Natural Porphyrins - Chlorophylls.
A Review of UV-Vis Spectrophotometric Measurement and Calculations.

Eugenia Făgădar-Cosma, Adriana Filiaș, Adrian Chiriac

a Institute of Chemistry Timisoara of Romanian Academy, Bd. M. Viteazul 24, 300223-Timisoara, ROMANIA, e-mail: efagadar@yahoo.com
b West University of Timisoara, Faculty of Chemistry – Biology – Geography, Inorganic Chemistry Department, Str. Pestalozzi 16, 300115, Timisoara, ROMANIA

Received: 05 December 2005 Modified: 06 December 2005 Accepted: 10 December 2005

Summary

An exhaustive study comprising the UV-VIS spectrophotometric measurements and calculations of chlorophyll content in higher plants and algae is presented. Trichromatic and monochromatic equations even with the modified version of acidification step, using for pigment extractions different solvents, are reviewed. Keywords: UV-vis spectroscopy, chlorophylls, trichromatic equations, monochromatic equations, acid-corrected equations.

Introduction

Chlorophylls are greenish pigments which contain a porphyrin ring. This is a stable tetrapyrollic ring-shaped molecule with aromatic structure. Because the electrons move freely, the ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll "captures" the energy of sunlight.
There are several kinds of chlorophyll, the most important being chlorophyll "a". This is the molecule which makes photosynthesis possible, by passing its energized electrons on to molecules which will manufacture sugars. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll "a".

A second kind of chlorophyll is chlorophyll "b", which occurs only in green algae and in the plants. A third form of chlorophyll, called chlorophyll "c" is found only in the photosynthetic members of the Chromista as well as the dinoflagellates.

The differences between the chlorophylls of these three major groups can easily be put into evidence by registering their UV-Vis spectra [1].

The two major types of chlorophyll, named \( \textit{a} \) and \( \textit{b} \), differ only slightly, in the composition of a sidechain (in \( \textit{a} \) it is \(-\text{CH}_3\), in \( \textit{b} \) it is \(-\text{CHO}\)).

The chemical IUPAC nomenclature of Chlorophyll a and b is given below:

\[
\text{Phytyl} \quad \left(13^{\text{R}},17^{\text{S}},18^{\text{S}}\right)-3-(8\text{-ethyl}-13^2\text{-methoxycarbonyl}-2,7,12,18\text{-tetramethyl}-13'-\text{oxo}-3\text{-vinyl}-13^1\text{-}13^2\text{-}17,18\text{-tetrahydrocyclopenta}\left[\text{at}\right]\text{-prophyrin}-17\text{-yl})\text{propionate}, \quad \text{(Pheophytin a),}
\]

or as the magnesium complex (Chlorophyll a).

\[
\text{Phytyl} \quad \left(13^{\text{R}},17^{\text{S}},18^{\text{S}}\right)-3-(8\text{-ethyl}-7\text{-formyl}-13^2\text{-methoxycarbonyl}-2,12,18\text{-trimethyl}-13'\text{-oxo}-3\text{-vinyl}-13^1\text{-}13^2\text{-}17,18\text{-tetrahydro-cyclopenta}\left[\text{at}\right]\text{-prophyrin}-17\text{-yl})\text{propionate},
\]
(Pheophytin b), or as the magnesium complex (Chlorophyll b).

Both of these two chlorophylls are very effective photoreceptors because they contain a network of alternating single and double bonds, and the orbitals can delocalize establishing the structure. Such delocalized polyenes have very strong absorption bands in the visible regions of the spectrum, allowing the plant to absorb the energy from sunlight.

![Absorption spectra of pure chlorophyll a and b](image)

The different sidegroups in the 2 chlorophylls 'tune' the absorption spectrum to slightly different wavelengths, so that light that is not significantly absorbed by chlorophyll a, at, say, 460nm, will instead be captured by chlorophyll b, which absorbs strongly at that wavelength. Thus these two kinds of chlorophyll complement each other in absorbing sunlight.

Plants can obtain all their energy requirements from the blue and red parts of the spectrum, however, there is still a large spectral region, between 500-600nm, where very little light is absorbed. This light is in the green region of the spectrum, and since it is reflected, this is the reason plants appear green.

Chlorophyll a is a green dye and its absorption spectrum shows that it absorbs strongly in the red and blue-violet regions of the visible spectrum. Because it absorbs red and blue-violet light, the light it reflects and transmits appears green. Chlorophyll absorbs so strongly that it can mask other less intense colors.

Some of these more delicate colors (from molecules such as carotene and
quercetin) are revealed when the chlorophyll molecule decays in the autumn, and the woodlands turn red, orange, and golden brown. Chlorophyll can also be damaged when vegetation is cooked, since the central Mg atom is replaced by hydrogen ions. This affects the energy levels within the molecule, causing its absorbance spectrum to alter. Thus cooked leaves change color - often becoming a paler, insipid yellowy green [2].

**Detection and analysis methods for chlorophyll control**

The spectroscopic methods for quantitative determination of chlorophylls a and b are suitable for quick, routine analysis of the pigment content. The precision of spectroscopic method depends on the type of device used, the ability to determine with precision the absorbance maxima, and the accuracy of the absorption coefficient used for the calculation [3, 4]. It is undoubtedly that HPLC methods generally offer better resolving power, but are usually more laborious, being carried out with different types of columns and different organic solvents.

Fluorometry is another highly sensitive method to determine chlorophyll concentration, but disadvantages of fluorometry [5] comprise problems of the quenching of chlorophyll a fluorescence by β-carotene and other accessory pigments and the dependence of chlorophyll fluorescence on temperature. The transfer of energy is highly efficient and can only occur from a pigment absorbing at a shorter wavelength to one absorbing at a longer wavelength.

The fluorescence of chlorophyll a may correspond to the same yield of light absorbed by chlorophyll b. T.W. Goodwin suggests that the light absorbed by pigments other than chlorophyll a is transferred to chlorophyll a which then sensitizes photosynthesis. Experiments have indicated that it is necessary to have the simultaneous excitation of chlorophyll a and another pigment in order to have efficient photosynthesis. Accessory pigments are believed to effect the efficiency of photosynthesis by transferring their excitation energy by this resonance transfer to fluorescent chlorophyll a [6].

Chromatography is another technique for separating chemical substances. The separation of colored compounds on paper strips requires the use of a porous material such as paper, silica gel and alumina. The paper is a self-supporting adsorbent since it is relatively stiff and hangs together [7]. For chlorophyll, up to eight colored spots can be observed. In order of decreasing Rf values they are carotenes (two spots, orange), chlorophyll a (blue-green), the xanthophylls (four spots, yellow) and chlorophyll b (green) [6].
**MATERIALS AND METHODS**

*General solvent extraction method for measuring the concentration of chlorophyll in leaves*

A well defined weight fresh leaf tissue are cut into small pieces (about 1 mm wide) with scissors or razor blade. The pigment is extract by grinding the cut tissue for 5 minutes in 100 ml of the chosen solvent (80% acetone, 85% acetone, 100% acetone, methanol, N,N-dimethylformamide, diethylether etc.) in a mortar and pestle.

Than the homogenate is transferred to a Buchner funnel and filtered the extract using the vacuum. The filtered extract is transferred to a 100-ml volumetric flask and make up to volume with solvent.

The optical density (absorbance) of the extract is measured with the UV-Vis apparatus. The measurements of optical density are made at the lengthwaves where maximum absorption of chlorophyll \(a\) and \(b\) occur. The concentration of chlorophyll \(a\) and \(b\) is then calculated by one of the presented above formula.

In course of extraction procedure some reactions produced chlorophylls degradations could occur and must be avoided.

*Chlorophyll degradation*

During storage chlorophyll encounters many elements which cause degradation. Chlorophyll \(a\) is formed about five times faster than chlorophyll \(b\). At room temperature in vitro chlorophylls will form isomeric chlorophylls \(a\) and \(b\), which are formed in vivo by heating. An in vitro sample will experience isomerization in a few hours or rapidly if alkali is added at room temperature. Chlorophyll \(a\) and chlorophyll \(b\) each have three isomers with magnesium bonded to three different pairs of pyrrole nitrogen atoms. These isomers exist as tautomers rather than as resonant hybrids [30].

Also, the original chlorophyll sample may undergo oxidation and produce oxidized products. The isomers and oxidized products account for the chlorophyll-like products that are separable from chlorophyll on chromatographic analysis and they absorb at slightly different wavelengths. Oxygen attack on the isocyclic carbon C-10 also breaks down the pigment. This process causes oxidation of C-10 to hydroxy, then a breaking of the ring to form a variety of purpurins and chlorins. Further oxidation of these derivatives occurs through complete scission of the isocyclic ring, then oxidation of tetrpyrrole occurs [27]. Other derivatives such as pheophytins \(a\) and \(b\), pheophorbides \(a\) and \(b\), and pyroderivatives are caused by acidification, hydrolyzation and heat treatment, respectively [28].
Reduction occurs not only by oxidation, but also by changes in temperature, pH and light. Heating degrades the structure and instigates chemical reactions as well as further oxidation. The pH changes during storage and processing leads to chlorophyll degradation. Chlorophyll stability varies as a function of pH. A pH between 6 and 7 is critical. For spinach the critical pH is between 6.7 and 7 [31].

Strong light bleaches chlorophyll solutions [19]. In vitro, photobleaching causes chlorophyll breakdown where the chlorophyll solution is irreversibly bleached by light in the presence of oxygen.

Chlorophyll a and b have reaction rates on the same order. The order of magnitude is larger than that of pheophytins and pheophorbides which are more stable. The difference relates to the magnesium attached to the chlorophyll a and b excited triple state molecules. Light studies during senescence show that the ratio of chlorophyll a to other chlorophylls decreases while held in darkness. In this environment, direct photochemical degradation of the pigment occurs.

**RESULTS AND DISCUSSION**

**Measuring Chlorophyll by UV-Vis Spectrometry**

There are three main specific methods for measuring chlorophyll. Spectrophotometric analysis of chlorophyll pigments by UV-Vis measurements were developed in the 1930's and 1940's [8].

Richards and Thompson (1952) introduced a trichromatic technique that was supposed to measure chlorophylls a, b, and c. Trichromatic equations attempted to remove interferences of the other chlorophylls by taking the measurement values at the maximum absorption wavelength for each chlorophyll. During time a number of modifications have been made to these equations with the purpose of better estimating of the chlorophylls [9-11]. When these equations are ultimately compared with concentrations of chlorophyll obtained using physical separation techniques such as HPLC, paper or thin-layer chromatography, it is found that the degree of correspondence is low. Apparently the trichromatic equations are no substitute for physical separation techniques. In addition, these equations do not deal with the degradation products of chlorophyll.

The trichromatic "chlorophyll a" is better presented as chlorophyll a minus most of the interference of other chlorophylls and is still used in oceanographic research, where degradation products are less of a problem. Lorenzen (1967) and Moss (1967) introduced an acidification step in a
monochromatic method to circumvent the interference by chlorophyll degradation products [12]. When chlorophylls are acidified, the magnesium ion is lost from the porphyrin ring, resulting in the production of a phaeophytin. Lorenzen (1967) produced equations capitalizing on the fact that the ratio of pure chlorophyll $a$ after acidification to that before was 1.7 [12]. If the sample contained pure phaeophytin, then the absorbance would not change, and the ratio would be 1.0. Acid ratios between 1.0 and 1.7 would therefore indicate the amount of degradation products in the sample, and the estimate of chlorophyll could thus be corrected.

In natural waters, the acid ratio, and therefore the resulting estimate of chlorophyll $a$, varies considerably depending on, not only the relative concentrations of chlorophyll $a$ and phaeophytin, but also the concentrations and behavior after acidification of chlorophylls $b$, phaeophorbides, chlorophyllides, bacteriochlorophylls, and perhaps phycobiliprotein pigments.

Numerous modifications have been made to this technique, including changing the normality of the acid, the absorbance wavelengths, the time between acidification and the reading of the value, and the extracting solvent. Despite major modifications in the technique, the resultant value is always called chlorophyll $a$, even though each methodological change alters the estimated chlorophyll $a$ value. For example, other pigments, particularly chlorophyllide $a$, cannot be spectrophotometrically distinguished from chlorophyll $a$.

These magnesium-containing degradation products of chlorophyll can comprise a significant proportion of the total pigments. Hallegraeff (1976) found magnesium-containing degradation products, on some dates, to increase over 50% of what would have been estimated as chlorophyll $a$. As well as a number of other authors, he emphasized that the acidification technique really eliminates only the interference of non-manganese-containing pigments [13].

That is the reason why numerous authors state that the only method for measuring chlorophyll $a$ accurately is using some separation procedure such as HPLC. Any other method produces only an estimate of the chlorophyll concentration.

An alternative to the use of physical separation techniques and the distress of choosing the “proper” spectrophotometric equation is to report the amount of total chlorophyll pigments [14]. This is the estimation of all chlorophyll pigments and degradation products that absorb at 665 nm. This measurement descends of the Odum’s (1958) monochromatic chlorophyll $a$ equation [15]. Golterman and Clymo’s equation uses the extinction coefficients of Strickland and Parsons (1963) in 90% acetone which are probably the most popular extinction coefficients and solvent. Their equation is:
Total Chlorophyll = $11.0 (Abs_{665} - Abs_{550}) \frac{V}{V_p}$ (1)

where V is the volume filtered (L), v is the volume of extract (ml), and p is the pathlength (cm). Using values for total chlorophyll pigments rather than either the trichromatic equations or the acid-corrected equations get around the problem of interference by ignoring it. It is simply a measure of absorbance at 665 nm [14, 16].

Comparisons of the total chlorophyll concentration with trichromatic chlorophyll a calculated by the Parsons and Strickland (1963) equations using data from the Ohio-NEFCO program have correlations with the trichromatic chlorophylls of greater that 0.99 with a slope of 1. Herve and Heinonen (1982) also reported no significant differences between the Parsons and Strickland (1963) and the "Proposed Norsk Standard" chlorophyll equation, which is identical to that for total chlorophyll pigments [17, 18].

Absorption spectra of the various chlorophylls depend on the solvent used. Kundt’s rule states that the longer the refractive index of the solvent, the longer the wavelength of the absorption maxima [19]. Lichtenthaler & Wellburn derived equations (2) and (3) in order to determine the amounts of chlorophyll a and b in individual leaves using a 100% acetone extract [20].

Similar equations exist for other solvents such as methanol and diethyl ether. The coefficients in equations (2) were determined for pure chlorophyll a and b in 100% acetone by finding the locations of their peaks in the red region (645 nm for chlorophyll b, 662 nm for chlorophyll a) and the values of their absorption coefficients at these peaks.

Assuming that the absorbance by one pigment does not influence the absorbance by the other, the total absorbance in a mixture of chlorophyll a and b at the two peaks can be calculated as the sum of the individual absorbances of each pigment. This gives rise to two equations which can be solved simultaneously to give the concentration of each pigment in μg/ml of extract as shown in equations (2) below. $C_a$ is the concentration of chlorophyll a and $C_b$ is the concentration of chlorophyll b. These equations are valid for standard square cuvettes with a path length of 1cm.

$$C_a = 11.75 A_{662} - 2.35 A_{645}$$

$$C_b = 18.61 A_{645} - 3.96 A_{662}$$ (2)

A constant conversion factor exists for absorbances in square and round cuvettes, the absorbances in equations (2) and (3) measured with round cuvettes will all be scaled by a constant factor. Thus, for calculating the ratio of chlorophyll a : chlorophyll b, this factor cancels out and thus absorbance readings from round cuvettes will give the same results for the ratio as those obtained from square cuvettes.

The spectroscopic methods for quantitative determination of chlorophylls a and b
are suitable for quick, routine analysis of the pigment content. The precision of spectroscopic method depends on the type of device used, the ability to determine with precision the absorbance maxima, and the accuracy of the absorption coefficient used for the calculation [3, 4].

The most used equations for chlorophylls content in plants, using as extraction solvent 90% acetone are given below:

“SCOR-UNESCO (1966)” 90% acetone [10]:

\[
\begin{align*}
\text{CHL}_a (\mu g/mL) &= 11.64 A_{665} - 2.16 A_{645} + 0.10 A_{630} \\
\text{CHL}_b (\mu g/mL) &= -3.94 A_{663} + 20.97 A_{645} - 3.66 A_{630} \\
\text{CHL}_c (\mu g/mL) &= -5.53 A_{663} - 14.81 A_{645} + 54.22 A_{630}
\end{align*}
\]

Jeffrey and Humphrey (1975) 90% acetone [11]:

\[
\begin{align*}
\text{CHL}_a (\mu g/mL) &= 11.85 A_{664} - 1.54 A_{647} - 0.08 A_{630} \\
\text{CHL}_b (\mu g/mL) &= -5.47 A_{664} + 21.03 A_{647} - 2.66 A_{630} \\
\text{CHL}_c (\mu g/mL) &= -1.67 A_{664} - 7.60 A_{647} + 24.52 A_{630}
\end{align*}
\]

Jeffrey and Humphrey (1975) with Humphrey (1979) 90% acetone (chromophyte modification) [11, 21]:

\[
\begin{align*}
\text{CHL}_a (\mu g/mL) &= 11.47 A_{664} - 0.40 A_{630} \\
\text{CHL}_c (\mu g/mL) &= 24.36 A_{630} - 3.73 A_{663}
\end{align*}
\]

Lorenzen (1965) Chl\text{a} corrected for pheopigments 90% acetone [12]:

\[
\begin{align*}
\text{CHL}_a (\mu g/L) &= [26.73 (A_{665}^o - A_{665}^a)] v / V \\
\text{Pheo} (\mu g/L) &= [26.73 (A_{665}^a - A_{665}^o)] v / V
\end{align*}
\]

where: \(A_{665}^o\) and \(A_{665}^a\) are absorption at 665nm before and after acidification, 
\(v\) = volume of the pigment extract, 
\(V\) = volume of the water filtered 
26.73 is an absorption coefficient correction for the ratio of these pigments with pure chlorophyll.

In 80% acetone extract solution absorbance was measured at 647 nm and 663 nm, these being the absorbance maxima for chlorophyll a and chlorophyll b. Absorbance values were used to calculate pigment concentrations using standard extinction equations reported by Lichtenthaler [22]:

\[
\begin{align*}
\text{chlorophyll } a (\text{mg} \cdot \text{L}^{-1}) &= (12.25 \cdot A_{663} - 2.79 \cdot A_{647}) \cdot D \\
\text{chlorophyll } b (\text{mg} \cdot \text{L}^{-1}) &= (21.5 \cdot A_{647} - 5.1 \cdot A_{663}) \cdot D \\
\text{chlorophyll } a+b (\text{mg} \cdot \text{L}^{-1}) &= (7.15 \cdot A_{663} + 18.71 \cdot A_{647}) \cdot D
\end{align*}
\]

where: 
A is absorption at given wavelengths, 
D - thickness of the used cuvette (cm).

Two registered UV-Vis spectra of mixtures of different concentrations of chlorophylls a and b extracted in 80% acetone, from wheat seedlings [23-25] are given below:
In N,N-dimethylformamide used as extraction solvent, the absorbance have to be measured at 664 nm for chlorophyll a and at 647 nm for chlorophyll b. The amount of chlorophyll was calculated according to Moran [26], giving concentrations of chlorophyll in mg/dm³ (mg/l). The equations are:

Chlorophyll a (mg/l): \( C_a = 12.64A_{664} - 2.99A_{647} \)  
Chlorophyll b (mg/l): \( C_b = -5.60A_{664} + 23.26A_{647} \)  

For determining the concentrations of Chl a, Pheo a and Car of PS II RC from their extracts in 80% acetone, for each wavelength the absorption \( A_\lambda \) of the extract is given by:

\[ A_\lambda = C_{chl}e_{chl,\lambda} + C_{pheo}e_{pheo,\lambda} + C_{car}e_{car,\lambda} \]  

(9)

So, if the molar extinction coefficients of the three pigments are known at three different wavelengths, the concentrations \( c \) of the three pigments in the extract can be determined. Because for some preparations contamination of Chl b can not always be totally excluded, we present an additional method including this pigment.

The extraction procedure of PS II RC preparations from plants in 80% acetone was described in detail before [27]. Chl a, Chl b, Pheo a and Car were obtained from such extracts with 99.9% purity by reversed phase HPLC with 100% methanol as mobile phase.

The peak fractions were collected, dried by flushing with nitrogen gas and resolubilized in 80 % acetone, after which their absorption spectra were recorded on a Cary 219 spectrophotometer. The spectra of Chl a, Chl b and β-Car were normalized to published extinction coefficients in 80% acetone (86.3 mM⁻¹cm⁻¹ at 663 nm for Chl a, 134.14 mM⁻¹cm⁻¹ at 460 nm for Chl b and 140 mM⁻¹cm⁻¹ at 454 nm for β-Car – data from Lichtenthaler [22], based on which we obtained extinction coefficients of these pigments in 80% acetone at all recorded wavelengths [27] for the spectra of Chl a and β-Car in 80 % acetone after normalization according to these extinction coefficients.
With the extinction coefficients of Chl $a$, Pheo $a$ and $\beta$-Car being known at all wavelengths between 370 and 720 nm, equation (1) can now be solved by using three key wavelengths (or four if the concentration of Chl $b$ is to be estimated as well). The best result is obtained when at each of these wavelengths the total absorption is dominated by a different pigment.

For Method 1 it was chosen for 431 nm (where the absorption of Chl $a$ dominates the total absorbance), 412 nm (Pheo $a$ absorption dominates), 480 nm ($\beta$-Car absorption dominates) and 460 nm (to get an idea on possible Chl $b$ contamination), whereas for Method 2 we chose for 663 nm (Chl $a$ absorption dominates), 535–551 nm (Pheo $a$ absorption dominates) and again 480 nm ($\beta$-Car). Equation (9) gives the following equations for Method 1:

$$
\begin{align*}
    c_a &= -1.709 A_{412} + 11.970 A_{431} - 2.998 A_{460} - 5.708 A_{480} \\
    c_p &= +10.280 A_{412} - 8.380 A_{431} + 2.456 A_{460} - 1.233 A_{480} \\
    c_c &= -0.430 A_{412} + 0.251 A_{431} - 4.376 A_{460} + 13.216 A_{480} \\
    c_b &= -0.171 A_{412} - 0.230 A_{431} + 11.871 A_{460} - 13.248 A_{480}
\end{align*}
$$

(10)

For Method 2 these equations were calculated:

$$
\begin{align*}
    c_a &= +11.577 A_{663} - 76.994 A_{535-551} + 0.624 A_{480} \\
    c_p &= +0.020 A_{663} + 132.505 A_{535-551} - 1.150 A_{480} \\
    c_c &= -0.146 A_{663} - 4.054 A_{535-551} + 8.311 A_{480}
\end{align*}
$$

(11)

In these equations, $c$ is given in $\mu$M and $A_\lambda$ in cm$^{-1}$; the suffices $a$, $p$, $c$ and $b$ stand for Chl $a$, Pheo $a$, $\beta$-Car and Chl $b$, respectively.

**In diethyl ether** by using acid-corrected equations, the measurements of the absorbances of the solution in a 1 cm cell against a diethyl ether blank are to be made at 660.4 nm, 642.0 nm, 667.2 nm and 654.4 nm (these being the absorbance maxima in diethyl ether for chlorophyll $a$, chlorophyll $b$, phaeophytin $a$, and phaeophytin $b$, respectively). In addition measurements at 649.8 nm and 628.2 nm, for corrections to the remaining diluted solution add one crystal of oxalic acid and after dissolution and mixing it must remeasure the absorbances at the same wavelengths.

"Delta $A$" is the difference between the absorbances between the absorbance at the respective wavelengths, before and after addition of oxalic acid. The concentration of the individual compounds in micromoles per litre from can be calculated by using the following equations:

$$
\begin{align*}
    \text{Chlorophyll } a &= 17.7 \Delta A (660.4 \text{ nm}) + 7.15 \Delta A (642.0 \text{ nm}) \\
    \text{Chlorophyll } b &= 19.4 \Delta A (642.0 \text{ nm}) - 2.92 \Delta A (660.4 \text{ nm})
\end{align*}
$$

(12)

Convert the figures in micro moles per litre to percentages using the following equations:

255
Expression of results in leaf area units, can be expressed:

\[
\text{PC (mg m}^{-2}\text{)} = \frac{V}{1000} \times \frac{A}{A} \times \text{PC (mg L}^{-1}\text{)}
\]

(14)

where:
- PC is the pigment concentration (mg L\(^{-1}\)),
- V - 80% acetone volume used,
- A - leaf tissue segments area (m\(^2\)).

Besides higher plants, the chlorophyll content measured in algae must specify the level of chlorophyll c, as presented in the below equations were:

\[
\text{OD}_{664} = \text{Abs}_{664} - \text{Abs}_{750}
\]

(15)

For Chlorophyll c containing organisms in 90\% acetone:

Chl a = 11.47 OD\(_{664}\) - 0.4 OD\(_{630}\)

Chl c = 24.36 OD\(_{630}\) - 3.73 OD\(_{664}\)

(16)

And in 80\% Methanol:

Chl a = 12.66 OD\(_{665}\) - 0.5 OD\(_{635}\)

Chl c = 31.25 OD\(_{635}\) - 5.79 OD\(_{665}\)

(17)

For Chlorophyll b containing organisms in 90\% Acetone:

Chl a = 11.93 OD\(_{664}\) - 1.93 OD\(_{647}\)

Chl b = 20.36 OD\(_{647}\) - 5.5 OD\(_{664}\)

(18)

And in 80\% Methanol:

Chl a = 16.5 OD\(_{665}\) - 8.3 OD\(_{650}\)

Chl b = 33.8 OD\(_{650}\) - 12.5 OD\(_{665}\)

(19)

There is a trichromatic calculation (90\% Acetone) for determining chlorophylls in a mixed phytoplankton assemblage in which both chlorophyll b and c containing organisms are present.

Chl a = 11.85 OD\(_{664}\) - 1.54 OD\(_{647}\) - 0.08 OD\(_{630}\)

Chl b = -5.43 OD\(_{664}\) - 21.03 OD\(_{647}\) - 2.66 OD\(_{630}\)

Chl c = -1.67 OD\(_{664}\) - 7.60 OD\(_{647}\) - 24.52 OD\(_{630}\)

(20)

*** Chl c calculation may lead to results which are 24\% too low

More recently, the following equations have been determined for 100\% Methanol [28]:

Chl a = 16.29 OD\(_{665}\) - 8.54 OD\(_{652}\)

Chl b = 30.66 OD\(_{652}\) - 13.58 OD\(_{665}\)

(21)

The following equations have been determined for 85\% acetone [29]:

\[
\text{mg chlorophyll a/g tissue} = 1.07 \times (O.D. \_663) - 0.094 \times (O.D. \_644)
\]

\[
\text{mg chlorophyll b/g tissue} = 1.77 \times (O.D. \_644) - 0.280 \times (O.D. \_663)
\]

(22)

The constants used in these calculations have been determined empirically.
Conclusions

A study comprising the UV-VIS spectrophotometric measurements and calculations of chlorophyll content in higher plants and algae is presented. Trichromatic and monochromatic equations even with the modified version of acidification step, using for pigment extractions different solvents: acetone, diethyl ether, methanol, N,N-dimethylformamide are reviewed.

References


257