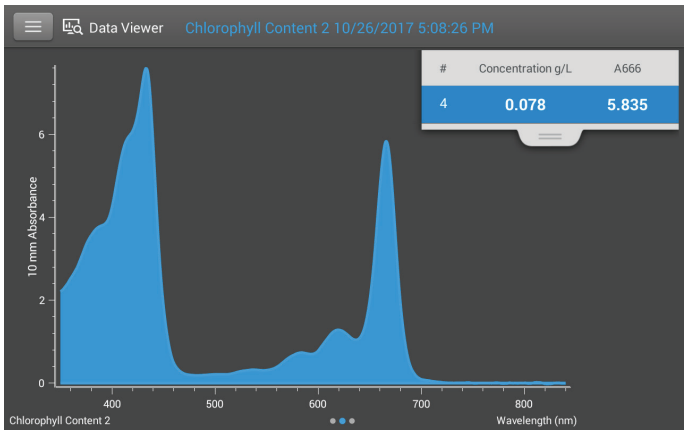
chlorophyll *a*



chlorophyll *b*

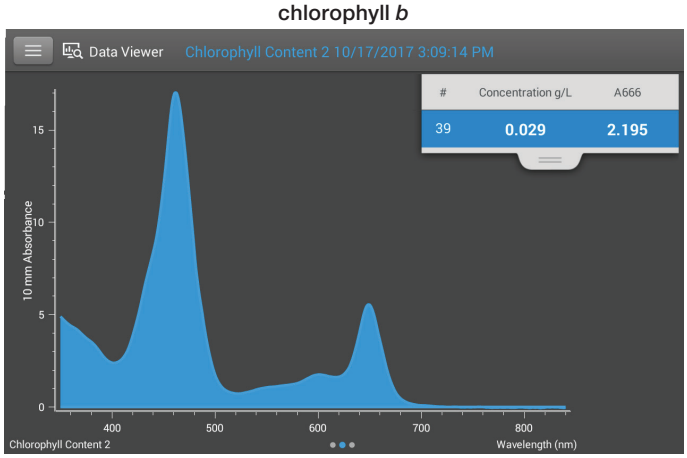


Figure 1. Absorbance spectrum of chlorophyll *a* and chlorophyll *b*. Chlorophyll *a* displayed major absorbance peaks at 433 nm and 666 nm (top). Chlorophyll *b* displayed major absorbance peaks at 462 nm and 650 nm (bottom).

Methods

Sample Preparation

Various solvents can be used to extract chlorophyll *a* and chlorophyll *b*. Porra et al. (1989) discuss assays of chlorophyll *a* and chlorophyll *b* suspended in various solvents including dimethylformamide, methanol, and 80% aqueous acetone. Barnes et al. (1991) describe the use of dimethyl sulfoxide (DMSO) for the extraction and determination of chlorophyll *a* and chlorophyll *b*. Based on the findings of Barnes et al., and the low toxicity and low vapor pressure of DMSO, we determined DMSO was an appropriate solvent for chlorophyll *a* and chlorophyll *b* measurements on the NanoDrop One/One^C pedestal.

Pure chlorophyll *a* from spinach (Sigma-Aldrich® Product # C5753) and pure chlorophyll *b* from spinach (Sigma-Aldrich Product # C5878) were each dissolved in 100% DMSO. Half-fold serial dilutions for each suspension were prepared using DMSO. The samples were stored in amber tubes at –20°C until measured.

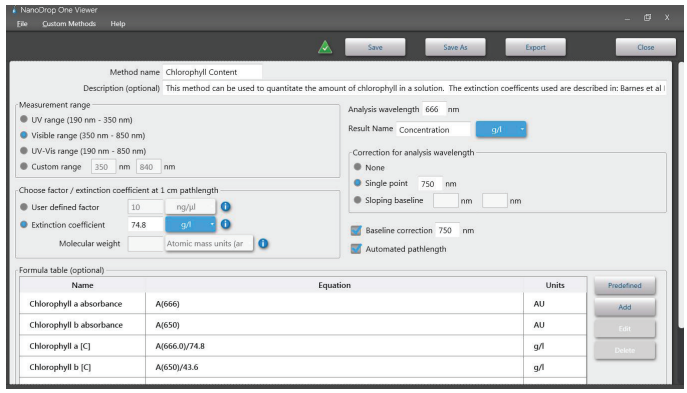


Figure 2. Chlorophyll Content custom method created to quantify chlorophyll *a* and chlorophyll *b* samples suspended in 100% DMSO.

Chlorophyll Quantification Custom Method

The NanoDrop One/One^C PC Viewer Custom Method application allows a user to specify how to calculate and report results. The Custom Method application was used to create a user-defined custom method to quantify chlorophyll *a* and chlorophyll *b*.

For this study, the following custom method parameters were used:

Wavelength range	Visible (350–850 nm)
Extinction coefficient	74.8 g/L
Analysis wavelength	666 nm
Correction for analysis wavelength	750 nm
Baseline correction	750 nm
Automated pathlength	On

Custom formulas were entered in the Formula table to report the absorbance at the analysis wavelength for chlorophyll *a* and chlorophyll *b*, and to calculate the concentration of pure chlorophyll *a* and pure chlorophyll *b*. Additional formulas were added to report the concentration of chlorophyll *a*, chlorophyll *b*, and chlorophyll *a*+*b* in samples containing a mixture of chlorophyll *a* and chlorophyll *b*. The formulas were taken from Barnes et al. (1991) and the peak locations were determined in the UV-Vis application (Figure 1).

The custom method was loaded in the Custom Method application on the NanoDrop One/One^C local control. The custom method was run to measure each chlorophyll *a* and chlorophyll *b* serial dilution in triplicate.

Note: For this study, the NanoDrop One/One^C UV-Vis application was used to identify the major peaks of chlorophyll *a* and chlorophyll *b* to determine the analysis

wavelength. If you are using a solvent other than DMSO, we recommend using the UV-Vis module to identify the maximum peaks for your samples, and modifying the custom method accordingly.

Custom Method Download

1. Navigate to www.thermofisher.com/nanodrop
2. On the left, select "NanoDrop Software Download"
3. Choose the "NanoDrop One/One^{C}" tab
4. Select "Local Control Software Download Instructions"
5. Scroll to "How to add a NanoDrop One/One^{C} Custom Method file" and click on "Chlorophyll Content Method"
6. Unzip the custom method file and copy the .method file to a USB device and then follow the online "Instructions for uploading a Custom Method to the instrument from a USB device".

Performance Data

Each chlorophyll *a* and chlorophyll *b* dilution was measured using the user-defined custom method described above. Each sample was measured in triplicate to assess reproducibility (Tables 1 and 2). Standard deviation values in all cases were below 0.002 Abs (10 mm equivalent).

Chlorophyll *a*	A(666) (n=3)	Conc. (g/L) (n=3)	Std. dev.
Sample 1	12.096	0.162	0.001
Sample 2	5.841	0.078	0.000
Sample 3	3.270	0.044	0.000
Sample 4	1.705	0.023	0.000

Table 1. Chlorophyll *a* content. The table displays calculated results for dilutions of chlorophyll *a* measured in triplicate.

Chlorophyll *b*	A(650) (n=3)	Conc. (g/L) (n=3)	Std. dev.
Sample 1	10.342	0.237	0.001
Sample 2	5.455	0.125	0.002
Sample 3	2.852	0.065	0.001
Sample 4	1.474	0.034	0.000

Table 2. Chlorophyll *b* content. The table displays calculated results for dilutions of chlorophyll *b* measured in triplicate.

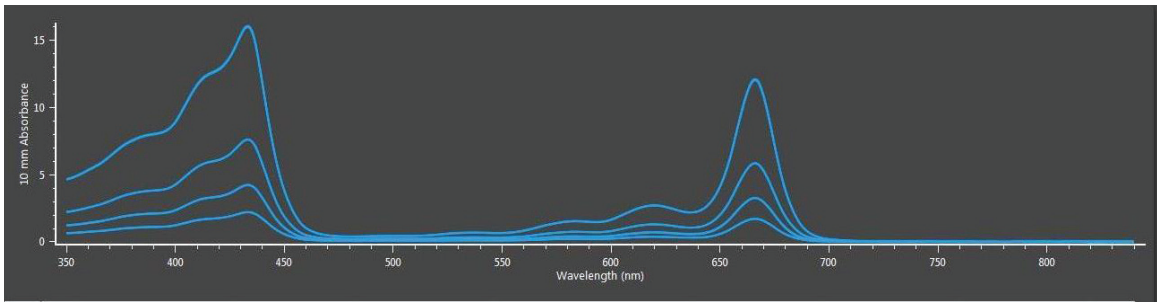


Figure 3. Custom method absorbance spectrum for each sample measurement of Chlorophyll *a* from spinach, Sigma-Aldrich Product # C5753, suspended in 100% DMSO. Chlorophyll *a* displayed major absorbance peaks at 433 nm and 666 nm.

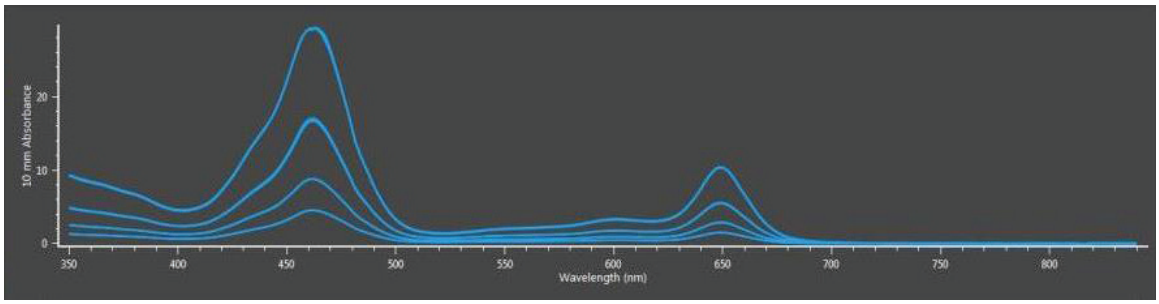


Figure 4. Custom method absorbance spectrum for each sample measurement of Chlorophyll *b* from spinach, Sigma-Aldrich Product # C5878, suspended in 100% DMSO. Chlorophyll *b* displayed major absorbance peaks at 462 nm and 650 nm.

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Conclusion

This study demonstrates the NanoDrop One/One^{C} UV-Vis Microvolume Spectrophotometer can be used to accurately quantify chlorophyll *a* and chlorophyll *b* using a user-defined custom method. The low standard deviation indicates there was a high agreement between replicate measurements. This validates the NanoDrop One/One^{C} Spectrophotometer can quantify samples accurately and reproducibly. The ability to measure chlorophyll *a* and *b* using a custom method serves as a valuable tool for research and the advancement of science.

References

1. J.D. Barnes, L. Balaguer, E. Manrique, S. Elvira, and A.W. Davison (1991). A reappraisal of the use of DMSO for the extractions and determination of chlorophylls a and b in lichens and higher plants. *Environmental and Experimental Botany.* Vol. 32. No. 2, 85–100.
2. R.J. Porra, W.A. Thompson and P.E. Kriedemann. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy (1989). *Biochimica et Biophysica Acta*. 975, 384–394.

Further Assistance and Technical Support

For further assistance, contact NanoDrop technical support at nanodrop@thermofisher.com or visit thermofisher.com/nanodrop.

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