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An alternative approach to absorption measurements of aquatic particles retained on filters

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Abstract

We made modifications to the procedure used for absorption measurements of aquatic particles retained on glass-fiber filters that extend the procedure's application range to "case 2" waters with high suspended sediment content and allow the determination of phytoplankton pigment absorption in situations where the standard solvent extraction method is not effective.

The first result was achieved by combining light-transmission and light-reflection measurements (the latter made possible by the use of a commercially available integrating-sphere attachment for the dual-beam spectrophotometer) so as to remove the spurious contribution to the measured absorption caused by sample backscattering. The second result was obtained by bleaching the sample with a NaClO solution—a method that proved satisfactory even with water-soluble pigments (e.g. the important class of the phycobilins) and solvent-resistant Chlorophyceae. This process also allows for depigmentation of the particle suspension, and thus it was used to evaluate the empirical expression for converting the filter-retained sample absorbance to the equivalent particle suspension value.

Both features of the modified procedure have been positively tested through measurements carried out on solvent-resistant phytoplankton species and on samples of inorganic suspended sediment.

Light-transmission measurements on particles retained in glass-fiber filters (Yentsch 1962) are convenient for determining the light absorption spectrum of particle suspensions consisting mainly of natural phytoplankton and organic and inorganic detritus (Gordon and Morel 1983).

The procedure has two basic advantages: particles can be concentrated so that instrumental accuracy requirements can be met regardless of the high dilution occurring in situ, and information is provided on the absorption of the cells in vivo. The main problem in data analysis arises from the large modification of light transmission due to multiple scattering by the filter, which results in an overestimate of particle absorption relative to suspension state (Butler 1962). Several empirical expressions have been derived for converting the absorption of filter-retained particles (for convenience also referred to as "sample" in the following) to the equivalent absorption of particle suspension. A review on this subject is given by Cleveland and Weidemann (1993).

Kishino et al. (1984, 1985) proposed a procedure for discriminating absorption by phytoplankton pigments from absorption by detritus based on measurements performed before and after pigment extraction by methanol. Although some doubts remain about the effectiveness of the procedure (Bricaud and Stramski 1990) and its validity range shows some limitations (little or no effect on some algal species), solvent extraction is the method generally used to identify pigment absorption in natural phytoplankton populations.

It is current practice to minimize the loss of forward-scattered light, which causes an overestimate of the sample absorption, by using a large detector placed directly against the sample. Elimination of the spurious absorption similarly resulting from backscattering by the sample can be achieved by placing the sample inside an integrating sphere (Frei et al. 1975). Probably because it requires special equipment, this method has found few practical applications (Maske and Haardt 1987). In routine light transmission measurements of filter-retained samples, the effect of scattering is globally corrected for in an approximate way (*see below*) that is not always satisfactory.

We describe a modification of the standard light-transmission method through the use of a commercially available "integrating-sphere" attachment to a standard dual-beam spectrophotometer. Our modification permits correcting for sample backscattering and thus extends the application field of the method. We also provide an alternative procedure for phytoplankton depigmentation with more general validity than the solvent extraction method as well as a procedure for converting the measured absorbance of the filter-retained particles to the equivalent particle suspension absorption that is believed to have a sounder physical basis than currently used routines.

Measurement outline

The dual-beam spectrophotometer with the integrating-sphere attachment (Fig. 1) permits measurements of filter-retained particle samples in both the transmission mode and the reflection mode. The two beams, referred to as sample beam and reference beam, cross the sphere through ports A1, A2 and B1, B2. The result of the measurement is the ratio of radiant fluxes incident on the

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detector placed inside the sphere that are induced by the sample and reference beam fluxes, i.e. $\rho(\lambda) = \Phi_{sb}(\lambda)/\Phi_{rb}(\lambda)$, where Φ is the radiant flux and λ is the wavelength. A list of notation is provided.

To measure in the transmission mode, we place the sample-filter set on port A1, with sample side facing the beam, while a reference filter is placed on port B1. Ports A2, B2 are closed by reflecting Spectralon (99% efficiency) plates.

The result of the measurement in the transmission mode is the ratio

$$\rho_T = \frac{T_s{}^p M T_f{}^d}{T_f{}^p}. \quad (1)$$

T is the transmittance, i.e. the fraction of the incident flux that is transmitted (dimensionless), the factor M (dimensionless) accounts for any multiple reflection between the particle layer and the supporting filter that would result in an increase of the transmitted light ($M \sim 1$ when backscattering by the sample is negligible), the subscripts s and f indicate sample and filter, and the superscripts p and d indicate normally incident parallel light and incident diffuse light. The wavelength dependance of the parameters is omitted to simplify the notation.

Since the glass-fiber filter is a highly diffusing medium, it can be assumed that $T_f{}^d = T_f{}^p = T_f$, which simplifies Eq. 1 to

$$\rho_T = T_s{}^p M. \quad (2)$$

For measuring in the reflection mode ports A1 and B1 are open, the sample-filter set is placed on port A2 (sample side facing the beam), and the reference filter is placed on port B2. Absorbing (99.5%) black boxes are placed behind both the sample-filter set and the reference filter.

The result of the measurement in the reflection mode is

$$\rho_R = \frac{R_{sf}{}^p}{R_f{}^p}. \quad (3)$$

R is the reflectance, i.e. the fraction of the incident flux that is backscattered (dimensionless), and the subscript sf indicates the sample-filter set. Because of the diffusive properties of the glass-fiber filter, it can be assumed that $R_f{}^d = R_f{}^p = R_f$.

Notation

Φ_{sb}, Φ_{rb}	Radiant flux on the detector due to the sample beam or to the reference beam, W
$T_f{}^p$, $T_f{}^d$	Transmittance of the filter (i.e. fraction of incident flux that is transmitted) for parallel or diffuse incident light, dimensionless
$T_s{}^p$, $T_s{}^d$	Tranmittance of the filter-retained particle sample for parallel or diffuse incident light, dimensionless
M	Factor accounting for multiple reflection between sample and supporting filter, dimensionless
$R_f{}^p$, $R_f{}^d$	Reflectance of the filter (i.e. fraction of the incident flux that is reflected) for parallel or diffuse incident light, dimensionless
$R_s{}^p$, $R_s{}^d$	Reflectance of the filter-retained particle sample for parallel or diffuse incident light, dimensionless
ρ_T	Φ_{sb}/Φ_{rb} for measurements in the transmission mode
ρ_R	Φ_{sb}/Φ_{rb} for measurements in the reflection mode
A_s	Absorbance [i.e. $\log(1/T)$] of the filter-retained particle sample, dimensionless
τ	$T_s{}^p/T_s{}^d$
P	Fraction of the flux entering the sphere that is backscattered into the entrance port, dimensionless
a_s, a_f	Fraction of incident flux absorbed by the filter-retained particle sample or by the filter on a single throughway, dimensionless
$a_s{}^*$	Fraction of the incident flux absorbed by the filter-retained particle sample (including the contribution of the backscattering by the supporting filter), dimensionless
A_{sus}	Absorbance of the particle suspension, dimensionless
α	Specific absorption of the particle suspension, m^{-1} per unit particle concn
X	Ratio of filtered volume to filter clearance area, m
C	Particle concentration, mass m^{-3}
λ	Wavelength, nm

Interpretation scheme

Standard procedure—The standard procedure for determination of filter-retained particle absorption is based on interpretation of the single light-transmission measurement through Eq. 2. Normally, one sets $M = 1$ (i.e. any multiple reflection occurring at the sample–filter interface is regarded as part of the complex set of radiation transfer phenomena going on within the sample-filter system whose net effect is absorption amplification and which are corrected for empirically by means of expressions such as Eq. 15 and 16). The sample absorbance is computed as

$$A_s = \log\left(\frac{1}{\rho_T}\right). \quad (4)$$

Actually, Eq. 4 yields an overestimate of the sample absorbance due to the spurious contribution of light scattering by the sample (backscattering as well as forward scattering not collected by the detector) that needs to be corrected for. The correction generally applied consists of subtracting the absorbance value at 750 nm from the absorbance spectrum given by Eq. 4 and depends on the assumption that the phytoplankton absorption is negligible at this wavelength and the scattering is not wavelength-dependent.

This operation is not supported by a fully satisfactory physical justification. In fact, natural phytoplankton always includes some detritus whose 750-nm absorbance is low but not negligible, so that the observed $A(750)$ is the result of both scattering and absorption—the relative importance depending on the amount of detritus and the

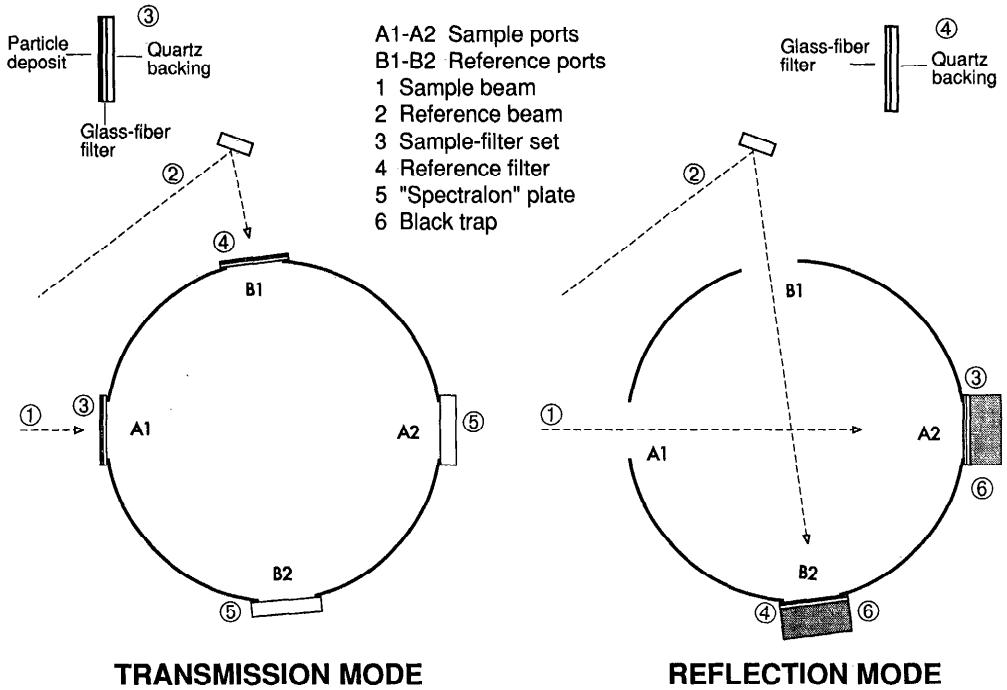

Fig. 1. Schematic view of the integrating-sphere attachment to the Perkin-Elmer L19 dual-beam spectrophotometer.

experimental setup. In addition, both scattering and absorption by detritus increase with decreasing wavelength, as λ^{-1} and exponentially, respectively (Prieur and Sathyendranath 1981; Maske and Haardt 1987), while scattering by phytoplankton is roughly inversely proportional to absorption (Sathyendranath et al. 1989).

In practice, the correction performed by subtracting the 750-nm value from the absorbance spectrum is adequate for measurements carried out on samples of oceanic waters (case 1 water, e.g. Gordon and Morel 1983) because the focus is on pigment absorption. The detritus content—mostly organic matter—is relatively low (except for strong oligotrophic environments) and covaries with phytoplankton concentration.

The situation may change a great deal in coastal waters (case 2) due to the presence of widely varying amounts of suspended inorganic sediment (from river outlets, bottom resuspension, urban and industrial effluents) characterized by backscattering coefficients considerably higher than that of phytoplankton (Prieur and Sathyendranath 1981). For coastal waters with high suspended sediment content, the interpretation of absorption measurements, carried out on filter-retained particle samples by the light-transmission method, requires a more effective procedure than simple subtraction of the $A_s(750)$ bias.

Proposed procedure—Our proposed method for determining filter-retained particle absorption uses the data provided by the double transmission-reflection measurement (Eq. 1 and 3). The observation that, for the selected experimental conditions (Fig. 1), the radiation measured in the reflection mode is just the backscattered radiation of the measurement in the transmission mode (except for a minor difference examined at the end of this subsection) suggests a procedure that corrects for the effect of the sample backscattering. This procedure is based on the radiation balance equation for the sample-filter set:

fraction transmitted into the sphere (= TR)
+ fraction backscatttered (= BK)
+ fraction absorbed by the filter (= AF)
+ fraction absorbed by the sample (= AS) = 1. (5)

The terms of Eq. 5 are

$$TR = T_s^p M T_f = \rho_T T_f \quad (6)$$

$$BK = R_{sf} = \rho_R R_f \quad (7)$$

$$AS = a_s + a_s R_f M T_s^p \tau + a_s T_s^p M^2 T_f^2 \tau + \ldots \quad (8)$$

$$AF = a_f T_s^p M + a_f T_s^p T_f M P + \ldots . \quad (9)$$

a is absorption due to an incident unitary parallel light beam on a single through-way, $\tau = T_s^p/T_s^d$ and P is the fraction of the flux entering the sphere that is diffused back into the entrance port (Goebel 1967). Equations 6 and 7 are derived from Eq. 1 and 3. The second term in the right-hand side of Eq. 8 is the absorption due to the light fraction backscattered by the filter. The third and second terms in the right-hand side of Eq. 8 and 9, respectively, which represent the absorption caused by the light diffused by the sphere back into the entrance ports, can be neglected. The minor quartz backing absorption is included in the AF term. Thus, inserting Eq. 6–9 into Eq. 5 yields

$$a_s = \frac{1 - \rho_T T_f - \rho_R R_f - a_f T_s^p M}{1 + R_f M T_s^p \tau}. \quad (10)$$

Because $a_f = 1 - (T_f + R_f)$ and $\rho_T = T_s^p M$ (Eq. 2), Eq. 10 can be rewritten as

$$a_s = \frac{1 - \rho_T + R_f(\rho_T - \rho_R)}{1 + R_f \rho_T \tau}. \quad (11)$$

The filter reflectance, R_f, is obtained from a measurement in the reflection mode, with a reference filter (backed with a black trap) against port A2 and a Spectralon reflecting plate closing port B2. The factor τ is determined by repeating the transmission measurement with the sample-filter set position inverted (sample side facing the sphere cavity). Note that the multiple reflection factor, M, does not need to be evaluated, since it is no longer present in the resolving Eq. 11. The sample absorption, a_s, is converted to the sample absorbance

$$A_s = \log\left(\frac{1}{1 - a_s}\right) \quad (12)$$

and then to the equivalent particle suspension absorbance, A_{sus}, by means of the empirical correlation [$A_{sus}(\lambda)$, $A_s(\lambda)$]) (*see below*).

Actually, there is a range of scattering angles close to 90° for which the transmitted light in the transmission mode and the backscattered light in the reflection mode do not enter the integrating sphere. This range depends on the thickness of the sample-filter set and on the size of both the light beam and the sphere entrance port. The corresponding light loss results in an underestimate of R_f in Eq. 11 (the other terms are unaffected, as can be checked following the stream of equations leading to Eq. 11). In the present experimental configuration, the R_f underestimate is small (i.e. about −1% for a perfectly diffusing filter). Also, the error is partially compensated for in Eq. 11 because R_f is present in both numerator and denominator of the equation.

Some concern might arise about the value assigned to the R_f term in the denominator of Eq. 11, which represents the light fraction reflected by the filter in the sample-filter assembly, because a fraction of the particles retained in the filter is interspersed with the filter's fibers and thus may cause appreciable modification of the measured reference filter reflectance.

This concern no longer subsists if the particle suspension absorbance is correlated with the global sample absorption, i.e. the addition of the direct and filter-backscattered light absorption

$$a_s^* = a_s[1 + (\rho_T R_f \tau)], \quad (13)$$

because the empirical correlation [$A_{sus}(\lambda)$, $a_s^*(\lambda)$] takes into account any change induced by the particle deposit on the value of R_f. The only requirement is that this modification does not depend significantly on particle type. The second-order effect due to variations in the filter reflectance within the filter's batch has been found negligible. Obviously, the numerical constants in the expression for the correlation [$A_{sus}(\lambda)$, $a_s^*(\lambda)$] depend on the filter type. Using this scheme to determine the equivalent particle suspension absorbance simplifies the measurement because τ need not be determined.

Sample depigmentation

Standard procedure—The method most frequently used for the depigmentation of natural phytoplankton samples retained on filters was developed by Kishino et al. (1984, 1985) and achieves pigment extraction by immersion in methanol (30–60-min treatment). Similar solvent extraction procedures have been adopted by others. Maske and Haardt (1987) washed each filter twice with an acetone-methanol mixture. Hoepffner and Sathyendranath (1991, 1992) extracted the pigments by passive flow of filter solvents consisting of a 90% acetone solution or a mixture of 90% acetone and DMSO (6 : 4 volume ratio); extraction times were of the order of 25–30 min.

Although widely applicable to marine phytoplankton, solvent extraction presents significant limitations, because some pigment types are not extractable (e.g. the phycobilins, *see* Bricaud and Stramski 1990) and the procedure is ineffective with some phytoplankton species (e.g. Chlorophyceae, whose thick cellulose cell-wall hinders solvent penetration). Chlorophyceae are not frequent in the open sea but form an important fraction of the phytoplanktonic population in estuarine and freshwaters. Also, solvent extraction by immersion may cause loss of loose particles from the filter.

Proposed procedure—We have developed a depigmentation process with more general validity by using the oxidizing agent sodium-hypochloride (NaClO). The standard procedure setup consists of placing a few drops of a 15% NaClO solution (1% active Cl) on the sample retained in the filter held horizontal against a 1-mm-thick quartz backing (10 drops for a 10-cm^2 filter). Complete bleaching of the phytoplankton pigments throughout the filter takes 1–3 min, depending on cell type.

The NaClO absorption is very low over the wavelength range of interest. The standard amount used for bleaching the sample displays a flat absorbance of ~0.008 in the 400–750-nm range. The computation of the corresponding minor correction to the data can be avoided by adding the same NaClO amount to the reference filter.

The peculiar action of NaClO, such that physical removal of the pigments is not required as it is with solvent extraction, permits bleaching of the phytoplankton suspension (1–5 ml of a 50% NaClO solution per liter of suspension, 5–10-min treatment time). This feature permits us to establish a new—and possibly better—procedure for determining the correlation between absorbance of the suspension and of the filter-retained particle sample (*see below*).

Extensive testing showed that the NaClO treatment offers a number of distinct advantages over solvent extraction: it acts rapidly and effectively on algal types resistant to solvents, including the important Chlorophyceae species; it completely bleaches water-soluble pig-

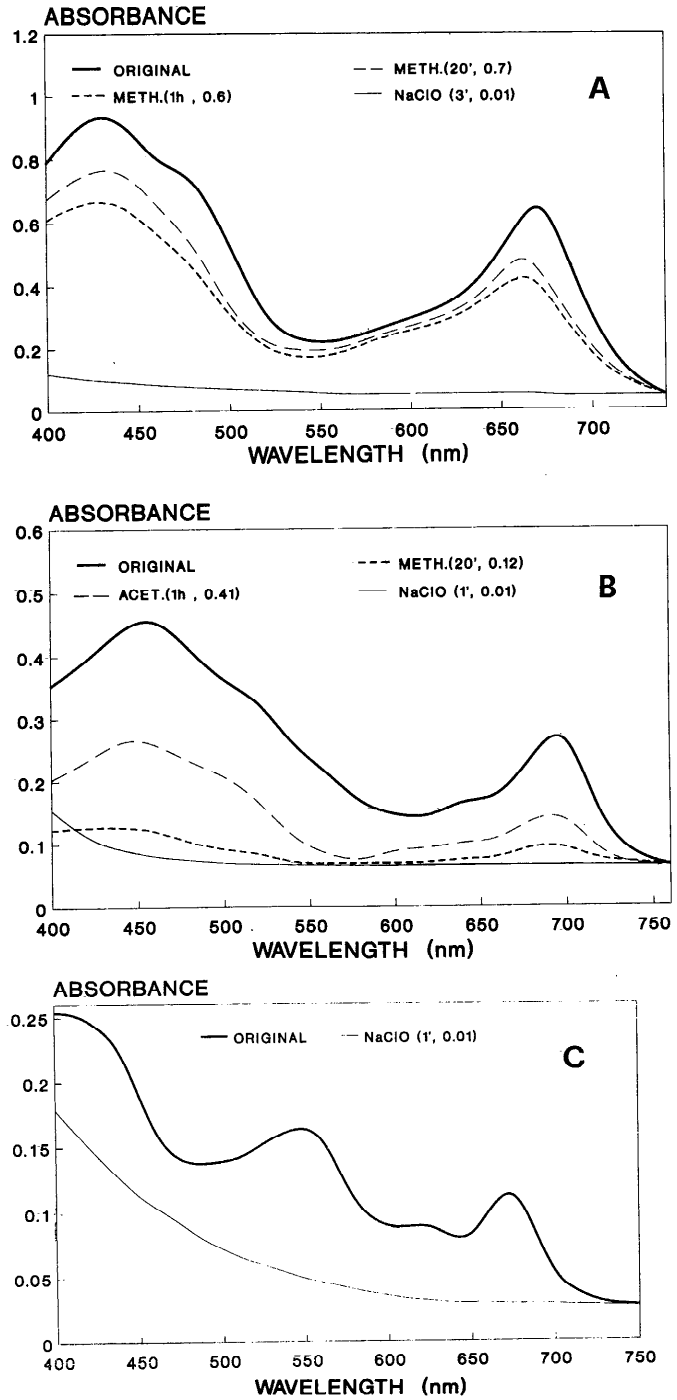

Fig. 2. Depigmentation efficiency of NaClO, methanol, and acetone for (A) a chlorophycean (*Scenedesmus obliquus*), (B) a diatom (*Phaeodactylum tricornutum*), and (C) a blue-green. Numbers in parentheses are the treatment time and the efficiency index r_{675} (i.e. the ratio of the heights of the Chl *a* peaks at 675 nm after and before depigmentation).

ments contained in cyanobacteria (such as phycocyanin and phycoerythrin); and it does not cause loss of matter from the filter. In the presence of very loose deposits that might be slightly displaced by the drops of bleaching agent, the NaClO is added directly to the phytoplankton suspension before filtration.

Figure 2 presents results of comparative tests of the NaClO effectiveness carried out by measuring in the transmission mode laboratory cultures of a freshwater chlorophycean (*Scenedesmus obliquus*) and of a diatom (*Phaeodactylum tricornutum*), as well as natural phytoplankton collected in Lake Maggiore (Italy), dominated by blue-greens and characterized by a strong phycoerythrin 555-nm peak. Acetone and methanol extraction was carried out by passive filtration. The degree of depigmentation, evaluated as the ratio r_{675} of the height of the residual 675-nm chlorophyll *a* peak in the depigmented sample absorption spectrum to the height of the same peak in the absorption spectrum of the original sample, is also shown in Fig. 2.

As is the case with solvent extraction, the NaClO action cannot be assumed to be perfectly selective; some bleaching of the organic detritus may also occur. Transmission-reflection measurements carried out on the algal species mentioned above have, however, shown that the $A_s(750)$ value is not appreciably reduced by the NaClO treatment, which indicates that bleaching of the detritus derived from photosynthetic pigments is not important.

Oxidative bleaching has been used by Doucha and Kubin (1976) in the determination of algal absorption spectra. Their procedure, which consists of a 30-min treatment by peracetic acid at 40°C and at high irradiance, does not seem to have found other applications.

Conversion of the experimental result to suspension absorbance

Standard procedure—The sample absorbance, $A_s(\lambda)$, obtained from measurements carried out on particles retained in the filter (Eq. 4) is converted to the value, $A_{sus}(\lambda)$, corresponding to an aqueous suspension with the same material thickness (per unit beam cross-section). Once this conversion is performed, the specific absorption coefficient, $\alpha(\lambda)$, of the substance is computed as

$$\alpha(\lambda) = 2.3\frac{A_{sus}(\lambda)}{XC}. \quad (14)$$

X is the ratio of the filtered volume to the filter clearance area, and C is the particle concentration.

Mitchell and Kiefer (1984, 1988) correlated the results of light transmission measurements performed on phytoplankton particles either retained in GF/C glass-fiber filters or suspended in a bovine serum solution and obtained the empirical function $\beta = f(A_s)$, where $\beta = A_{sus}/A_s$. Mitchell (1990), by extending the study to other filter types, determined the expression

$$A_{sus}(\lambda) = 0.392A_s(\lambda) + 0.665A_s^2(\lambda). \quad (15)$$

Cleveland and Weidemann (1993) measured the transmission of samples from 48 phytoplankton cultures of seven different species by a similar approach (GF/F filters, aqueous suspension) and obtained the correlation

$$A_{sus}(\lambda) = 0.378A_s(\lambda) + 0.523A_s^2(\lambda), \quad (16)$$

which is in substantial agreement with Eq. 15. Both expressions were established over the $A_s(\lambda)$ range from 0 to 0.4. Other expressions for the conversion from $A_s(\lambda)$ to $A_{sus}(\lambda)$ were similarly derived from measurements carried out by others (e.g. Kishino et al. 1985; Bricaud and Stramski 1990; Hoepffner and Sathyendranath 1992).

In these measurements—all performed by the standard light-transmission method—the correction for light scattering was carried out by subtracting the absorbance value measured at 750 nm from the absorbance spectrum.

Proposed procedure—Our proposed procedure consists of a sequence of four measurements: transmission-reflection measurement on the filter-retained particle sample, which yields the sample absorbance (Eq. 12) after the spurious contribution due to backscattering by the sample is removed; the same measurement on the sample depigmented by NaClO; transmission measurement on a particle suspension sample with the same material thickness (e.g. particle mass per unit area crossed by the spectrophotometer beam); and the same measurement on the particle suspension depigmented by NaClO.

The last two measurements are performed with a 10-mm-long plane-parallel quartz cell filled with an aqueous suspension of a laboratory culture of phytoplankton against port A1 of the integrating sphere. An identical reference cell containing the culture-filtered water is placed against port B1. The phytoplankton suspension is progressively diluted so as to uniformely cover the absorbance range of interest; the same dilution is applied to the content of the reference cell. This configuration corrects for the contribution of the absorption of any yellow substance present in the sample.

Depigmentation of the filter-retained particle sample permits selective determination of phytoplankton pigment absorbance by subtracting the values measured before and after pigment neutralization. Because the lower bleached sample absorbance undergoes higher amplification through multiple scattering by the filter, it is reduced to the value corresponding to the measured absorbance of the original sample before carrying out the subtraction. This can be shown to be the same as multiplying $A_s(\lambda)$ by $[1 - \eta(\lambda)]$, where $\eta(\lambda) = A^B_{sus}(\lambda)/A_{sus}(\lambda)$, with $A_{sus}(\lambda)$ given by Eq. 17 (the index B refers to the bleached sample).

Subtracting the absorbances measured before and after bleaching the particle suspension yields the real in vivo phytoplankton pigment absorption, and corrects for particle backscattering as well as for the radiation forward-scattered by the particles that is not collected by the detector. Subtraction of the particle suspension absorbances can be performed directly because there is no differential signal amplification.

Thus, the sequence of the four measurements yields the function $[A_{sus}(\lambda), A_s(\lambda)]$, which correlates the absorbance of particles in water suspension and retained on the filter, having eliminated contributions due to radiation scattering. Note that such a scheme cannot be followed if the standard solvent extraction method is used because this process does not operate on the phytoplankton suspension.

The correlation between suspension absorbance and filter-retained particle absorption $[A_{sus}(\lambda), a_s^*(\lambda)]$ is obtained in a similar way.

Experimental details

The following paragraphs present some characteristic features of the experimental equipment and procedure; for other aspects that can be considered standard practice, the reader is referred to the recent literature (e.g. Mitchell 1990; Cleveland and Weidemann 1993).

The dual-beam spectrophotometer was provided with a 60-mm-diameter, barium sulfate coated integrating-sphere attachment (Fig. 1). The ratio of apertures to sphere surface is ~7.5%. The sphere is equipped with a side-on photomultiplier and a PbS cell, permitting measurements in the spectral range from 200 to 2,500 nm. Correction for the difference in the beam efficiencies is performed automatically. For this experiment, the unit was operated in the wavelength range from 350 to 750 nm, with 1-nm spectral band width and 1-nm spectral increments.

Particle suspensions were filtered under 20 mm of Hg vacuum through Whatman GF/F glass-fiber filters (0.7-μm retention efficiency) with 10.8-cm^2 clearance area. The sample-filter set and the reference filter were cut into 10-mm-wide strips, placed on 1-mm-thick quartz backings, wetted to saturation by distilled water (because of the type of samples measured, i.e. a freshwater alga and river sediments), and clamped to the A1, B1 (transmission mode) or A2, B2 (reflection mode) ports of the integrating sphere, with the sample side facing the beam.

Quartz backings were used to increase the mechanical strength of the specimens as well as to retard dehumidification and to facilitate uniform bleaching of the filter by the NaClO solution. Because identical backings were placed on both the sample-filter set and the reference filter with the same geometry with respect to the light beam, the overall effect of the backing on the measurements was negligible (as verified by tests carried out—somewhat more laboriously—without backings). The measurements were performed within 10 min (usually 5 min) of filtration.

Results and discussion

The first step in validating the proposed experimental procedure was to determine the $[A_{sus}(\lambda), A_s(\lambda)]$ correlation according to the standard routine outlined above (Fig. 3). The filter-retained sample absorbances were derived by Eq. 4 from measurements performed in the transmission mode. The 140 data points displayed are the values recorded with 12.5-nm increments over the wavelength range 400–750 nm for five samples taken from a laboratory culture of the freshwater chlorophycean, *S. obliquus,* and characterized by 440-nm absorbance, $A_s(440) = 0.17, 0.30, 0.41, 0.43$, and 0.60. The absorbance value measured at the 750-nm wavelength was subtracted from both $A_{sus}(\lambda)$ and $A_s(\lambda)$. Least-squares fitting yielded the correlation

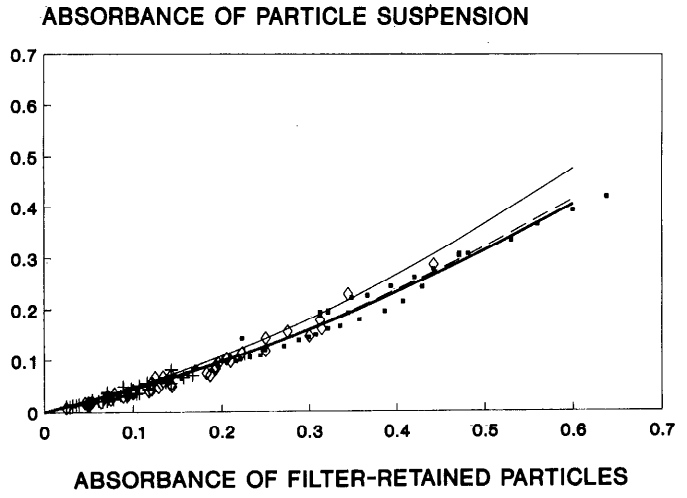

Fig. 3. Experimental correlation between particle suspension absorbance and filter-retained particle absorbance. The data points are the result of measurements carried out in the transmission mode on samples of a *Scenedesmus obliquus* culture. Different symbols are used for the wavelength subintervals 400–500(■), 500–600(+), and 600–700(◇) nm. The thick solid line is the least-squares fit to the data (Eq. 17). The thin solid line represents the fit obtained by Mitchell (1990) and the dashed line that by Cleveland and Weidemann (1993) (i.e. Eq. 15 and 16).

$$A_{sus}(\lambda) = 0.406A_s(\lambda) + 0.519A_s^2(\lambda). \quad (17)$$

The plot of Fig. 3 suggests that the correlation extends above the value $A_s = 0.4$ recommended as the upper limit by Mitchell (1990). The curve of Eq. 17 is very close to the fit obtained by Cleveland and Weidemann (1993), our Eq. 16, the maximum deviation over the common absorbance range (i.e. 0–0.4) being <4%. The better match with Eq. 16, rather than with the fit obtained by Mitchell (1990), our Eq. 15, is physically significant, since our experimental conditions (e.g. water suspension of particles, GF/F filter) were closer to those of the measurements of Cleveland and Weidemann (1993).

The regular distribution about the fit line of the data points belonging to the three subintervals 400–500, 500–600, and 600–700 nm (denoted by different symbols in the figure) seems to exclude any significant dependance of the correlation on wavelength. Also, this evidence is in agreement with the observations of Cleveland and Weidemann (1993). The "hystheresis" effect observed by Bricaud and Stramski (1990), which generates a sort of loop in the data corresponding to the ascending and descending slope of absorption maxima, was not noted. The dispersion of the points in each wavelength subinterval, which might suggest loop patterns, was due to dispersion of the five sets of data.

The good agreement among the $[A_{sus}(\lambda), A_s(\lambda)]$ correlations expressed by Eq. 15, 16, and 17 increased our confidence that appropriate care was taken in performing the measurement, particularly with regard to experimental error. This led to the second step, i.e. determining the $[A_{sus}(\lambda), A_s(\lambda)]$ and $[A_{sus}(\lambda), a_s^*(\lambda)]$ correlations derived from the transmission-reflection measurements, as outlined above. The results obtained from the previous set of *S. obliquus* samples yielded the least-squares fits

$$A_{sus}(\lambda) = 0.423A_s(\lambda) + 0.479A_s^2(\lambda), \quad (18)$$

and

$$A_{sus}(\lambda) = 0.054a_s^*(\lambda) + 0.321a_s^{*2}(\lambda), \quad (19)$$

the dispersion of the data points about the fit lines being comparable to that displayed in Fig. 3.

Because the measured absorbance amplification with respect to the suspension value is predominantly due to multiple scattering by the filter, it is reasonable to expect that Eq. 18 and 19 could be used to infer the equivalent suspension value of the absorbance measured for filter-retained particles of various sources (phytoplankton, organic and inorganic sediment), provided that the spurious absorbance component caused by sample scattering is removed. This assumption was tested through measurements performed on inorganic suspended sediment particles (*see below*).

The third step in validating the experimental procedure proposed here consisted of a series of transmission-reflection measurements carried out on filter-retained particle samples taken from algae cultures and from water collected in situ. The results of the measurements were converted to suspension absorbances by Eq. 17, 18, and 19 and then to specific absorption coefficients by Eq. 14. The results of three series of tests carried out on phytoplankton samples, inorganic suspended sediment samples, and samples consisting of a mixture of phytoplankton and inorganic sediment so as to simulate a typical coastal water composition are reported below.

The phytoplankton tests were performed on four samples obtained by filtering known amounts of a moderately aged culture of *S. obliquus,* i.e. with a detritus content higher than that of the culture used to derive the $[A_{sus}(\lambda), A_s(\lambda)]$ correlation, so as to uniformly cover the range $0.14 < A_s(440) < 0.42$. The transmission-reflection measurements were carried out before and after NaClO bleaching. The $A_s(750)$ bias was subtracted from the data of the transmission measurement before conversion to $A_{sus}(\lambda)$ by Eq. 17. Subtraction of the bleached sample from the original sample data to yield the phytoplankton pigment absorbance was carried out after conversion of both data sets to the equivalent suspension values.

The specific absorption coefficients obtained by averaging the values measured for the four samples are plotted in Fig. 4A (total absorption) and Fig. 4B (pigment absorption) over the wavelength range from 400 to 725 nm. The dashed lines delimit the error band of the spectrum yielded by Eq. 18 that was computed from the dispersion of the data about the mean values in terms of standard deviation ($+1\sigma$, -1σ). The error bands of the spectra

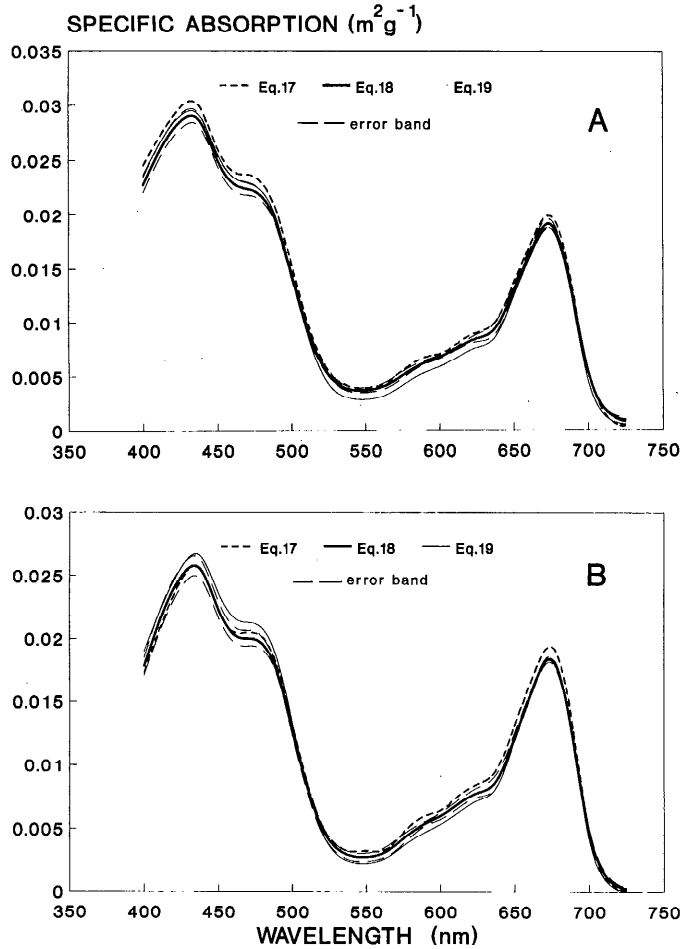

Fig. 4. Spectra of specific absorption coefficients obtained from measurements performed on samples of a laboratory culture of *Scenedesmus obliquus*. A. Total absorption. B. Pigment absorption. The error band refers to the spectrum yielded by Eq. 18.

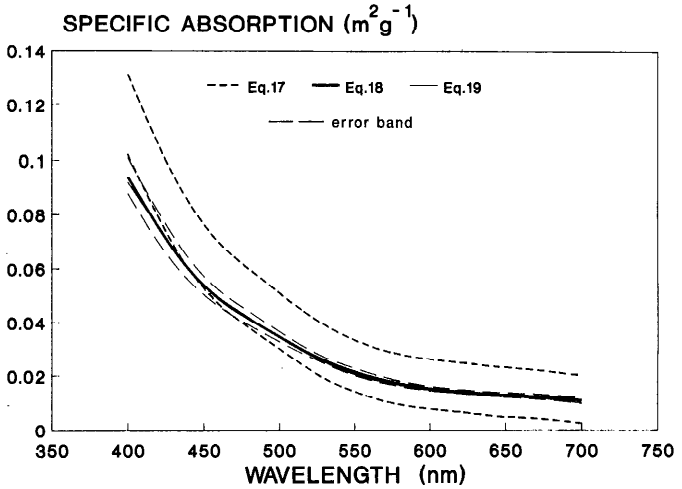

Fig. 5. Spectra of specific absorption coefficients of inorganic suspended sediment, obtained from measurements performed on samples of River Toce water. The error band refers to the spectrum yielded by Eq. 18.

obtained through Eq. 17 and 19 have somewhat larger and similar amplitudes, respectively.

The agreement among the three specific absorption spectra is within the combined error limits over the whole wavelength range, confirming that subtracting the $A_s(750)$ bias is a valid procedure for phytoplankton-dominated case 1 waters. Thus for this water type, the double transmission-reflection measurement does not seem to achieve any appreciable improvement with respect to the standard light-transmission measurement.

The situation is different in the case of sediment-dominated case 2 waters. Combining light-transmission and light-reflection measurements permits the determination of sediment absorption, having removed the backscattering contribution. Instead, the transmission measurement by itself does not provide sufficient information to accurately determine suspended sediment absorption because the assumptions on which the $A_s(750)$ bias subtraction is based (i.e. no absorption at the near-infrared wavelength, wavelength-independent scattering) are not valid.

We performed a series of tests on inorganic suspended sediment to provide a quantitative analysis of this situation. The samples were obtained by filtering water collected from the River Toce that flows from the western Alps into Lake Maggiore and the River Trebbia that flows from the Apennines to the River Po. The two rivers were selected because of the difference in the geological features of their basins, which could imply the formation of sediments with different optical properties. The measured suspended sediment concentrations were 4 and 5.35 g m^{-3}, respectively. The phytoplankton content was <0.02 mg m^{-3} Chl *a*. Since this minor contamination had no effect on the tests, the samples were measured without preliminary bleaching (this explains the small irregularities displayed by the aborption spectra).

Four samples, prepared by filtering 250, 500, 1,000, and 1,500 ml of water of the River Toce and characterized by $A_s(440)$ values uniformly distributed in the 0.1–0.5 range, were measured in the transmission and reflection modes. The absorbances yielded by Eq. 18 and 19 were converted to specific absorption coefficients by Eq. 14 and are averaged and plotted in Fig. 5 over the 400–700-nm range. The error band of the specific absorption coefficient spectrum given by Eq. 18 is delimited by dashed lines ($+1\sigma$, -1σ). The data interpretation partitions the measured $\rho_T(750)$ between absorption and backscattering contributions into two almost equal parts.

The same set of data was converted to absorbance by Eq. 4, assuming that the whole measured $\rho_T(750)$ was due to suspended sediment absorption or suspended sediment scattering. The specific absorption coefficients were derived by Eq. 17 and 14 (upper and lower plots in Fig. 5).

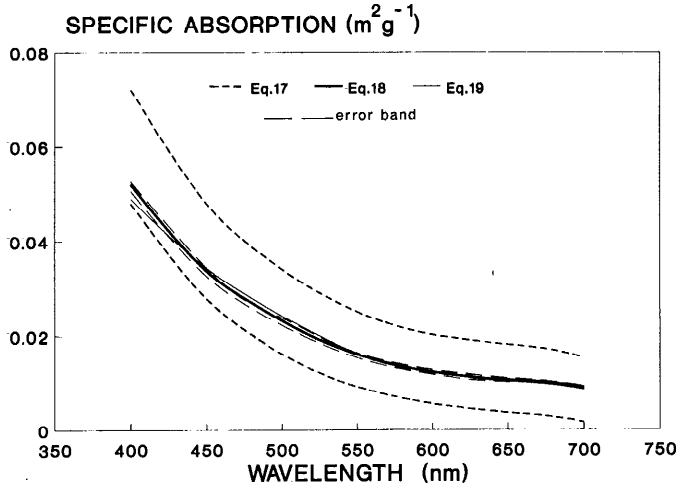

Fig. 6. As Fig. 5, but of River Trebbia water.

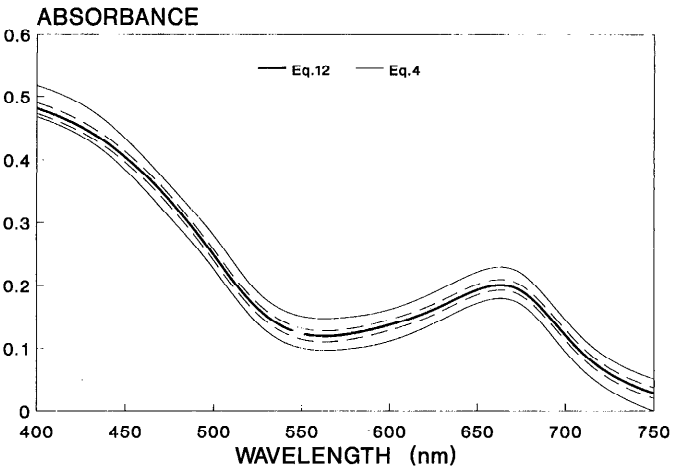

Fig. 7. Absorbance spectra obtained from measurements performed on a sample that simulates the composition of case 2 water with medium sediment load. Dashed lines delimit the error band of the spectrum yielded by Eq. 12.

If we assume no contribution by sediment scattering at 750 nm, the specific absorption coefficients are overestimated over the entire wavelength range (by 30% at 440 nm and by 60% at 675 nm). If we assume no sediment absorption at 750 nm, there is a severe underestimate in the medium-to-high wavelength range. Note that the deviation from the specific absorption coefficient spectra yielded by the transmission-reflection measurement (Eq. 18 and 19) is well outside the experimental error band.

The results similarly obtained from measurements performed on three samples of the River Trebbia water, with nominally equal sediment content, are plotted in Fig. 6. Probably because of the different mineralogic composition, the Trebbia sediment exhibits lower absorption and higher backscattering than the Toce sediment. As a consequence, the difference between the results of the double transmission-reflection and single transmission measurements is larger.

To test the proposed method on typical case 2 water with medium sediment load, we performed measurements on samples prepared by filtering 500 ml of water from the River Toce plus 50 ml of a *S. obliquus* culture, so as to have about equal contributions to the 440-nm absorbance. The absorbance spectra obtained from Eq. 4, with and without subtracting the $A_s(750)$ value (lower and upper thin lines), and from Eq. 12 are plotted in Fig. 7. The standard practice of subtracting the $A_s(750)$ bias from the result of the transmission measurement underestimates the absorbance by 15, 35, and 25% at the wavelengths of 440, 550, and 670 nm, respectively, well outside the error band of the result of the transmission-reflection measurement.

Reproducibility tests were carried out to evaluate the error sources that affect the various stages of the measurement. A partial list is presented in Table 1. The quoted errors are estimates of the standard deviation of the absorbance, $\sigma(A)$, as computed from a series of transmission measurements. Similar error values were evaluated from the results of reflection measurements.

The results of these tests were used to optimize the

Table 1. Error sources affecting various stages of the measurement. $\sigma(A)$—estimate of the standard deviation of the measured absorbance.

Tests performed	$\sigma(A)$
Wetting the filter to saturation	0.005
Measuring the filter from 2 to 10 min after wetting	0.01
Changing the filter within the same batch	0.006
10-min time series on a filter-retained sample	0.015
Measuring different portions of the same particle-retaining filter	0.007
10-min time series on the particle-suspension sample	0.002

Table 2. Estimates of the standard deviation of the specific absorption coefficient at the typical wavelengths of 440, 550, and 675 nm (percent values). $TR + RE$ = transmission-reflection; $TR - A750$ = transmission, 750-nm absorbance removed; TR + $A750$ = transmission, 750-nm absorbance not removed.

Sample	Measurement	Wavelength (nm) 440	550	675
R. Toce	$TR + RE$	4.0	7.0	10.0
	$TR - A750$	15.0	13.0	37.0
	$TR + A750$	9.0	8.0	12.5
R. Trebbia	$TR + RE$	3.5	6.0	8.0
	$TR - A750$	7.0	11.0	23.0
	$TR + A750$	9.0	15.5	21.0
Scenedesmus obliquus	$TR + RE$	3.0	8.5	4.0
	$TR - A750$	4.0	12.5	6.0
S. obliquus pigments	$TR + RE$	5.0	17.0	2.5
	$TR - A750$	7.5	17.0	4.0

experimental procedure. Having done this, we assessed the global error of the measurement of the filter-retained particle absorbance spectrum by comparing independent determinations. For instance, measuring four samples obtained by filtering equal volumes of the River Trebbia water, with absorbance varying from 0.5 to 0.1 in the 400–700-nm range, yielded the errors $\sigma(A) = 0.008$–0.003 (transmission-reflection measurement) and $\sigma(A) = 0.014$–0.007 [transmission measurement, $A(750)$ bias subtracted]. The lower uncertainty of the transmission-reflection measurement is probably due to compensation of errors in the double measurement.

An evaluation of the overall error of the final parameter yielded by the measurement (i.e. the specific absorption coefficient) is presented in Table 2, which lists error estimates computed from the dispersion of the single determinations about the mean spectra displayed in Figs. 4–6. These errors combine the contributions from instrument, volume filtration, filter batch, filter wetting and dehumidification, NaClO bleaching, numerical constants of the correlations expressed by Eq. 17, 18, and 19, and data interpretation schemes. The values listed in the table are estimates of the standard deviation of the single measurement, σ, divided by the mean value and expressed as percentage at the characteristic wavelengths of 440, 550, and 675 nm (maximal and minimal Chl *a* absorption; high, medium, and low suspended sediment absorption). The consistently lower error of the results of the transmission-reflection measurements could be taken as another indication of the validity of the proposed procedure.

The small error of the suspended sediment absorption (River Toce water) obtained from the absorbance spectra of samples with X values (Eq. 14) varying over a factor of 6 and the lack of evidence for any trend with X in the results support the validity of the assumption that Eq. 18 and 19 also apply to nonchlorophyllous particles.

Similarly, estimated error bands ($+1\sigma$, -1σ) are added to the specific absorption coefficient spectra (dashed lines in Fig. 4, 5, 6, and 7) to help determine whether the difference between the results of the transmission-reflection and transmission measurements is statistically significant. For the considered cases, no meaningful difference was found between the specific absorption spectra obtained by means of the $[A_{sus}(\lambda), A_s(\lambda)]$ and $[A_{sus}(\lambda), a_s^*(\lambda)]$ correlations (Eq. 18 and 19).

In conclusion, the alternative approach proposed for absorption measurements of aquatic particles retained on glass-fiber filters, based on the double transmission-reflection measurement, appears to be a reliable procedure. This procedure has the advantage of also operating on samples with significant amounts of light-scattering particles (as one might expect to find in sediment-loaded coastal water environments), where the standard light-transmission method is likely to produce inaccurate estimates of particle absorption. Actually, the correction for sample backscattering, as it is performed by the transmission-reflection measurement, is a prerequisite for the precise evaluation of the specific absorption coefficient of suspended sediment that is needed to provide input data to multicomponent models of sea color (Sathyendranath et al. 1989). The procedure requires only a commercially available integrating-sphere attachment for the dual-beam spectrophotometer and a modest increase in experimental effort. The reproducibility of the results is satisfactory; for the cases considered, it is better than that of the results of the standard light-transmission measurement.

The phytoplankton depigmentation by NaClO bleaching has proven to be simple, rapid, and highly effective even in situations (water-soluble pigments, resistant algal cell-walls) where solvent extraction fails. An interesting feature of this process is that it also acts on particle suspensions. Additional studies are desirable for a better understanding of the NaClO action on chlorophyll-protein complexes.

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