Chapter 6
Optical Characteristics of Phytoplankton

This chapter focuses on the optical properties of different phytoplankton communities. Standard ocean optic protocols (version-4) were followed for filtration of water samples and Kishino’s (1985) method was adopted for de-pigmentation of the algal particles. While computing total particulate absorption, path-length amplification (β) was calculated using the coefficients recommended by Kahru and Mitchell (1998). Results revealed interesting facts such as waters dominated by cyanophytes provide erroneous information on pigment composition of the phytoplankton communities. Kishino’s method of de-pigmentation actually provides pigment absorption rather than phytoplankton absorption and thus the package effect is minimized. Water samples with mixed phytoplankton population, diatom dominated population and dinoflagellate dominated population showed observably distinct absorption characteristics. Similarly, optical features of waters with massive *Noctiluca* bloom and those without bloom were clearly distinguished. Although *Noctiluca* is a heterotrophic dinoflagellate with no pigment of its own, it showed characteristic features of *Pedinomonas* (containing Chlorphyll-a and b) which forms symbiotic association with *Noctiluca*.
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Natural waters are physical-chemical-biologically complex media comprising living and non-living materials that may be present in aqueous solution or in aqueous suspension. Suspended material that influence the optical properties of water are broadly classified into algal and non-algal particles. Non-algal particulate matter comprises of white sands of coral beach, continental dust and clay depending on the geological structure and composition of the adjacent land. Action of water currents brings in sediments from the bottom into suspension. Further, the outflow of muddy rivers and estuaries, large tidal excursions, winds and volcanic eruptions also brings sediments into suspension (Motwani et al., 2013 and references therein). Seasonal and regional variation in phytoplankton species composition and distribution greatly affects the optical properties of the water.

Acquiring information about various optical constituents from ocean colour requires understanding of the optical characteristics of these constituents present in dissolved and particulate form. Phytoplankton is most important among the optical constituents as the chlorophyll-α concentration varies with both inherent as well as apparent properties (Kiefer and Austin, 1974 and Morel and Smith, 1974) and phytoplankton being the primary producers has great ecological importance. An optical characteristic of water varies largely with photosynthetic capacity of the cell which is its inherent property (Yentsch, 1962; Bricaud et al., 1995). Many authors such as Yentsch and Phinney, 1989; Nelson et al., 1993; Cleveland, 1995, have shown that a non-linear relationship exists between light absorption coefficient and chlorophyll-α concentration. This non-linearity is due to influence of size, shape of the cell as well as the presence of accessory pigment in it (Morel and Bricaud, 1981; Spinrad and Brown, 1986). Recent studies have shown that absorption by phytoplankton varies from one site to other as well as at varying depths. These variations are caused by taxonomic changes within the phytoplankton communities, cell size and pigment composition (Mitchell and Kiefer, 1988; Milla‘n-Nu’n“ez et al., 1998). Presently, remote sensing instruments provide large number bio-optical information but knowledge of variations in absorption coefficient with site and species composition is needed to develop better models and algorithms (Milla et al., 2004).
6.1 Methodology:

6.1(a) Sample Collection:

Water samples were collected using Niskin bottles from various study sites categorized into various stations. Three (sometimes two, depending on the turbidity of water) depths with 100% light intensity (surface), 50% light intensity and 1% light intensity with respect to surface PAR (Photosynthetically Active Radiation) at every station. Light intensities in the vertical column of water were measured using Satlantic® hyperspectral underwater radiometer. The water sample in the niskin bottle was shaken well to re-suspend the larger particles that may settle during the course of acquisition and then this sampled water was sub-sampled into opaque carboys that were then kept in cool conditions. Samples were filtration immediately after bringing them back to the laboratory.

6.1(b) Filtration (Scott et al., 2003):

The Whatman® GF/F filters with pore size of 0.7 µm and diameter 25 mm were used for concentrating the particulate matter on the filter paper. Figure 6.1 (b-1) shows the arrangement of the filtration assembly for total and phytoplankton absorption. Following precautions were taken while filtering the samples:

- The filtration was carried out in dim light conditions to prevent photodegradation of the pigments.
- A very low vacuum (~125 mm Hg) was maintained while filtration so that the phytoplankton cells or the pigments do not burst due to pressure.
- The volume of water Filtered ($V_f$) was adjusted so that the optical densities of the filtered samples, relative to the blank filter should be greater than 0.005 and less or equal to 0.25 at 675 nm whereas at 440 nm the optical densities should be less than or equal to 0.4.
- The vacuum was turned off immediately after completing the filtration so that the filter paper does not turn dry.
The wet filters were placed in the Fisher Histoprep™ tissue capsules and stored in the Liquid nitrogen immediately after the filtration to freeze the cells and prevent any changes in the physiological state of the cell due to the stress.

The inner diameter (D_t) of the filtration cup (the diameter that contains the concentrated particles on the filter) and the volume of water filtered were recorded for later analysis.

![Figure 6.1 (b-1): Filtration assembly for Total and phytoplankton absorption](image)

6.1 (c) Determination of spectral optical density of the sample filters (Scott et al., 2003):

Dual beam spectrophotometer (Shimadzu® UV-2450) (figure 6.1 (c-1)) was used for measuring the optical densities (O.D.) of the samples. Before the spectrophotometer was switched on, the cuvette holder was replaced by an instrument specific integrated sphere (the filter holder) for efficient capturing of the scattered photons. Then the instrument was turned on and allowed to initialize and warm up for 30 minutes for stabilizing the intensity of light from its source. To check the performance of instrument, initial instrument baseline was run and stored.

Two pre-soaked and water saturated blank filters were mounted (one for the sample beam, and one for the reference beam) on the filter holder. The reference baseline scan was then run and stored. For measuring absorption by particles concentrated on the filters, baseline should be spectrally flat and its noise must be <0.01 O.D., and noise
<0.005 O.D. is strongly recommended. The same blank filters were also scanned as sample and stored.

Meanwhile the frozen samples (filter papers) were removed from the liquid nitrogen and placed on a drop of filtered sea water in petri-dishes to ensure its hydration. The samples were allowed to thaw for approximately 5 min and kept in dark until each filter was analyzed.

The blank filter from the sample holder was then replaced by the sample filter and sample O.D. was measured and saved. The reference filter is hydrated repeatedly as it dries out with time.

![UV-VIS Dual beam spectrophotometer](image)

Figure 6.1 (c-1): UV-VIS Dual beam spectrophotometer used for measurement of optical densities

**6.1 (d) De-pigmentation of particulate matter (Scott et al., 2003):**

The most widely used method of extracting pigments by using methanol (Kishino et al., 1985) was adopted for de-pigmentation of the particular samples collected on the filter paper. The sample and blank filters were removed from the spectrophotometer and again placed on the filtration system. Blank filters were treated exactly as sample filters.10 ml of slightly warmed 100 % methanol was gently poured down from the sides of the filter funnel. Care was taken that the settled detritus is not re-suspended
while pouring the methanol. Methanol was passively filtered through the sample. This passive filtration with methanol was repeated thrice with varying volumes as 10ml, 10ml, and finally with 5ml to ensure complete de-pigmentation of all the algal particle on the filter paper.

Similarly, the filtration cups were rinsed three times with 10 mL, 10 mL and 5 mL of filtered sea water (filtered through cellulose membrane filter paper with mesh size of 0.2 µm). The blanks were also rinsed with filtered sea water after methanol extraction to minimize filter dehydration during spectrophotometric analysis.

6.1 (e) Spectrophotometric measurements of de-pigmented absorption spectra (Scott et al., 2003):

The OD spectra of the de-pigmented samples were measured in the spectrophotometer in the same way as it was done with the total particulate samples. These absorption spectra were typically characterized by absence of the chlorophyll-a peak at 675nm. In some spectra chlorophyll-a absorption peak was present at 675 nm, indicating incomplete de-pigmentation. Therefore the methanol extraction procedure was repeated for quality control.

6.1 (f) Data processing and analysis (Scott et al., 2003):

To compute particle absorption in suspension from O.D. measured through spectrophotometer, for which the particles were concentrated on a GF/F filter, it is necessary to appropriately adjust the optical path length. This includes substituting the geometric optical path length of the particles in suspension, and a scaling factor, $\beta$, accounting for the increase in the optical measurement path by scattering within the filter sample. The geometric absorption path length ($l_g$) of the filtered material in suspension is given by:

$$l_g = \frac{V_f}{A_f}$$
Where: \( V_f \) is the volume of water filtered and \( A_f \) is the area of the filter paper containing concentrated particles, calculated from the diameter \( D_f \) of the part of each filter that contained the particles.

Scattering of light within the GF/F filter increases the absorption path length. The absorption coefficient of filtered particles must be corrected for path length amplification and the equivalent absorption coefficient in \( \text{m}^{-1} \) in suspension is computed as:

\[
a_p(\lambda) = \frac{2.303 A_f}{\beta V_f} \left[ [\text{OD}_{fp}(\lambda) - \text{OD}_{bf}(\lambda)] - \text{OD}_{\text{null}} \right]
\]

Where: \( \text{OD}_{fp}(\lambda) \) is the measured optical density of the sample filter, \( \text{OD}_{bf}(\lambda) \) is the optical density of a fully hydrated blank filter, and \( \text{OD}_{\text{null}} \) is a null wavelength residual correction from the infrared where particle absorption is minimal, as explained in the following section.

**6.1 (g) Null point correction of particle absorption spectra (Scott et al., 2003):**

Null point correction is done to correct for residual offsets in the sample filter relative to the reference, and for scattering artifacts due to particle loading. For this a mean O.D. of 10 nm interval (e.g. 790 nm to 800 nm) was used as the null value to minimize the introduction of noise in the null correction procedure.

**6.1 (h) Path length amplification correction (Scott et al., 2003):**

To correct for the path length increases due to multiple scattering in the filter, the \( \beta \) was estimate empirically through a quadratic function:

\[
\beta = \left[ C_1 + C_2 [\text{OD}_{fp}(\lambda) - \text{OD}_{\text{null}}(\lambda)] \right]^{-1}
\]

Here \( C_1 \) and \( C_2 \) are coefficients of least squares regression fits of measured data. Based on the species composition of our sample these coefficients were chosen as recommended by Kahru and Mitchell (1998). This equation is as follows:
β = [0.31*OD\text{null}] + [0.57*(OD\text{null})^2]

6.1 (i) De-Pigmented particle and phytoplankton absorption coefficients (Scott et al., 2003):

OD for the de-pigmented particles was also calculated using the same method for null and β correction as used for the particle absorption. The spectral absorption coefficient for phytoplankton pigments can be computed as the difference between particulate and de-pigmented estimates:

\[ a_\phi(\lambda) = a_p(\lambda) - a_d(\lambda) \]

Here \(a_\phi(\lambda)\) is the value of absorption coefficient for phytoplankton at a particular wavelength; \(a_p(\lambda)\) is particle absorption coefficient at a particular wavelength and \(a_d(\lambda)\) is the spectral absorption coefficient for de-pigmented particles. The phytoplankton absorption was normalized for chlorophyll-\textit{a} absorption at 443nm.

6.2 Results and discussions:

Measurements for total particulate absorption, which includes detritus absorption and phytoplankton absorption measurements, were carried out from October 2009 to April 2012 for coastal water off Veraval. The results were classified into various seasons viz. fall inter monsoon (October to November), winter monsoon (December to March) and inter monsoon (April to May). Annual and seasonal variations in Total, detritus and phytoplankton absorption were compared and studied. In addition absorption measurements were also carried out for bloom and non bloom regions of northern Arabian Sea.

6.2 (a) Annual and seasonal variations in optical properties of phytoplankton:

To study annual variations in optical characteristics, phytoplankton absorption was measured during fall inter monsoon season of November 2009, October 2010 and October 2011. The results of these measurements are shown in plates 6.1, 6.2 and 6.3.
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(annexure 6.1, 6.2, 6.3 and 6.4). The spectral features for phytoplankton absorption spectra measured during November 2009 were not sharp as compared to those of October 2010 and 2011, due to dominance of *Trichodesmium* in the waters. *Trichodesmium* is a cyanophyte with phycocyanin and phycoerythrin as the major pigments (apart from chlorophylls). These water soluble pigments are incompletely extracted through methanol. Thus in this case the absorption spectra provide erroneous information about the pigment composition of the phytoplankton communities present in the water. Phytoplankton absorption features were most prominent (in terms of magnitude and sharpness of the spectral curves) for the samples collected during October 2011 as compared to those of November 2009 and October 2010. This indicates that the chlorophyll-\(a\) concentration in the cells is higher and the pigment package effect is less.

Variations in optical characteristics of phytoplankton were measured during October 2011 to April 2012 to study variations in their absorption during fall inter monsoon, winter monsoon and spring inter monsoon season. A strong seasonal variation in optical properties of phytoplankton was observed (see plate 6.3 to 6.4). Absorption by phytoplankton was highest during winter monsoon season (December 2011 to March 2012). Phytoplankton absorption was same for surface and at the depth with 50% light level (middle depth) in majority of the stations studied, indicating absence of significant variations in pigment concentrations. Although, phytoplankton cells showed significant variations in cell sizes, in the vertical column of water (see section 5.2 (d) of chapter 5). The spectral peaks were sharp, indicating less self shading. This may be due to higher chlorophyll-\(a\) concentrations in relation to cell sizes. Soret band and secondary peak of chlorophyll-\(a\) were sharpest in winter monsoon. This observation stands in support with microscopic studies showing maximum diversity during winter monsoon season (detailed in chapter 4). It was also observed that phytoplankton absorption was higher for off shore (far coast) stations (station 1; abbreviated as ‘stn1’ in the plates) as compared to the near shore stations (station 2; abbreviated as ‘stn2’ in the plates) due to presence of sediments in the near shore stations which enhanced scattering of light in those regions. Variations in phytoplankton absorption coefficient can be attributed to three main factors: 1) properties of the cell such as cell surface, cell size, shape of the cell and composition as well as distribution of pigments within the cell; 2) environmental light field; 3) interaction of phytoplankton cell with the surrounding light.
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(Sathyendranath et al., 1987; Mitchell and Kiefer, 1988; Babin et al., 1993; Stuart et al., 1998). In the method adopted for de-pigmentation, methanol was used to extract pigments as described by Kishino et al., 1985. The re-measured absorption spectra actually do not represent the phytoplankton absorption but ‘pigment absorption’ as de-pigmented algal cells remain on the filter paper along with the non-algal particles. While subtracting the absorption by detritus from that of total particulate matter, the contribution of the cell and cellular constituents (those insoluble in methanol) to the absorption spectra was also deduced.

6.2 (b) Variations in optical characteristics of different phytoplankton groups:

According to Jeffrey 1997, some set of pigments are unique to taxonomic groups of phytoplankton. As shown in Figure 6.2 (b-1) this uniqueness in pigment composition was clearly observed by difference in the optical behavior of the absorption spectra that were dominated by different phytoplankton groups such as mixed population, diatoms and dinoflagellates. Figure 6.2 (b-1) - (a) represents the absorption spectra of mixed phytoplankton population from all sampling depths of coastal waters of Veraval, Gujarat coast, during winter monsoon period. Figure 6.2 (b-1) - (b) and (c) represents absorption spectra of phytoplankton population dominated by diatoms and dinoflagellates respectively, from various sampling depths of coastal waters of Veraval, Gujarat coast, for the same season (winter monsoon) and almost of the same size class (measured through microscope). In general, pigment composition of diatoms is constituted by chlorophyll-\(a\), \(c_2\), \(\beta,\beta\)-carotene, fucoxanthin, diatoxanthin and diadinoxanthin. Along with chlorophyll-\(a\) and \(c_2\), Chlorophyll-\(c_1\) is also usually present in diatoms and may be replaced by chlorophyll-\(c_3\) in some species while those of photosynthetic dinoflagellates have chlorophyll-\(a\), \(c_2\), \(\beta,\beta\)-carotene, peridinin, dinoxanthin, diadinoxanthin and peridinin derivatives Jeffrey 1997. Heterotrophic dinoflagellates with endosymbionts have optical properties based on the pigment composition of phytoplankton group associated (the endosymbiont) with/engulfed within the host dinoflagellate. The absorption spectra shown in figure 6.2 (b-1)-(c) represents that of photosynthetic dinoflagellate (Prorocentrum).
Figure 6.2 (b-1): Variations in phytoplankton absorption characteristics for (a) Mixed phytoplankton population (b) population dominated by diatoms and (c) population dominated by dinoflagellates

6.2 (c) Phytoplankton absorption in bloom and non bloom regions:

Phytoplankton absorption was measured for bloom and non bloom regions during the open ocean cruise (Cruise Id: SS286) in March 2011. Figure 6.2 (c-1) shows observable difference in the absorption characteristics of phytoplankton from bloom and non-bloom regions of the Arabian Sea. The non bloom region consisted of mixed phytoplankton population with maximum diversity of diatoms. *Noctiluca scintillans* is known to form massive blooms in north eastern part of Arabian Sea during winter monsoon period. *Noctiluca scintillans* is a heterotrophic dinoflagellate with no pigments of its own. *Pedinomonas sp.* grows as a symbiotic associate within the *Noctiluca* where former provides food to *Noctiluca* and the later provides shelter to *Pedinomonas*. *Pedinomonas* is a chlorophyte with chlorophyll-\(a\) and chlorophyll-\(b\) as major photosynthetic pigments. Peaks at 443nm are typical chlorophyll-\(a\) absorption peaks seen for waters from both bloom and non-bloom regions whereas, the distinguished shoulders at 465nm seen only for the waters of the bloom region are due to chlorophyll-\(b\) absorption (from *Pedinomonas*).
The aim of studying optical properties of the phytoplankton was to obtain information on the pigment types present in the cells in varying conditions. Relation between pigment concentrations and absorption coefficient is not linear and straightforward due to similarity in the spectral response of some pigments which makes their identification through absorption characteristics difficult. Also phytoplankton cells scatter the photons which can be wrongly considered as absorption, although, use of an integrated sphere minimizes this problem (Shibata et al., 1954; Bricaud et al., 1983).

6.3 Conclusion:

Absorption spectra for waters dominated by cyanophytes (observed during November 2009), *Trichodesmium* in this case, were not sharp and provided erroneous information about the pigment composition. Phytoplankton absorption was observed to be higher during winter monsoon season of 2011-2012 (dec2011 to March 2012) corresponding with the greater phytoplankton diversity in the coastal regions off Veraval. Sharp peaks of Chlorophyll-*a* absorption for most spectra revealed that chlorophyll-*a* concentration in the cells was higher and pigment package effect was less due to methanol extraction method adopted for de-pigmentation which does not remove phytoplankton cells from the suspended matter/detritus. Therefore detritus spectra also include the spectral
properties of cell and cellular components that are insoluble in methanol. These components along with proteins associated with pigments contribute to the package effect. Distinct absorption features for waters dominated by different phytoplankton communities such as mixed population, diatoms and dinoflagellates could be seen due to presence of different pigment compositions in these dominating groups. Prominent shoulder of chlorophyll-\textit{b} absorption at 465nm was seen during winter monsoon season in north eastern Arabian Sea due to presence of \textit{Pedinomonas} in symbiotic association with \textit{Noctiluca} which formed a massive annual bloom in this region. The detailed estimation of pigment compositions from the absorption spectra is discussed in the next chapter (section 7.2).
Plate 6.1: Phytoplankton Absorption (Phyto) in coastal areas off Veraval during 17th to 19th November 2009 (S – Surface with 100% light, M – Middle depth and D – Deepest sampling depth with 1% light; 100ml and 50ml are the volumes of sample filtered)
Plate 6.2: Phytoplankton Absorption (Phyto) in coastal areas off Veraval during 4th to 7th October 2010
(S – Surface with 100% light, M – Middle depth and D – Deepest sampling depth with 1% light; 100ml and 50ml are the volumes of sample filtered)
Plate 6.3: Phytoplankton Absorption (Phyto) in coastal areas off Veraval from October 2011 to April 2012 (S – Surface with 100% light, M – Middle depth and D – Deepest sampling depth with 1% light; 250ml, 150ml and 300ml are the volumes of sample filtered)
Plate 6.4: Phytoplankton Absorption (Phyto) in coastal areas off Porbander on 14th March 2012 (S – Surface with 100% light and 300ml is the volume of sample filtered)
Plate 6.5: Phytoplankton absorption (Phyto) in open ocean regions of Arabian Sea from 7th March 2011 to 19th March 2011