Short Communication

New Spectrophotometric Equations for Determining Chlorophylls a, b, c_1 and c_2 in Higher Plants, Algae and Natural Phytoplankton

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Summary

New equations are presented for spectrophotometric determination of chlorophylls, based on revised extinction coefficients of chlorophylls a, b, c_1 and c_2. These equations may be used for determining chlorophylls a and b in higher plants and green algae, chlorophylls a and $c_1 + c_2$ in brown algae, diatoms and chrysomonads, chlorophylls a and c_2 in dinoflagellates and cryptomonads, and chlorophylls a, b, and $c_1 + c_2$ in natural phytoplankton.

Spectrophotometric equations currently used for determining chlorophylls a and b in higher plants (Arnon 1949; Vernon 1960), chlorophylls a and c in algae (Humphrey and Jeffrey in Jeffrey and Haxo 1968) and chlorophylls a, b and c in phytoplankton (Parsons and Strickland 1963; SCOR-UNESCO 1966) are based on extinction coefficients of Mackinney (1941) and Smith and Benitez (1955) for chlorophylls a and b, and that of Jeffrey (1963) for chlorophyll c (Table 1). Jeffrey (1968, 1969) subsequently found that chlorophyll c was a mixture of two components (c_1 and c_2), which polymerize when purified (Jeffrey and Shibata 1969) to give lowered intensities of absorption bands and therefore lowered extinction coefficients. Each component of chlorophyll c has now been carefully crystallized to prevent polymerization, and extinction coefficients determined (Jeffrey 1972). The new extinction coefficients for chlorophylls c_1 and c_2 are approximately twice the former value for unresolved chlorophyll c.

Table 1 *Extinction coefficients of chlorophylls used to derive spectrophotometric equations in current use*

Chlorophyll	Extinction Coefficients in diethyl ether at red maximum $(l \cdot gm^{-1} cm^{-1})$			
	Mackinney (1941)	Smith and Benitez (1955)	Strain et al. (1963)	Jeffrey (1963)
chlorophyll a	90.1	102.0	96.0	
chlorophyll b	54.9	59.0	59.0	
chlorophyll c				15.8

Table 2 *Extinction coefficients of chlorophylls used to derive the new equations*

Chlorophyll	Extinction Coefficients at the red maximum $(l \cdot gm^{-1} cm^{-1})$		
	Diethyl ether	100% acetone	90% acetone
chlorophyll a*	98.07 (660.7 nm)	88.15 (662.7 nm)	87.67 (664.3 nm)
chlorophyll b*	62.00 (643.3 nm)		51.36 (646.8 nm)
chlorophyll c_1+		39.2 (629.1 nm)	44.8 (630.6 nm)
chlorophyll c_2+		37.2 (629.6 nm)	40.4 (630.9 nm)

* JEFFREY unpublished
+ JEFFREY (1972)

Thus determinations of chlorophyll c using the old equations give results approximately twice as high as those indicated by the present work. Extinction coefficients for crystalline chlorophylls a and b have been also redetermined for a variety of solvents most frequently used for making pigment assays (JEFFREY in preparation). Extinction coefficients of these highly purified preparations of chlorophylls a, b, c_1 and c_2 at their red maxima, are given in Table 2. These new values for a and b are almost identical to the most careful recently determined coefficients of STRAIN and co-workers (1963), (Table 1). The early MACKINNEY values used in the original ARNON (1949) higher plant equations are some 8% lower than the new values of both STRAIN and JEFFREY. The VERNON values, based on the SMITH and BENITEZ coefficients, which were also used in the algal $a + c$ and phytoplankton $a + b + c$ equations, are about 5% higher than the STRAIN and JEFFREY values. There are independent reasons for believing that these values are a few percent too high (JEFFREY, in preparation).

The new sets of equations have therefore been written using the revised extinction coefficients of JEFFREY for chlorophylls a, b, c_1 and c_2 (Table 2). Since the extinction coefficients of chlorophylls c_1 and c_2 differ only by 5% and 10% in 100% and 90% acetone respectively, and because of the smallness of the errors involved, equations utilizing chlorophylls c_1 and c_2 were derived for 1:1 mixtures of these two pigments. In the many algal species which have been examined which contain both chlorophylls c_1 and c_2, the most common proportions of chlorophylls c_1 and c_2 are $c_2 : c_1 = 1 : 0.7$ and $c_2 : c_1 = 1 : 1$ (JEFFREY, unpublished). The equations may be employed to assay a variety of mixtures of chlorophylls which are typically found in higher plants, green algae, brown algae, diatoms, chrysomonads, dinoflagellates and cryptomonads (JEFFREY 1968, 1969, 1972), and natural mixtures of these algae. We present here equations derived for chlorophyll estimation in 90% and 100% acetone. Results are expressed in $\mu g \cdot ml^{-1}$ (using 1 cm cell path-length). The symbol E_x denotes the extinction at wavelength x.

1. **Higher plants and green algae containing chlorophylls a and b**
   (solvent 90% acetone)
   Chlorophyll a $\quad= 11.93\ E_{664} - 1.93\ E_{647}$
   Chlorophyll b $\quad= 20.36\ E_{647} - 5.50\ E_{664}$

2. **Diatoms, chrysomonads and brown algae containing chlorophylls a, and c_1 and c_2 in equal proportions** (solvent 90% acetone)
   Chlorophyll a $\quad= 11.47\ E_{664} - 0.40\ E_{630}$
   Chlorophylls $c_1 + c_2 = 24.36\ E_{630} - 3.73\ E_{664}$

3. **Dinoflagellates and cryptomonads containing chlorophylls a and c_2**
   (solvent 100% acetone)
   Chlorophyll a $\quad= 11.43\ E_{663} - 0.64\ E_{630}$
   Chlorophyll c_2 $\quad= 27.09\ E_{630} - 3.63\ E_{663}$

4. **Mixed phytoplankton populations containing chlorophylls a and b and equal amounts of chlorophylls c_1 and c_2** (solvent 90% acetone)
   Chlorophyll a $\quad= \quad 11.85\ E_{664} - \quad 1.54\ E_{647} - 0.08\ E_{630}$
   Chlorophyll b $\quad= -5.43\ E_{664} + 21.03\ E_{647} - 2.66\ E_{630}$
   Chlorophylls $c_1 + c_2 = -1.67\ E_{664} - \quad 7.60\ E_{647} + 24.52\ E_{630}$

The recovery of chlorophyll a from known mixtures using all four equations was usually in the range of 98—102%. The recovery of chlorophyll b from mixtures of $a + b$, using the $a + b$ equations, was from 98—106%. The recovery of chlorophyll c from mixtures of $a + c$, using both sets of $a + c$ equations, averaged 95—104% when chlorophyll c was present in amounts greater than one-fifth the chlorophyll a concentration. Decreasing proportions of chlorophyll c with respect to chlorophyll a gave increasing errors in recovery, due to the swamping of the small absorption band of chlorophyll c by the relatively large absorption band of chlorophyll a at the wavelength of estimation (630 nm). Likewise in mixtures of $a + b + c_1 + c_2$ the recovery of b and c using the fourth set of equations, were in the range 95—110%, where b and c were each greater than one quarter the a concentration. However, in mixtures increasingly overloaded with chlorophyll a recoveries of b and c were significantly over estimated. Very low concentrations of all pigments in mixtures (less than $0.2\ \mu g \cdot ml^{-1}$) also gave large errors in recovery (up to 60%).

The preparative methods used for obtaining the revised extinction coefficients of chlorophylls a and b, the sensitive tests for purity of the chlorophylls, and data on the accuracy and precision of the equations will be presented in detail elsewhere. Also the effects on accuracy of common degradation products of chlorophyll a (chlorophyllide a, pheophytin a and pheophorbide a) which occur in natural phytoplankton populations (JEFFREY 1974) will be discussed.

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