Commonwealth of Australia

Copyright Act 1968

Notice for paragraph 49 (7A) (c) of the Copyright Act 1968

Warning

This material has been provided to you under section 49 of the Copyright Act 1968 (the Act) for the purposes of research or study. The contents of the material may be subject to copyright protection under the Act.

Further dealings by you with this material may be a copyright infringement. To determine whether such a communication would be an infringement, it is necessary to have regard to the criteria set out in Division 3 of Part III of the Act.

For further information contact the Copyright Coordinator on ext. 34272 or at copyright@csu.edu.au
Using Optical Fibre Sensing for Measuring Chlorophyll-Related Pigments in Turbid Water

Yeremias Bunganaen

B.A. and S1 (IKIP Yogyakarta)
Grad. Dip. Sc. (UNE, Armidale, NSW)
M.Sc. Stud. (UNE, Armidale, NSW)

A thesis submitted to Charles Sturt University for the degree of Doctor of Philosophy

School of Science and Technology

Wagga Wagga, NSW, 2678
Australia
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE OF CONTENTS</td>
<td>ii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xiii</td>
</tr>
</tbody>
</table>

## Chapter 1: Thesis Aim and Overview .............................................. 1

## Chapter 2: Optical Characteristics and Measurement of Colour in Turbid Water ........................................ 5

2.1. Water .................................................................................. 5
  2.1.1. Water Quality and Water Colour .................................... 6
  2.1.2. Measurement of Water Colour ....................................... 8

2.2. Spectro-Optical Properties of Fresh Waters .............................. 11
  2.2.1. Fundamental Optical Properties ................................... 11
  2.2.2. Absorption Properties of Clear Water .............................. 15
  2.2.3. Reflection Properties of Clear Water .............................. 18

2.3. Spectro-Optical Properties of Natural Waters ............................ 26
  2.3.1. Colour in Natural Waters ............................................ 26
  2.3.2. Spectro-Optical Properties of Chlorophyll-Related Pigments in Water ........................................ 27
  2.3.3. Measurement of Absorption ......................................... 34

## Chapter 3: Optical Fibre Sensing .................................................. 36

3.1. Optical Fibre Theory ................................................................ 36
  3.1.1. Internal Reflection .................................................... 36
  3.1.2. Fibre Construction ..................................................... 41
Chapter 4: Measurement of Chlorophyll Pigments in Turbid Water Using the Direct-Absorption Technique

4.1. Introduction ........................................................................................................... 68

4.2. Preparation of Standard Samples ......................................................................... 68
  4.2.1. Chlorophyll Pigments ..................................................................................... 68
  4.2.2. Suspended Sediments ..................................................................................... 71

4.3. Optical Instrumentation ......................................................................................... 72
  4.3.1. Regulated Light Source ................................................................................ 73
  4.3.2. Sample Cell ..................................................................................................... 74
  4.3.3. Spectrometer .................................................................................................. 75
  4.3.4. Spectrometer Performance – the Maximum Absorbance Limit .................. 77

4.4. Results .................................................................................................................... 79
  4.4.1. Absorbance of Chlorophyll-Related Pigments in Clear Water .................... 79
  4.4.2. Absorbance of Chlorophyll-Related Pigments in Turbid Water ............... 87

Chapter 5: Measurement of Chlorophyll Pigments in Turbid Water Using FEFA Technique

5.1. Introduction ........................................................................................................... 92

5.2. Optical Fibre Preparation and Sample Cell ........................................................ 93

5.3. Optical Instrumentation ......................................................................................... 95

5.4. Results .................................................................................................................... 96
  5.4.1. Absorbance of Chlorophyll-Related Pigments in Clear Distilled Water ........ 96
  5.4.2. Absorbance of Chlorophyll-Related Pigments in Turbid Water .................. 104
Chapter 6: Investigating the Sensitivity of FEFA - a Theoretical and Experimental Parametric Study

6.1. Introduction ................................................................. 110

6.2. The Skip-Length Model .................................................. 111
  6.2.1. Depth of Penetration and Skip Length ............................. 111
  6.2.2. Displacement and the Effective Length of the Absorption Path in FEFA ...... 115
  6.2.3. Calculating Absorbance Using the Skip-Length Model ...................... 117

6.3. Initialising the Skip-Length Model ..................................... 119
  6.3.1. Refractive Index of Distilled Water
         Containing Chlorophyll-Related Pigment ................................ 119
  6.3.2. Estimating Net Absorption Parameters $a_w$ and $a_s$ ....................... 121
         6.3.2.1. Parameter $a_w$ for Distilled Water ................................ 124
         6.3.2.2. Parameter $a_s$ for Water Containing Chlorophyll-Related Pigment .... 125

6.4. Experimental Parametric Measurements
and Comparison with Skip-Length Model .................................. 126
  6.4.1. Chlorophyll-Related Pigment Concentration ........................... 127
  6.4.2. Optical Fibre Interaction Length ...................................... 130
  6.4.3. Optical Fibre Core Diameter .......................................... 132
  6.4.4. Light Launch Angle .................................................... 134

6.5. Estimating the Sensitivity of FEFA to Suspended Sediment ............. 141

Chapter 7: Summary and Conclusions ....................................... 144

References .......................................................................... 148

Appendix 1: Relationship Between % Brix and Refractive Index ............... 167
DECLARATION

I .................................................... JEREMIAS ONANANGI .................................................................

Hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgment is made in the acknowledgments.

Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

31 July 2002

Signature ................................. Date .............................
ACKNOWLEDGMENTS

It was impossible to complete this thesis without support, guidance and encouragement of many others, such as family members, sponsors, teachers, friends and associates. Therefore, I would like to express my genuine thanks to those who have helped and supported me throughout my research and writing time.

A heartfelt and earnest thanks to Assoc. Prof. David W. Lamb for his supervision and overall contribution towards the project as principal supervisor. I am grateful to him for his patience, intelligence, assistance and constant encouragement throughout the years. My sincere gratitude goes also to Prof. Gerry A. Woolsey for his theoretical and technical knowledge as a co-supervisor as well as Dr. John Louis and Dr. Rod L. Oliver.

I also wish to express my thanks to Dr Stuart Helliwell for his technical advice in relation to the use of ultrasound sonication (Sonifier 450, Branson Ultrasonics, Dandury, CT, USA) for extracting chlorophyll-related pigments from plant sample (i.e. "spinach"). Moreover, I wish to particularly acknowledge Mr. Geoff White for his technical assistance in all matters related to the conduct of all experiments.

A sincere thanks is also extended towards Assoc. Prof. Kevin Robards as a Head of School of Science and Technology, clerical staff, academic staff, research student and all members of School of Science and Technology who have contributed to this work by discussion, interest and more importantly by providing a constructive and healthy academic environment in which to study and to stay in Wagga Wagga. I also expressed my deep appreciation to Prof. Alistair Robertson for his efforts in securing me financial to remain at Charles Sturt University (CSU) for the last two months of this project.
My heartfelt thanks and earnest appreciation is expressed to the all financial sponsors from the Indonesian Government, especially, the staff of the Local Project Implementation Unit of Development for Undergraduate Education (LPIU - DUE), University of Nusa Cendana, Kupang (Drs. M. J. Pella MSc., Drs Y. Sugi MSc., Drs. P. R. Manggut MA, Ir. D. Adar MSc., and others). This financial support funded my tuition and living allowance and associate for a period of three years and six months.

Finally, my sincere thanks to my parents (Alphonsus I. Bunganaen and Margaretha T. Samon) and my parents-in-law (Johanes B. Soemardi and Theresia Sri Sutakti), my beloved wife (Aluisya Gansaga Sri Yuniana Sumartanti) and children (Christian Bunganaen, Margaretha Theresia Bunganaen, Maria Oktavia Bunganaen, Alexander Bunganaen), my all the relatives (brothers and sisters) including those who have passed-away, and my friends and colleagues, for their understanding, continuous encouragement and prayers. This thesis was a family endearment, therefore from the bottom of my heart, please receive my heartfelt and earnest acknowledgments on the successful result of this work. "GOD and LEWOTANA BLESS YOU ALL".
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Distribution of water on earth, and the time taken for</td>
<td>6</td>
</tr>
<tr>
<td>2.2</td>
<td>Standard solution equations: colour = m a + b used for</td>
<td>10</td>
</tr>
<tr>
<td>2.3</td>
<td>The specific absorption coefficient [(\bar{\alpha}), (m(^2) g(^{-1}))] and</td>
<td>29</td>
</tr>
<tr>
<td>3.1</td>
<td>Sample characteristics of PMMA and PFP polymer optical fibres</td>
<td>44</td>
</tr>
<tr>
<td>6.1</td>
<td>Measured refractive index of chlorophyll-related pigments</td>
<td>120</td>
</tr>
<tr>
<td>6.2</td>
<td>Multimode PCS optical fibres used in the parametric study</td>
<td>127</td>
</tr>
<tr>
<td>6.3</td>
<td>Input parameters used to theoretical predict relative absorbance</td>
<td>128</td>
</tr>
<tr>
<td>6.4</td>
<td>Input parameters used to calculate relative absorbance versus fibre</td>
<td>130</td>
</tr>
<tr>
<td>6.5</td>
<td>Input parameters used to calculate relative absorbance versus PCS fibre</td>
<td>132</td>
</tr>
<tr>
<td>6.6</td>
<td>Input parameters used to calculate absorbance versus light</td>
<td>136</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 2.1: Interaction of beam of light with a thin layer of aquatic medium ............... 12
Figure 2.2: The geometrical relations underlying the volume scattering function. .......... 13
Figure 2.3: (a) Field radiation at a point (P) in a space; (b) Field radiation at .......... 16
Figure 2.4: Reflection and refraction of light at the boundary between air and .......... 18
Figure 2.5: Reflectance of water surface as a function of zenith angle of light .......... 19
Figure 2.6: The reflectance of clear water as a function of wavelength .................. 20
Figure 2.7: Absorption coefficient (a, m⁻¹) as a function of wavelength for .......... 22
Figure 2.8: Scattering coefficient (b, m⁻¹) as a function of wavelength for .......... 23
Figure 2.9: Diffuse attenuation coefficient (c, m⁻¹) as a function of ....................... 24
Figure 2.10: Spectrum of the temperature dependency of the absorption ................ 25
Figure 2.11: The specific absorption coefficient (\( \tilde{a} \), m² g⁻¹) spectra of some .......... 33
Figure 2.12: The schematic of the spectrophotometer in normal mode for ............... 34

Figure 3.1: Basic optical fibre structure ......................................................... 37
Figure 3.2: Path of light beam travelling from medium with refractive index, \( n_0 \), ........ 37
Figure 3.3: Optical fibre will only receive light rays incident within a cone ............... 38
Figure 3.4: Diagram of one cycle of the zigzag light path of a propagating .......... 40
Figure 3.5: Comparison of singlemode and multimode step-index and .................. 42
Figure 3.6: The break-point temperature sensor (extracted from: Rogers, 1999) .......... 45
Figure 3.7: Schematic diagram of basic optical fibre sensor ............................... 45
Figure 3.8: Schematic comparison between (a) intrinsic, and (b) extrinsic sensor .......... 46
Figure 3.9: The Diagram of intrinsic Mach-Zehnder fibre optic sensor ......................... 47
Figure 3.10: Schematic diagram of the set-up white-light intrinsic FFPI sensor ............... 49
Figure 3.11: The shift in the centre of the central fringe of the white light ................. 50
Figure 3.12: (a) An extrinsic optical-fibre absorption sensor. Radiation is ................. 51
Figure 3.13: Extrinsic FFPI sensor with a cavity formed by the air gap between .......... 54
Figure 3.14: The Simplest configuration of the extrinsic Fabry-Pérot interferometric ..... 55
Figure 3.15: Principle of the extrinsic Optical FFPI for electric-field ....................... 56
Figure 3.16: An evanescent field is generated from total internal reflection ............... 57
Figure 3.17: Basic configuration of fibre-optic based evanescent field .................... 59
Figure 3.18: Configuration of the FEFA cell used by Simhony et al. (1988) ............... 64
Figure 3.19: The absorption spectra of water for various fibre immersion .................. 64
Figure 3.20: Schematic diagram of the optical system and the optical mask used .......... 65
Figure 3.21: The evanescent absorbance spectrum of methylene blue (5 x 10^-6 M) ....... 66
Figure 3.22: Variation of evanescent absorbance with unclad fibre length for .......... 66
Figure 4.1: Photograph of (a) the "domestic" spinach, (b) small pieces of .................. 69
Figure 4.2: Photograph of a laboratory spectrophotometer ..................................... 70
Figure 4.3: Photograph of (a) clay and (b) silt sediment ....................................... 72
Figure 4.4: Schematic diagram of the complete apparatus for measuring .................... 73
Figure 4.5: Series Q lamp housing with integrating collimating optics ....................... 73
Figure 4.6: Schematic diagram of a sample cell ..................................................... 74
Figure 4.7: Photograph of three of the sample cells used. Length are: ....................... 74
Figure 4.8: Schematic diagram of Ocean Optics SD1000 spectrometer unit ............... 76
Figure 4.9: Photograph of Ocean Optics SD1000 spectrometer and ......................... 76
Figure 4.10: Absorbance spectrum corresponding to the signal/noise limit of .......... 78
Figure 4.11: Raw digital spectrum of Ocean Optics SD1000 spectrometer ................. 78
Figure 4.12: Absorption spectra of chlorophyll-related pigments in clear .......... 80
Figure 4.13: Apparent shift of wavelength of BLUE absorption peak with .......... 82
Figure 4.14: Relationship between apparent wavelength of RED absorption .......... 83
Figure 4.15: Absorption spectra of 0.83 mg/L chlorophyll-related pigments in .......... 84
Figure 4.16: Relationship between BLUE peak absorbance wavelength of .......... 85
Figure 4.17: Relationship between absorbance at 670 nm and concentration of .......... 86
Figure 4.18: Relationship between absorbance at 670 nm and sample cell .......... 86
Figure 4.19: Selected absorbance spectra of 0.830 mg/L chlorophyll-related .......... 88
Figure 4.20: Relationship between absorbance at 670 nm of 0.830 mg/L .......... 89
Figure 4.21: Representative absorbance spectra of 0.83 mg/L chlorophyll .......... 90
Figure 4.22: Relationship between wavelength of RED absorption peak .......... 91

Figure 5.1: Schematic diagram of pure core silica (PCS) step-index optical fibre .......... 93
Figure 5.2: Length of prepared PCS optical fibre for evaluation of the FEFA .......... 94
Figure 5.3: Schematic diagram of “perpex” sample cell ........................................ 94
Figure 5.4: Photograph of perpex sample cell with PCS optical fibre inserted .......... 95
Figure 5.5: Schematic diagram of the complete apparatus for evaluating .......... 96
Figure 5.6: FEFA spectra of chlorophyll-related pigments in clear distilled .......... 98
Figure 5.7: FEFA spectrum obtained by using the same sample of ......................... 99
Figure 5.8: Relationship between wavelength of BLUE absorption peak and .......... 100
Figure 5.9: Relationship between wavelength of RED absorption peak and .......... 101
Figure 5.10: Relationship between absorbance at 670 nm (RED peak) and .......... 102
Figure 5.11: Relationship between absorbance at 670 nm and FIL using .......... 102
Figure 5.12: Selected FEFA spectra of 4.15 mg/L chlorophyll-related pigments .......... 105
Figure 5.13: Relationship between absorbance at 670 nm (RED peak) of .......... 106
Figure 5.14: Relationship between wavelength of BLUE absorbance peak region .......... 107

xi
Figure 5.15: Relationship between wavelength of RED absorbance peak region ............ 107
Figure 5.16: Relationship between absorbance at 670 nm of 4.15 mg/L .................. 108
Figure 6.1: An electromagnetic wave undergoes reflection and refraction at .......... 111
Figure 6.2: The light incident on the tip of the PCS optical fibre and propagates .... 114
Figure 6.3: Photograph of the ATC Sugar/Brix Refractometer .......................... 120
Figure 6.4: Relationship between chlorophyll-related pigment concentration .......... 121
Figure 6.5: Relationship between light transmission and launch angle for .......... 122
Figure 6.6: Absorbance at 670 nm as a function of chlorophyll-a pigment .......... 123
Figure 6.7: Absorbance as a function of acetone concentration in clear freon ........ 128
Figure 6.8: Absorbance at 670 nm of chlorophyll-a pigment in clear distilled ...... 129
Figure 6.9: Absorbance at 664 nm of methylene blue as a function of fibre .......... 131
Figure 6.10: Absorbance at 670 nm of 4.15 mg/L chlorophyll-a pigment in clear .... 131
Figure 6.11: A propagating ray suffers a greater number of internal reflections ...... 133
Figure 6.12: Absorbance as a function of optical fibre (OF) core radius .......... 133
Figure 6.13: Additional modification to the FEFA apparatus to allow variation, .... 134
Figure 6.14: Absorbance at 670 nm of 4.15 mg/L chlorophyll-a pigment in clear .... 135
Figure 6.15: Absorbance of methylene blue as a function of light launch angle .... 136
Figure 6.16: Measured absorbance at 670 nm of 4.15 mg/L chlorophyll-a .......... 139
Figure 6.17: Measured absorbance at 670 nm of 4.15 mg/L chlorophyll-a .......... 140
This thesis describes an optical fibre-based technique, known as fibre-evanescent-field absorption (FEFA), and its investigation as a potential method for measuring colour in water samples containing suspended material without the need for sample filtering. A simple FEFA apparatus, assembled using off-the-shelf components was constructed and its performance in measuring absorption of chlorophyll-related pigments in the presence of suspended clay particles compared to bulk-absorption measurements typically conducted in water quality laboratories. The FEFA technique was found to be capable of measuring the wavelength-dependent absorption of milligrams per litre (mg/L) of dissolved chlorophyll-related pigments in the presence of up to 2.5 g/L of suspended clay particles. These concentrations of suspended clay particles were found to preclude the use of bulk absorption measurements due to complete beam attenuation via scattering.

A mathematical model of the FEFA apparatus, based on the skip-length theory, was also derived to investigate methods of increasing the sensitivity of the FEFA technique. Increasing fibre interaction length, reducing the fibre diameter and using light-launch angles approaching the critical angle of the fibre-water interface were all found to significantly increase the sensitivity of the FEFA apparatus to detecting pigment absorption. Model calculations also demonstrated that the insensitivity of the FEFA technique to suspended material could be explained by the average distance between particles in suspension exceeding the penetration depth of the radiation travelling along the optical fibre.

It was subsequently shown that concentrations of suspended material would have to exceed 1 kg/L, two-thousand times higher than that encountered in turbid inland rivers in Australia, in order for the suspended material to influence the pigment absorption measurements. In addition to a general discussion of results some considerations of using the FEFA technique as the basis of a field-portable in-situ device for measuring water colour are also presented.
Chapter 1

Thesis Aim and Overview

The aim of this thesis is to investigate an optical fibre-based technique for measuring the colour of water in the presence of suspended sediment. An optical fibre technique, known as Fibre-Evanescent-Field Absorption (FEFA), will be investigated for its potential to measure the absorbance of pigments in water in the presence of suspended sediment.

Seven-tenths of the earth's surface is covered by ocean water. A considerably smaller portion of the earth's surface is covered by fresh water although it is fundamentally significant in the survival of terrestrial life (Moss, 1998; Wetzel, 2001). Abnormal suspended sediment fluctuations, increasing amounts of dissolved nutrients, the incidence of toxic algal blooms, and the introduction of foreign organic and inorganic constituents are major global issues in water quality (Webb and Walling, 1992; Sweeting, 1994; McStay et al., 1995; Lamb and O'Donnell, 1996; McFeeters, 1996; King et al., 2000; Falconer, 2001).

On a global scale, considerable time and money is being invested in water quality monitoring to meet the information requirements of decision makers (Cotruvo, 1987; Daly
et al., 1995; Gough and Ward, 1996; Ramanathan, 2001). A significant proportion of water-quality research is based on developing more efficient, cost-effective means of assessing and monitoring water resources (McFeeters, 1996).

Parameters used to quantify water quality are many and varied and will depend on the nature of the water under observation. In the context of routine monitoring of waterway health, these parameters will usually include turbidity, suspended solids, algal cell concentration and colour. Turbidity is a term used to describe the lack of water clarity (Kirk, 1994), and is caused by suspended matter such as clay, silt, finely divided organic and inorganic matter, soluble organic compounds, plankton and other macroscopic organisms (APHA, 1992; Nebbache et al., 2001). Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted through water. Turbidity is measured in nephelometric turbidity units (NTU). The dry weight of suspended solids, filtered from a water sample, and recorded in mg/L is used to quantify suspended solids (or SS).

Water colour is primarily measured by the Hazen method. This involves the direct comparison of the colour of water samples with standard Platinum-Cobalt (Pt-Co) solutions. The colour is recorded in Pt units, where 1 mg/L of Pt-Co = 1 TCU (true colour unit) (APHA, 1992; Cuthbert and del Giorgio, 1992; Hongve and Åkesson, 1996). Measurements of water colour conventionally distinguish between true colour which represents that caused by dissolved matter, and apparent colour which also includes the effects of suspended solids (APHA, 1992). In practise, turbidity is removed by filtering prior to optical analysis (Wetzel and Likens, 2000).

Colour in natural water is predominantly attributed to the presence of natural metallic ions (i.e. iron, manganese), humus and peat materials, plankton (i.e. microscopic algae) and industrial waste. Chlorophyll-related pigments are a major source of "natural" water colour (Davies-Colley and Close, 1990; Barale, 1991). The concentration of chlorophyll-a in natural water was reported to vary in depth, for example, 0.055 mg/L at 4 m to 0.010 mg/L at 12 m (Ganf and Oliver, 1982). The spectral characteristics of numerous clear (non-turbid) water types have been investigated in the laboratory such as coastal waters and mountain lakes (McConnell and Sigler, 1959; Kirk, 1980a; Caraco, 1986; Davies-Colley et
al., 1986), and in sea ice of polar regions (Zeebe et al., 1996). However, unlike these waters, inland river systems such as those in Australia's Murray-Darling Basin have widely varying levels of suspended solids (Bain et al., 1996). Suspended material significantly influences the spectral characteristics of water (Oliver, 1990). An estimation of water colour in inland rivers requires an understanding of the influence of the suspended matter on the spectral characteristics.

Optical fibre sensing provides many advantages as a measuring tool in the field, especially for in-situ monitoring. One of the most convenient aspects of optical fibre sensing is the fact that the modulated signal can be transmitted from the sensing region without recourse to electrical connection (Culshaw, 1988; López-Higuera, 1998). Although optical fibres are fragile, they are small in size, low in weight, chemically inert and easy to manipulate (McStay et al., 1995). Furthermore, optical fibre sensors can readily be used in a distributed array with a single remote monitoring station.

Current methods of measuring water colour include spectrophotometric measurements of absorbance. These techniques, hitherto referred to as bulk absorption, involve directing an unbound beam of probing radiation through the water and measuring the wavelength-dependent attenuation via absorbance. However, any suspended sediment will attenuate the probing radiation via scattering and confound the measurement of absorbance. Bulk absorption techniques therefore require that the water must be filtered to remove any suspended sediment prior to the measurement of absorbance, a process which usually necessitates measurements be conducted in the laboratory (Wetzel and Likens, 2000).

Such a technique could then allow in-situ measurements of water colour to be completed without the need for sample filtering. To this end, a simple FEFA apparatus, based on off-the-shelf components, has been designed and assembled to provide a comparison with traditional bulk absorption measurements. Because of the potential importance of this investigation to the measurement of water quality in the context of environmental monitoring, the water-borne pigment used in this work is chlorophyll and the suspended sediment is finely-ground clay, both of which would be found in typical inland waterways such as the Murrumbidgee River (in close proximity to this university).
Chapter 2 commences with an introduction to the measurement of water quality, in particular colour, some relevant optical terminology, and the spectro-optical characteristics of water in the presence of absorbing pigments and suspended sediment. Chapter 3 presents an introduction to relevant optical fibre theory, as optical fibres are central to the FEFA technique. In Chapter 4, a simple apparatus for measuring bulk absorption of chlorophyll-related pigments in the presence of suspended sediment is then described. This chapter also includes an outline of the process of preparing stock solutions of chlorophyll-related pigment and the preparation of sediment for suspension in the water. The effect of pigment concentration, sample cell length and the presence of suspended sediment on the bulk absorbance characteristics are recorded and discussed as the basis for comparison with the results of the FEFA technique. In Chapter 5, a FEFA apparatus is assembled for making the same measurements as those of the bulk absorption apparatus. The FEFA measurements are compared with the bulk absorption measurements and key advantages and disadvantages of the FEFA versus the bulk absorption techniques are identified and discussed.

In order to understand how the FEFA technique works, and to investigate factors which influence the sensitivity of the FEFA apparatus, a mathematical model of the FEFA apparatus based on the skip-length theory is derived in Chapter 6. The influence of pigment concentration on measured absorbance and the effect of varying parameters such as optical fibre length, fibre-core diameter and angle at which the probing radiation is launched into the optical fibre are calculated. Model predictions are then compared with actual measurements completed using the assembled FEFA apparatus. An insight into why the FEFA technique is insensitive to suspended sediment is also presented based on empirical calculations from the skip-length model. Finally, in Chapter 7 a general discussion of the results is presented. This also includes some considerations of the potential of the FEFA technique as the basis of a portable field device for measuring in-situ water colour.
2.1. Water

The earth's surface is dominated by water. Continuous ocean waters covers over seventenths of the earth's surface to an average depth of 3800 m. Ocean waters contain, on average, 35 g/L of salts. Fresh water contains less than 1 g/L of salts and, in contrast to ocean water bodies, fresh-water exists as small discrete bodies. Table 2.1 summarises the abundance and relative distributions of both fresh and ocean water bodies on the earth's surface (Moss, 1998; Wetzel, 2001). The long residence times associated with water circulation (Table 2.1), in particular through groundwater, forms the basis of water being classified as a non-renewable resource (for example, Murray Darling Basin Ministerial Commission, 1995). The residence time is defined as the volume of the water body concerned divided by the volume added to it in a given time (the unit is length$^2$/length$^3$.time$^{-1}$.)
Chapter 2: Optical Characteristics and Measurement of Colour in Turbid Water

The relatively small amounts of water that occur in freshwater lakes and rivers are fundamentally significant in the preservation and survival of terrestrial life. Of the 105,000 km$^3$ of precipitation that falls on land surfaces per year, the ultimate source of freshwater, only about one-third of it reaches the oceans as river discharge. The remaining two-thirds of the annual water supply is returned to the atmosphere by evaporation and plant transpiration (Wetzel, 2001).

<table>
<thead>
<tr>
<th>Category</th>
<th>Water volume (km$^3$)</th>
<th>Fraction of total (%)</th>
<th>Residence time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmosphere</td>
<td>$1.3 \times 10^4$</td>
<td>0.001</td>
<td>7 – 11 days</td>
</tr>
<tr>
<td>River channels</td>
<td>$1.2 \times 10^3$</td>
<td>0.0001</td>
<td>7 days</td>
</tr>
<tr>
<td>Freshwater lakes</td>
<td>$1.2 \times 10^5$</td>
<td>0.009</td>
<td>330 days</td>
</tr>
<tr>
<td>Saline lakes and inland seas</td>
<td>$1.0 \times 10^5$</td>
<td>0.008</td>
<td>1 – 4 years</td>
</tr>
<tr>
<td>Soil water</td>
<td>$6.6 \times 10^4$</td>
<td>0.005</td>
<td>?</td>
</tr>
<tr>
<td>Groundwater</td>
<td>$8.2 \times 10^5$</td>
<td>0.62</td>
<td>60 – 300 years</td>
</tr>
<tr>
<td>Ice caps and glaciers</td>
<td>$2.9 \times 10^7$</td>
<td>2.15</td>
<td>12 000 years</td>
</tr>
<tr>
<td>Ocean</td>
<td>$1.3 \times 10^9$</td>
<td>97.2</td>
<td>300 – 11 000 years</td>
</tr>
</tbody>
</table>

2.1.1. Water Quality and Water Colour

Water quality is just as important as water abundance in supporting life. In fresh waters, abnormal suspended sediment fluctuations, increasing amounts of dissolved nutrients, the incidence of toxic algal blooms, and the introduction of foreign organic and inorganic constituents are major phenomena which degrade water quality (Webb and Walling, 1992; Sweeting, 1994; McStay et al., 1995; McFeeters, 1996; Lamb and O'Donnell, 1996; and King et al., 2000; Falconer, 2001).

There are numerous parameters used to quantify the quality of fresh water (for example, Gabric et al., 1998 and Chang et al., 2001). Generally speaking, routine measures of water
quality will include turbidity, suspended solids (SS), algal cell concentration and colour. Turbidity is a term used to describe the lack of water clarity (Hilton, 1984; Kirk, 1994), and is caused by suspended matter such as clay, silt, finely divided organic and inorganic matter, soluble organic compounds, plankton and other macroscopic organisms (Davies-Colley and Close, 1990; Nebbache et al., 2001; Wetzel, 2001).

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted through water (Hilton, 1984; Han and Rundquist, 1998). Measurements of turbidity, although simple to perform, do not always yield significant correlations with concentrations of suspended materials as turbidity is influenced by particle size and refractive index (Davies-Colley and Close, 1990; Volten et al., 1998).

The most common method of measuring turbidity in water is by comparing the intensity of light scattered by the sample under defined conditions to that scattered by a standard reference suspension under the same conditions (Wetzel and Likens, 2000). Standard reference suspensions include silica, clay (i.e. Kaolin), and formazin polymers. The nephelometric method involves measuring the light scattered at right-angles to a probing beam of light (usually in the near-infrared wavelength range of 700-800 nm) using a cylindrical detector (Kirk, 1980b). Measurement units are nephelometric turbidity units (NTU) (Nolen et al., 1985; Koenings and Edmundson, 1991; APHA, 1992).

The amount of suspended solids in water is determined by filtering and oven-drying the suspended material. The concentration of suspended solids is recorded in mg/L (APHA, 1992).

The colour of water has three main attributes: hue, saturation (or colour purity), and brightness (Davies-Colley et al., 1988; Smith et al., 1997). Hue is connected with the subjective description of the colour, for example, as blue, green or green-yellow, and is determined by the spectral distribution of light energy. Saturation relies on the spread of light energy, with spectral lines producing the purest colours and uniform distributions of energy giving neutral greys, the least saturated colour.
Lastly, brightness depends on the amount of light energy as detected by human eye, (which is most sensitive to green light of wavelength, $\lambda = 555$ nm) which is determined by the reflectance coefficient (Davies-Colley et al., 1988; Kirk, 1994), the ratio of upwelling to downwelling light in the water. These fundamental aspects will be discussed in more detail in Section 2.2.

2.1.2. Measurement of Water Colour

The term “colour” is commonly used to describe true colour; the colour of water from which all turbidity has been removed. In contrast, the term “apparent colour” embraces not only colour due to substances in solution, but also suspended matter (APHA, 1992).

For over a century, water colour has been measured by visual comparison of water samples with various standard colour solutions (APHA, 1992; Hongve and Åkesson, 1996; Bukata et al., 1997). Europeans traditionally used methyl orange as the basis of their standard colour solutions (Pozdnyakov et al., 1998). The Hazen method, where the colour scale comprises a set of reference solutions prepared using hexachloroplatinate and cobalt ions in hydrochloric acid (Pt-Co solutions), is now widely used. These solutions are found to mimic the hues of most natural waters (Hongve and Åkesson, 1996).

The colour is measured in Pt-Co units where 1 mg/L = 1 TCU (true colour unit) (Cuthbert and del Giorgio, 1992; Hongve and Åkesson, 1996). However, this measurement does not relate well to the actual appearance of a water body to a human observer in the field. The platinum-cobalt scale, in particular, cannot cope with blue or green-hued lake waters. Although the particulate constituents of water obviously affect the appearance of a water body, the “true colour” (platinum-cobalt scale) refers only to the yellowness of filtered water samples (Davies-Colley et al., 1988).

Prior to measurement of (true) water colour, turbidity must be removed as any residual turbidity causes the apparent colour to be noticeably higher than the true colour (APHA, 1992). Turbidity is removed by filtering through a 47 mm Whatman GF/C glass fibre filter (Ghassemi and Christman, 1968; Kirk, 1976 and 1981; Weidemann and Bannister, 1986;
Koenings and Edmundson, 1991; King et al., 2000). However, the process of filtering has also been observed to affect the remaining water colour (APHA, 1992). For example, Hongve and Åkesson (1996) reported that the reduction in visible colour observed after rapid filtration of appropriate volumes of water is normally caused by separation of particles. An optimal method of removing turbidity without affecting colour has not been reported in the literature.

Although visual colour measurement techniques are still widely used, in the past two decades many researchers have changed to the spectrophotometric determination of colour. The absorption of light energy on passing through a water sample is measured at one or more wavelengths in the near ultraviolet (UV) and visible wavelength range of the electromagnetic spectrum. The most commonly used wavelength is 440 nm (Bricaud et al., 1981; Bowling et al., 1986; Davies-Colley and Vant, 1987; Cuthbert and del Giorgio, 1992; Kirk, 1994; Smith et al., 1997; and Shooter et al., 1998) as it is an important wavelength in aquatic plant production (440 nm is at the peak of the blue absorption band of chlorophyll-a). Other wavelengths include 270 nm (Davies-Colley and Close, 1990), 340 nm (Davies-Colley and Vant, 1987), 400 nm (Koenings and Edmundson, 1991), 420 nm, 430 nm, 405 – 450 nm (Cuthbert and del Giorgio, 1992), and 410 – 465 nm (Hongve and Åkesson, 1996).

Measured absorbance values can be converted to equivalent Pt-Co units using a standard curve created from spectrophotometric absorbance measurements of Platinum-Cobalt solutions. The standard curves of light absorption and Pt-Co concentrations generally take the linear form of

\[
\text{Colour (Pt-Co, mg L}^{-1}\text{)} = ma + b
\]  

(2.1)

where \( a \) is the absorption coefficient (\( m^{-1} \)) at the corresponding wavelength, \( m \) and \( b \) are the slope and intercept, respectively. Values of the parameter \( m \) and \( b \) have been measured for numerous wavelengths and selections of these are summarised in Table 2.2 (Cuthbert and del Giorgio, 1992). The coefficients of determination for all the equations presented in Table 2.2 exceeded 0.99. The absorption coefficient (\( a \)) is converted from the measured absorbance (\( D \)) by means of the following equation:

\[
(a) \ (m^{-1}) = 2.303 \ \frac{D}{r}
\]  

(2.2)

where \( r \) is the cell path length (m) (Kirk, 1994).
Table 2.2: Standard solution equations: colour = m a + b used for converting absorption coefficients (a, m⁻¹) to colour (Pt-Co, mg L⁻¹) at seven different wavelengths. The path length is 0.1 m (Extracted from Cuthbert and del Giorgio, 1992)

<table>
<thead>
<tr>
<th>Wavelength, λ (nm)</th>
<th>Slope, m</th>
<th>Intercept, b</th>
<th>Linear range (Pt-Co, mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>0.199</td>
<td>-0.213</td>
<td>0 – 15</td>
</tr>
<tr>
<td>310</td>
<td>0.354</td>
<td>-0.223</td>
<td>0 – 35</td>
</tr>
<tr>
<td>330</td>
<td>1.196</td>
<td>-0.400</td>
<td>0 – 100</td>
</tr>
<tr>
<td>365</td>
<td>1.960</td>
<td>-0.866</td>
<td>0 – 150</td>
</tr>
<tr>
<td>405</td>
<td>6.557</td>
<td>-0.148</td>
<td>0 – 400</td>
</tr>
<tr>
<td>420</td>
<td>16.024</td>
<td>-0.837</td>
<td>0 – 500</td>
</tr>
<tr>
<td>440</td>
<td>18.216</td>
<td>-0.209</td>
<td>0 – 500</td>
</tr>
</tbody>
</table>

From Table 2.2, in the widely-used wavelength of 440 nm as a standard for measuring colour (Kirk, 1976; Bricaud et al., 1981; Davies-Colley and Vant, 1987) Equation 2.1 becomes

\[
\text{Colour (Pt-Co, mg L}^{-1}\text{)} = 18.216 \times a_{440} - 0.209
\]

Equation 2.3 can be used for measuring water colour at low colour concentrations, although it requires modification for very low concentrations of colour (i.e. < 5 mg L⁻¹ Pt-Co colour, or \(a_{440} < 0.286\)). However caution must be exercised, as Cuthbert and del Giorgio (1992) found that the slope of the regression lines, represented in Table 2.2, varies so much between samples that calculations of colour from absorbance measurements at other than the specified wavelengths can produce large errors. Nevertheless, they still found that estimation of colour by relating absorption coefficients of water samples to those of standard solutions is a more precise method than the traditional visual comparative method. As with the earlier direct visual method, filtration of the samples is crucial before any spectrophotometric measurement (Hongve and Åkesson, 1996; Wetzel and Likens, 2000).

Alternatively, there is a single relationship between colour and absorption coefficient with wavelength (\(\lambda\)) (Cuthbert and del Giorgio, 1992; King et al., 2000) is given by

\[
\text{Colour (Pt-Co, mg L}^{-1}\text{)} = 5.19 \times 10^{-2} a e^{(0.415 + 0.028\lambda)}
\]
Equation 2.4 allows the calculation of Pt-Co units from absorption coefficient (a) at any wavelength between 300 and 440 nm, and has the determination coefficient of approximately 0.97 (Cuthbert and del Giorgio, 1992).

2.2. Spectro-Optical Properties of Fresh Waters

2.2.1. Fundamental Optical Properties

Light is a form of electromagnetic energy, which in itself can be considered as taking the form of discrete indivisible units referred to as quanta or photons. For the purposes of this thesis, light, and the associated optical properties of media with which it interacts, is confined to that portion of the electromagnetic spectrum termed the ultraviolet-visible-near infrared (UV-VIS-NIR) spectrum with a wavelength range from 200 – 900 nm.

The optical properties of aquatic medium for light of any given wavelength may be classed as inherent or apparent optical properties (Smith and Baker, 1981; Kirk and Oliver, 1995). Inherent optical properties (IOP) depend only on the substances of the aquatic medium itself, such as coloured dissolved organic matter and suspended material, and not on the nature of the light fields that pervade it (Kirk, 1994; Loisel and Morel, 1998). In contrast, an optical property is termed apparent if its operational value at a given point in a medium is dependent upon the light-field at that point. Apparent optical properties (AOP) can be related to inherent optical properties by means of radiative transfer theory (Kirk, 1994) and, similar with the IOP, are dependent on the dissolved and suspended material in the water (Smith and Baker, 1981; Maffione, 1998).

When a plane parallel layer of aquatic medium is illuminated at right angles by a parallel beam of monochromatic light, as depicted in Figure 2.1, the thin layer absorbs some of the incident light. Most of the light is transmitted without deviation from its original path. However, some is scattered although predominantly in a forward direction.
Chapter 2: Optical Characteristics and Measurement of Colour in Turbid Water

![Diagram](image)

Figure 2.1: Interaction of beam of light with a thin layer of aquatic medium.

The coefficients of absorption and scattering are two fundamental inherent optical properties. The fraction of the incident flux which is absorbed, divided by the thickness of the layer, is called the absorption coefficient, \( a \). The fraction of the incident flux which is scattered, divided by the thickness of the layer, is called the scattering coefficient, \( b \). The volume scattering function, \( \beta \), is defined as the radiant intensity in a given direction from a volume element, \( dV \), illuminated by a parallel beam of light, per unit of irradiance on the cross-section of the volume, and per unit volume as described in Figure 2.2.

The beam attenuation coefficient, \( c \), is the sum of the absorption and scattering coefficients (Gordon et al., 1975; Smith and Baker, 1981; Bricaud et al., 1983; and Buiteveld et al., 1994). The absorption, scattering and beam attenuation coefficients all have units of reciprocal length (\( m^{-1} \)).
The coefficients of absorption and scattering are two fundamental inherent optical properties. The fraction of the incident flux which is absorbed, divided by the thickness of the layer, is called the absorption coefficient, \( a \). The fraction of the incident flux which is scattered, divided by the thickness of the layer, is called the scattering coefficient, \( b \). The volume scattering function, \( \beta \), is defined as the radiant intensity in a given direction from a volume element, \( dV \), illuminated by a parallel beam of light, per unit of irradiance on the cross-section of the volume, and per unit volume as described in Figure 2.2.

The beam attenuation coefficient, \( c \), is the sum of the absorption and scattering coefficients (Gordon et al., 1975; Smith and Baker, 1981; Bicaud et al., 1983; and Buiteveld et al., 1994). The absorption, scattering and beam attenuation coefficients all have units of reciprocal length (m\(^{-1}\)).
In addition to the measurement of light absorption in terms of the absorption coefficient, \( a \), parameters such as absorptance, \( A \) and absorbance or optical density, \( D \), are also used. If \( \Phi_s \) is the radiant flux (energy or quanta per unit time) incident in the form of a parallel beam on some physical system, \( \Phi_s \) is the radiant flux absorbed by the system (i.e. aquatic medium with pathlength or thickness, \( r \)), then, by definition (Kirk, 1994) and by virtue of the Beer’s Law (Skoog and Leary, 1992; Hodgkinson et al., 1998):

\[
A = \frac{\Phi_s}{\Phi_o} \quad \text{(2.5)}
\]

where, radiant flux absorbed (\( \Phi_s \)) is expressed by

\[
\Phi_s = \Phi_o (1 - e^{-ar}) \quad \text{(2.6)}
\]

From Equations 2.5 and 2.6, the relationship between absorption coefficient (\( a \)) and absorptance (\( A \)) is then given by

\[
a = - \frac{1}{r} \ln (1 - A) \quad \text{(2.7)}
\]

Equation 2.7 applies only for a medium with dominant absorption (i.e. neglecting scattering). On the other hand, for a medium with dominant scattering, the relationship between scattering coefficient (\( b \)) and scatterance (\( B \)) is expressed by
Chapter 2: Optical Characteristics and Measurement of Colour in Turbid Water

\[ b = -\frac{1}{r} \ln (1 - B) \]  \hspace{1cm} (2.8)

Similarly, the relationship between beam attenuation coefficient (c) and attenuance (C) is then given by

\[ c = -\frac{1}{r} \ln (1 - C) \]  \hspace{1cm} (2.9)

Furthermore, the absorbance or optical density, D, is commonly defined using (Skoog and Leary, 1992)

\[ D = \log_{10} \frac{I_0}{I} \]  \hspace{1cm} (2.10)

The word “intensity” can be equated with radiant flux, \( \Phi \), where \( I \) is equal to the incident flux minus the absorbed flux (i.e. \( I = \Phi_0 - \Phi_a \)). Therefore, after employing some mathematical manipulations, and given that \( A = \Phi_a/\Phi_0 \), Equation 2.10 becomes

\[ D = -\log_{10} (1 - A) \]  \hspace{1cm} (2.11)

By using Equation 2.7, Equation 2.11 becomes

\[ D = \frac{a r}{\ln 10} \]  \hspace{1cm} (2.12)

Hence, the absorption coefficient can be calculated from the absorbance value (Equation 2.2), using

\[ a = \frac{D \ln 10}{r} = 2.303 \frac{D}{r} \]  \hspace{1cm} (2.13)

or absorbance can be written as

\[ D = 0.434 \ a \ r \]  \hspace{1cm} (2.14)

Following Beer's Law, the absorbance, \( D \), of a liquid is directly proportional to the concentration (\( c \)) of the absorbing species and the path length (\( r \)) through the solution (Skoog and Leary, 1992). This is shown mathematically by

\[ D = \alpha \ c \ r \]  \hspace{1cm} (2.15)

where \( \alpha \) is a proportionality constant known as the absorptivity or extinction coefficient (alternative symbol, \( k \)) of the absorbing species (see also Smith, 1968). Depending on the units used for \( c \) and \( r \), \( \alpha \) (or \( k \)) will generally have units of \( \text{L g}^{-1} \text{cm}^{-1} \) or \( \text{m}^2 \text{kg}^{-1} \).
2.2.2. Absorption Properties of Clear Water

The relevant apparent optical properties of the water column include the vertical attenuation coefficients (K) as a function of wavelength across the spectrum and also the reflectance (R) (Kirk, 1984; Kirk et al., 1998). The irradiance (E) at a point in the water body is defined as

\[ E = \frac{d\Phi}{dS} \]  \hspace{1cm} (2.16)

where \( \Phi \) is the radiant flux, defined earlier for Equation 2.5 and \( S \) is the surface area of the volume element in question. Irradiance has the units Watt m\(^2\). Downward irradiance (\( E_d \)) is the irradiance due to the downwelling light stream, and upward irradiance (\( E_u \)) is the irradiance due to the upwelling light stream. In other words, \( E_d \) and \( E_u \) are the values of irradiance on the upper and lower faces, respectively, of an imagery slice of a water body.

Radiance (L) at a point in space is the radiant flux (d\( \Phi \)) at that point (P) in a given direction (D) per unit solid angle (d\( \omega \)) per unit area (dA) at right angles to the direction of propagation. This field radiance is illustrated in Figures 2.3 (a) and (b). Surface radiance, which is the radiant flux emitted in a given direction per unit solid angle per unit projected area (dS \( \cos \theta \)) (or the apparent unit area as seen from the viewing direction) of a surface, is illustrated in Figure 2.3 (c). To indicate that radiance is a function of direction, i.e. of both zenith angle (\( \theta \), the angle between a given light direction and the upward vertical) and azimuth angle (\( \phi \), the angle between the vertical plane incorporating the light direction and some other specified vertical plane such as the vertical plane of the sun), it is commonly written as \( L(\theta, \phi) \), and has the unit of Watt m\(^2\) steradian\(^1\). Mathematically, radiance is expressed as

\[ L(\theta, \phi) = \frac{d^2\Phi}{dS \cos \theta d\omega} \]  \hspace{1cm} (2.17)
From Figure 2.3 (b), the relationship between irradiance and radiance can be derived. The projected area of the element surface is $dS \cos \theta$ and the corresponding element of solid angle is $d\omega$. Therefore, the radiant flux on the element of surface within the solid angle is

$$d\Phi = L(\theta, \phi) \, dS \cos \theta \, d\omega$$

(2.18)

From Equation 2.16 and 2.18, the irradiance can be written in terms of radiance as

$$E = L(\theta, \phi) \cos \theta \, d\omega$$

(2.19)

The total downward irradiance at the point in the surface is achieved by integrating Equation 2.19 with respect to solid angle over the whole upper hemisphere

$$E_d = \int_{0}^{\pi} L(\theta, \phi) \cos \theta \, d\omega$$

(2.20)

In the similar manner, the total upward irradiance can be formulated, except for the fact that $\cos \theta$ is negative due to the values of $\theta$ between 90 and 180°,

$$E_u = -\int_{\pi}^{2\pi} L(\theta, \phi) \cos \theta \, d\omega$$

(2.21)

The net downward irradiance is defined as the difference between the downward and the upward irradiance

$$E = E_d - E_u$$

(2.22)

By applying Equations 2.20 and 2.21 to Equation 2.22, then
\[
\tilde{E} = \int_{\Omega} L(\theta, \phi) \cos \theta \, d\omega
\]  

(2.23)

The scalar irradiance \(E_0\) is the integral of the radiance distribution at a point over all directions about the point, which is \(E_0 = \int_{\Omega} L(\theta, \phi) \, d\omega\). Scalar irradiance is therefore a measure of the radiant intensity (I, the radiant flux \(d\Phi\) per unit solid angle \(d\omega\)) at a point, which treats radiation from all directions equally.

In any absorbing and turbid scattering water body, all of these properties of the light field will change with depth \(z\). For example, irradiance generally decreases and reflectance increases with increasing depth. It is therefore convenient to describe properties, which indicate the rate change of both \(E_d\) and \(E_u\) with depth \(z\). Consequently, the following vertical attenuation coefficients are defined (Kirk, 1984 and 1994):

- **downward irradiance**

  \[
  K_d = -\frac{d \ln E_d}{dz} = -\frac{1}{E_d} \frac{d}{dz} E_d
  \]  

  (2.24)

- **upward irradiance**:

  \[
  K_u = -\frac{d \ln E_u}{dz} = -\frac{1}{E_u} \frac{d}{dz} E_u
  \]  

  (2.25)

- **net downward irradiance**:

  \[
  K_g = -\frac{d \ln (E_d - E_u)}{dz} = -\frac{1}{(E_d - E_u)} \frac{d}{dz} (E_d - E_u)
  \]  

  (2.26)

- **scalar irradiance**:

  \[
  K_0 = -\frac{d \ln E_0}{dz} = -\frac{1}{E_0} \frac{d}{dz} E_0
  \]  

  (2.27)

and radiance:

\[
K(\theta, \phi) = -\frac{d \ln L(\theta, \phi)}{dz} = -\frac{1}{L(\theta, \phi)} \frac{d}{dz} L(\theta, \phi)
\]

(2.28)

Generally these vertical attenuation coefficients themselves also vary with water depth \(z\), and they are often expressed as \(K(z)\).
2.2.3. Reflection Properties of Clear Water

When a parallel light beam crosses the interface between media that differ in refractive index (for example air and water or glass and water), reflection and refraction always occur as shown in Figure 2.4. For example, for a beam entering the smooth surface of a water body from the air at right angles to the plane of the water surface, the fraction reflected is given by

\[ r = \frac{I_r}{I_o} = \frac{(n_w - n_a)^2}{(n_w + n_a)^2} \]  \hspace{1cm} (2.29)

where \( I_r \) and \( I_o \) are the intensity of the incident beam and reflected beam respectively; \( n_w \) and \( n_a \) are the refractive indexes for water and air successively. The formula in Equation 2.29 can be used for calculating the total losses due to the reflection. In the general case, where the angle of incidence can vary (relative to the normal of the interface) as depicted in Figure 2.4, the surface reflectance, \( r \), of unpolarised light is given by Fresnel’s equation (see Pedrotti and Pedrotti, 1993)

\[ r = \frac{1}{2} \left( \frac{\sin^2(\theta_a - \theta_w) + \tan^2(\theta_a - \theta_w)}{\sin^2(\theta_a + \theta_w) \tan^2(\theta_a + \theta_w)} \right) \]  \hspace{1cm} (2.30)

where \( \theta_a \) is the angle of incidence and \( \theta_w \) is the angle of refraction. The angle, \( \theta_w \), can be determined from the angle, \( \theta_a \), and the refractive index of water, \( n_w \), using Snell’s Law:

\[ \frac{\sin \theta_a}{\sin \theta_w} = \frac{n_w}{n_a} \]  \hspace{1cm} (2.31)

\[ \text{Figure 2.4: Reflection and refraction of light at the boundary between air and water.} \]

The calculated percentage reflectance from a flat water surface as a function of zenith angle is graphically depicted in Figure 2.5, where \( n_a = 1 \) and \( n_w = 1.33 \). The reflectance remains
low, increasing only gradually up to zenith angle of approximately $50^\circ$ whereby it increases very rapidly from $50^\circ$ to $90^\circ$.

The reflectance ($R$) of a water body, alternatively known as the irradiance reflectance or irradiance ratio, is defined as the ratio of the upward to the downward irradiance (Morel and Prieur, 1977). It is mathematically expressed by

$$ R = \frac{E_u}{E_d} \quad (2.32) $$

The work of Kirk et al. (1998) and others have shown that the values of vertical attenuation coefficients for photosynthetically active radiation (PAR: 400 – 700 nm) in marine waters vary from about 0.06 m$^{-1}$ in sub-tropical and clear waters, to about 0.1 – 0.12 m$^{-1}$ in the coastal waters. The value of the reflectance at a specified optical depth, e.g. the depth ($Z_m$) in the middle of the euphotic zone where downward irradiance is reduced to 10% of that at the surface, can be used, together with the value of the vertical attenuation coefficient for downward irradiance, to estimate the absorption and scattering coefficients of the water (Kirk, 1994; Kirk and Oliver, 1995; Kirk et al., 1998; Shooter et al., 1998).
Chapter 2: Optical Characteristics and Measurement of Colour in Turbid Water

The spectral shape of irradiance reflectance in clear water as a function of wavelength in the range from 350 to 600 nm (Kirk et al., 1998) is depicted in Figure 2.6.

![Reflectance graph](image)

Figure 2.6: The reflectance of clear water as a function of wavelength (Extracted from Kirk et al., 1998).

Morel and Prieur (1977) shows that the reflectance (R) as a function of wavelength is approximately proportional to the ratio of the scattering coefficient to the absorption coefficient (b/a). The flat shape of the reflectance spectrum from about 425 to 475 nm in Figure 2.6 suggests that the high magnitude arises from a combination of a comparatively high concentration of scattering particles with low levels of absorption by either gilvin (Latin *gilvus*: pale yellow (Davies-Colley and Vant, 1987)) or phytoplankton. The reduction in reflectance as wavelength decreases below 390 nm is likely to be due to absorption in the blue region by land-derived gilvin (Kirk et al., 1998). Reflectance starts to increase sharply at wavelengths above approximately 580 nm, and is probably due to additional contributions the influence of Raman emission and chlorophyll fluorescence (Hoge et al., 1989; Kirk et al., 1998).

Irradiance reflectance for total PAR in oceanic waters at 5 m depth vary from about 2 to 5 % (Kirk, 1994). While Kirk et al. (1998) found that in the wavelength range from 350 to
700 nm, irradiance reflectance just beneath the surface of the ocean waters at six representative stations in the South Pacific, east of New Zealand in the vicinity of the Catham Rise, varied from about 0.1 to 2.0 %. The irradiance reflectance from 350 to 650 nm within the mid region of the euphotic zone was also observed to be in the range of 0.6 to 10.7 %. Bowling et al. (1986) found that the irradiance reflectance for (PAR) just below the surface of fresh water bodies in Tasmania varied from about 1.2 % down to 0.14 %.

Essentially all the light absorption that takes place in even the clearest of natural waters is attributable to four components of the aquatic ecosystem: the water itself, dissolved yellow pigments (gilvin or gelbstoff), the photosynthetic biota (phytoplankton, and macrophytes where present) and inanimate particulate matter (tripton) (Kirk, 1994).

Pure water, although it appears colourless, is actually closest to the colour we perceive as blue liquid. This blue colour is clearly conspicuous under sunny conditions in oceanic waters or in coastal waters, which are infertile and have little input from rivers. Water absorbs electromagnetic radiation strongly at wavelengths above 550 nm (orange and red light) and below 400 nm (UV). For example, at a wavelength of 680 nm, a 1-m thick layer of pure water will absorb approximately 35 % of incident light (Kirk, 1994). Conversely, water exhibits only weak absorbance in the blue and green wavelengths (400 – 500 nm) (Atlas and Bannister, 1980; Moss, 1998) which is why, waters with very little concentration of dissolved organic material, suspended matter or chlorophyll appears blue/turquoise in colour (472 – 500 nm) (Bukata et al., 1997).

It is very difficult to measure the absorption coefficient of pure water in the blue/green spectral region. Because of its very weak absorption. Nevertheless, Smith and Baker (1981) for example have published values based partly on their own measurement of vertical attenuation coefficient for irradiance in the clearest natural waters. Values of the absorption coefficient (a), are plotted as a function of wavelength in Figure 2.7.
In the wavelength range 350 - 550 nm in Figure 2.7, the absorption is very low (Morel and Prier, 1977; Hass and Davisson, 1977; Tam and Patel, 1979; Boivin et al., 1986). Absorption increases with increasing wavelength in the range of 550 - 760 nm, with shoulders at 510 nm, 600 nm and 660 nm. These shoulders are attributable to the existence of impurities in the water (Buiteveld et al., 1994). The absorption increases from 690 nm to a local maxima at approximately 740 nm (Pegau and Zaneveld, 1993), from which it decreases. The strong absorption in the UV region (200 - 320 nm) is likely caused by residual absorbing impurities in the water and Rayleigh scattering (Quickenden and Irvin, 1980).
The scattering coefficient (b) of pure water is plotted as a function of wavelength in Figure 2.8. The general shape of this graph is attributable to Rayleigh scattering (Quickenden and Irvin, 1980). The scattering by water of light in the ultraviolet wavelength range (Quickenden and Irvin, 1980) is mathematically described by the Einstein-Smoluchowski equation (Buiteveld et al., 1994) with scattering attributed to fluctuations in the dielectric constant caused by the random motion of the water molecules. In the visible wavelength range, the volume scattering at right angles (β(90)) and total scattering coefficients (b) are expressed respectively in Equations 2.33 and 2.34.

\[
\beta(90) = \left(\frac{2\pi^2 kT}{\lambda^2 \beta_\tau}\right) n^2 \left(\frac{\partial n}{\partial \rho}\right)_\tau \left(\frac{6 + 6\rho}{6 - 7\rho}\right)
\]

(2.33)

\[
b = \left(\frac{3\pi}{8}\right) \beta(90) \left(\frac{2 + \rho}{1 + \rho}\right)
\]

(2.34)

where:

- β(90) = volume scattering function at 90° incident angle (m⁻¹ sr⁻¹)
- k = Boltzmann constant (1.38 \times 10^{-23} \text{ JK}^{-1})
- T = absolute temperature (K)
\( \lambda \) = wavelength (m)

\( \beta_T \) = isothermal compressibility of water (Pa\(^{-1}\))

\( n \) = refractive index of water

\( \left( \frac{\partial n}{\partial p} \right)_T \) = pressure derivate of \( n \) at constant temperature (Pa\(^{-1}\))

\( \rho \) = depolarisation ratio for scattered light

\( b \) = total scattering coefficient (m\(^{-1}\))

The diffuse attenuation coefficient (c), the sum of the absorption (a) and scattering coefficients (b) (Smith and Baker, 1981), is plotted as a function of wavelength in Figure 2.9 for the clearest ocean water.

![Figure 2.9: Diffuse attenuation coefficient (c, m\(^{-1}\)) as a function of wavelength for clearest ocean water(Extracted from Smith and Baker, 1981).](image)

Numerous researchers have measured the temperature dependence of the absorption coefficient of pure water (Tam and Patel, 1979; Pegau and Zaneveld, 1993; Buitenveld et al., 1994). Buitenveld et al. (1994) observed the temperature-dependence of the absorption coefficient \( \left( \frac{da}{dT} \right) \) for the wavelength range 400 – 800 nm. Measurements were completed
using a submersible absorption meter and a temperature range from 2.5 °C to 40.5 °C. The
temperature dependence of absorption \( \left( \frac{\text{da}}{dT}, \text{m}^{-1} \text{°C}^{-1} \right) \) in pure water and wavelength, \( \lambda \) (nm)
is plotted in Figure 2.10. The profile of Figure 2.10 shows little change in \( \frac{\text{da}}{dT} \) with some
notable exceptions at wavelengths of approximately 450 nm, 606 - 608 nm, 662 - 666 nm
and from 698 nm to 742 nm. The greatest value of \( \frac{\text{da}}{dT} \) is 0.017 m\(^{-1}\)°C\(^{-1}\) at a wavelength of
742 nm, while at wavelengths below 580 nm the slope coefficient \( \frac{\text{da}}{dT} \) is nearly constant at
0.0012 m\(^{-1}\)°C\(^{-1}\) (Buiteveld et al., 1994). Pegau and Zaneveld (1993) however reported lower
values of \( \frac{\text{da}}{dT} \). For example, at wavelengths of 600 nm and 750 nm, the values of \( \frac{\text{da}}{dT} \) at
were given as 0.0015 m\(^{-1}\)°C\(^{-1}\) and 0.009 m\(^{-1}\)°C\(^{-1}\), respectively.

![Figure 2.10: Spectrum of the temperature dependency of the absorption coefficient (da/dT, m^{-1} °C^{-1}) for pure water](image)

(Extracted from Buiteveld et al., 1994).

The greatest effect of temperature on the absorption coefficient of water occurs at
frequencies near the harmonics of the O-H bond-stretching frequency (Pegau and Zaneveld,
1993). For example, the sensitivity of the absorption coefficient to temperature is greatest at the wavelength corresponding to the fourth harmonic region, the 750 nm absorption maximum. A small local maxima in $\frac{da}{dT}$ also exists in the region of the fifth harmonic region; 600 nm (Tam and Patel 1979). At wavelengths higher than 780 nm, $\frac{da}{dT}$ becomes negative (Figure 2.10) (Buiteveld et al., 1994). This is likely due to very small quantities of impurities such as gilvin or gelbstoff in the water (Pegau and Zaneveld, 1993). Sources of water colour due to dissolved and suspended material will be discussed later.

2.3. Spectro-Optical Properties of Natural Waters

2.3.1. Colour in Natural Waters

The colour of natural waters is generally caused by the presence of metallic ions (i.e. iron and manganese), humus and peat materials, plankton, weeds and industrial wastes (APHA, 1992). Chlorophyll-related pigments from plants are a major source of "natural" water colour (Barale, 1991) and the spectral characteristics of numerous types have been investigated in the laboratory for clear (non-turbid) water such as coastal waters and mountain lakes (McConnell and Sigler, 1959; Kirk, 1980a; Caraco, 1986; Davies-Colley et al., 1986), and in sea ice of polar regions (Zeebe et al., 1996).

The apparent visual colour of water such as in a lake, reservoir or river, results from sunlight scattered upward after it has passed through the water to various depths and undergone selective attenuation en route (Wetzel and Likens, 2000). In clear (non-turbid) water, blue dominates the true colour because of molecular scattering of light by the water and low absorption coefficient (Pearcy and Keene, 1974) as discussed earlier. Oceanic waters, for example, contain few suspended particles; hence, scattering is inversely proportional to the fourth power of the wavelength via Rayleigh scattering (Bedidi and Cervelle, 1993) as described earlier. In these waters, the underwater light field becomes yellower and greener as light fades with depth (Smith et al., 1973). However, the blue colour of water will change to yellow, brown or even black when the water contains high concentrations of dissolved organic matter (Shapiro, 1957; Bukata et al., 1997; Ahn, 1999). In open sea waters,
chlorophyll-related pigments of phytoplankton are a dominant contributor to surface colour (Sathyendranath et al., 1989; Barale, 1991).

Most of the colour of fresh waters (lake and river) results from dissolved organic matter, and its rapid, selective absorption of the shorter wavelength of the visible spectrum. As a result, light scattered from a water body is dominated in the green portion of the spectrum and, with increasing concentrations of organic matter, especially humic compounds, increasingly in the yellows and red wavelengths (Wetzel, 2001). Aquatic humus comprises typically 40 – 60% of the total dissolved organic carbon and accounts for 85 – 100% of the colour of fresh waters (Davies-Colley and Vant, 1987). Dissolved organic substances responsible for imparting a yellow colour to waters are also known as gelbstoff or gilvin (Kirk, 1976; Baker and Smith, 1982; Davies-Colley and Vant, 1987). These materials, in addition to influencing water colour by absorption, cause a shift in the transmittance of the apparent colour to longer wavelength (i.e. yellow and red) (Jerlov, 1968; Pearcy and Keene, 1974).

2.3.2. Spectro-Optical Properties of Chlorophyll-Related Pigments in Water

Aquatic plants contain photosynthetic pigments to allow them to intercept sunlight, and in the presence of water and carbon dioxide, carry on the fundamental process of photosynthesis to form starch and related substances, and to release oxygen in the water (Krauss, 1956; Hall and Rao, 1989). This process is summarised in Equation 2.35.

\[ \text{CO}_2 + \text{H}_2\text{O} \xrightarrow{\text{light}} (\text{CH}_2\text{O}) + \text{H}_2\text{O} + \text{O}_2 \]  

(2.35)

The three main pigment groups that determine the bio-optical properties of plants are the chlorophylls, the carotenoids and the phycobiliproteins (Rowan, 1989; Johnsen et al., 1994). Of these, one of the most important photosynthetic pigments, and often the most abundant in concentration, are chlorophyll molecules that are bound to an ensemble of different proteins to form the structurally complex photosystems (Zucchelli et al., 2002). One of the characteristically important features of chlorophyll in the process of photosynthesis is related
to the quenching of their photoexcited states by compounds with high electron affinity via the electron-transfer process (El-Khouly et al., 2001).

Chlorophyll-a is the primary photosynthetic pigment of all oxygen-evolving photosynthetic organisms (Stockner and Antia, 1986; Krause-Jensen and Sand-Jensen, 1998; and Umetsu et al., 1999) and is present in all algae and cyanobacteria and photosynthetic organisms other than the photosynthetic sulfur bacteria (Wetzel, 2001).

Chlorophyll-b, although common in higher plants, is found only in certain algae such as green algae (Gantt, 1975), euglenophytes and certain minor groups. Chlorophyll-b, -c and -d are a light-gathering pigment that transfers absorbed light energy to chlorophyll-a (Croce et al., 2001; and Umetsu et al., 1999) for primary photochemistry. Chlorophyll-c, consisting of three spectrally distinct components, is generally used as an accessory pigment. While, chlorophyll-d is a minor pigment component found only in certain aquatic plants (i.e. red algae).

Carotenoids are well-known physical quenchers of chlorophyll excited-state and reactive oxygen species (Fiedor et al., 2001), and protect the biochemical photosynthetic apparatus from excess light (Frank and Cogdell, 1996). Among the many carotenoids pigments, the carotenes are linear unsaturated hydrocarbons, and the xanthophylls are oxygenated derivatives of carotenos (Wetzel, 2001). As in the case of chlorophyll-b, the light energy absorbed by carotenoids and biliproteins is transferred to chlorophyll-a, leading to fluorescence and excitation of chlorophyll-a molecules. About 50% of the energy absorbed by carotenoids is initially transferred directly to chlorophyll-b, while the rest is transferred to chlorophyll-a (Croce et al., 2001). β-carotene is the most widely distributed of the caroten (Albrecht et al., 2001) and is replaced by α-carotene only in certain aquatic plants (i.e. green algae) (Kirk, 1994). The biliproteins are water-soluble, pigment-protein complexes occurring in the cyanobacteria (blue-green algae) and, to a lesser extent, in certain cryptophytes and red algae (Ó Carra et al., 1964; Bogorad, 1975; Gantt, 1975; Kirk 1994, Samsonoff and MacColl, 2001).
Aquatic chlorophyll-containing plants including planktonic algae, also known as phytoplankton, are regarded as a major contributor to absorption and scattering of light in most natural waters (Kirk, 1977). Therefore, investigating the absorption characteristics of such species provides a valuable insight into the optical characteristics of water-borne pigments.

Using a spectrophotometer, Davies-Colley et al. (1986) have measured the specific absorption coefficient ($\bar{a}$) and the specific scattering coefficient ($\bar{b}$) of chlorophyll-a produced by some species of aquatic algae taken from New Zealand freshwater bodies. These are measured for intact cells of algae and are summarised in Table 2.3 (Davies-Colley et al., 1986). The specific absorption coefficient ($\bar{a}$) is defined as the absorption coefficient divided by chlorophyll-a concentration, and the specific scattering coefficient ($\bar{b}$) is defined as the scattering coefficient divided by chlorophyll-a concentration. The units of $\bar{a}$ and $\bar{b}$ are m$^2$ g$^{-1}$.

Table 2.3: The specific absorption coefficient [$\bar{a}$, (m$^2$ g$^{-1}$)] and scattering coefficient [$\bar{b}$, (m$^2$ g$^{-1}$)] at selected wavelengths of algal cultures
(Extracted from Davies-Colley et al., 1986)

<table>
<thead>
<tr>
<th>Alga</th>
<th>Concentration of chlorophyll-a ($x 10^3$ g m$^{-3}$)</th>
<th>$[\bar{a}$, (m$^2$ g$^{-1}$)]</th>
<th>676 nm</th>
<th>550 nm</th>
<th>550 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenedesmus</td>
<td>229 ± 4</td>
<td>16.5</td>
<td>2.7</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Chlamydomonas</td>
<td>110 ± 2</td>
<td>12.1</td>
<td>3.0</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Pediastrum</td>
<td>119 ± 2</td>
<td>10.7</td>
<td>2.6</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Nostoc</td>
<td>75 ± 2</td>
<td>13.5</td>
<td>4.0</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Anabaena</td>
<td>174 ± 15</td>
<td>13.8</td>
<td>3.8</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Navicula</td>
<td>403 ± 15</td>
<td>9.8</td>
<td>3.2</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

Specific absorption coefficients ($\bar{a}$) for six alga cultures (Davies-Colley et al., 1986) are plotted as a function of wavelength in Figure 2.11.

A prominent peak in the specific absorption spectra appears at a wavelength of 676 nm for all species (Figure 2.11 [a-e]). This is attributable to chlorophyll-a, and is often referred to as the chlorophyll-a red peak (Mittenzwey et al., 1992; Shooter et al., 1998). Similarly, a
prominent absorption maximum occurs at a wavelength 430 nm; this is the chlorophyll-a blue peak (Moss, 1967). Ganf et al. (1989) also observed these prominent absorption features in suspensions of various Microcystis. The absorption peak at 676 nm is actually the result of the close superimposition of two absorption peaks; one at 672 nm and one at 683 nm. These are two slightly different forms of chlorophyll-a (Davies-Colley et al., 1986).

The broad shoulder in the absorption spectra from 450 nm to 490 nm in the spectra of green algae (Chlorophyceae) (Figure 2.11 [a], [b] and [c]) is the result of absorption by carotenoids (Paerl et al., 1983; Faust and Norris, 1985), while the shoulder at 651 nm is ascribed to chlorophyll-b (Bricaud et al., 1983; and Johnsen et al., 1994).

The absorption feature at 627 nm for blue green algae (Cyanophyceae) (Figure 2.11 [d] and [e]) is attributable to phycobilin (Ó hEocha, 1963; and Davies-Colley et al., 1986). The diatom Navicula (Bacillarophyceae) (Figure 2.11 [f]) lacks chlorophyll-b absorption peaks but has an absorption peak at approximately 630 nm caused by Chlorophyll-c (Lorenzen, 1967; and Bricaud et al., 1983). Secondly, the broad, inclined shoulder from 450 to about 560 nm is attributable to the carotenoid fucoxanthin (Davies-Colley et al., 1986; Stockner and Antia, 1986; Smith and Alberte, 1994; and Zeebe et al., 1996).
The absorption spectra of Figure 2.11 highlights the potential of identifying different phytoplankton on the basis of differences in the pigment composition of the plant cells. Indeed, Johnsen et al. (1994) isolated numerous pigment components by means of high-performance liquid chromatography (HPLC) and recorded visible absorption spectra (400 - 700 nm) in an attempt to characterise the in-vivo absorption spectra of 10 main classes, covering 31 species, of bloom-forming phytoplankton.

There is, however, an important issue, which must be recognised when measuring the optical characteristics of free chlorophyll-related pigments as opposed to in-vivo. The wavelengths of absorption peaks are generally shifted to shorter wavelengths when the free pigments are dissolved in organic solvents relative to their wavelengths in-vivo (Smith and Alberte, 1994). For example, the chlorophyll-a red peak is at 676 nm in-vivo, but occurs at at 670 nm in pyridine (Umetsu et al., 1999), at 665 nm in acetone (Marker, 1972; Tett et al., 1975; Bracaud et al., 1983; Davies-Colley et al., 1986; and Cinque et al., 2000), at 663 nm in
dimethyl sulfoxide (Shoaf and Lium, 1976), at 680 nm in glycerol (Cinque et al., 2000), and at 660 nm in diethyl ether (Umetsu et al., 1999; and Zucchelli et al., 2002).

2.3.3. Measurement of Absorption

Generally, the most widely used equipment for measuring light absorption is the spectrophotometer (for example: Skoog and Leary, 1992; Kirk, 1994; Cinque et al., 2000), which is commonly based on the optical direct absorption.

A schematic diagram of a typical dual-beam spectrophotometer is given in Figure 2.12. The apparatus consists of two monochromatic light beams, two transparent glass or quartz cells of known path length and a photomultiplier for measuring the intensity of the light beams after travelling through their respective cells. One of the cells, known as the sample cell, contains a solution or suspension of the pigmented material. The other cell, known as the blank cell, contains only the pure solvent or suspending medium. The intensities of the beams, which have passed through the blank cell and the sample cell, are taken to be proportional to I₁ and I₂, respectively. The absorbance (D) of the sample can be calculated by using Equation 2.10.

![Schematic of a dual-beam spectrophotometer](image)

*Figure 2.12: The schematic of the spectrophotometer in normal mode for samples with negligible scattering (Extracted from Kirk, 1994).*

Spectrophotometers comprising light detectors with a light-collection angle of only a few degrees will not detect the often significant amount of incident light scattered by the sample
at larger angles. In actual fact, these spectrophotometers measure the attenuation of the sample rather than the absorbance (Kirk, 1994). The simplest geometrical arrangement of a spectrophotometer which allows collection of a large proportion of the scattered light involves placing the cells close to a wide-view photomultiplier so that the photons scattered up to quite wide angles can be detected. Another technique that is often used with small-field spectrophotometers is to place a layer of scattering material (e.g. opal glass), behind the blank and sample cells (Kirk, 1994).

Instruments designed for determining the total absorption coefficient of natural waters in situ at a given wavelength are widely known as absorption meters. The simplest immersible absorption meters comprise a collimated light source and, at a suitable distance, a detector with a wide angle of acceptance (Kirk, 1994). An alternative employs a point light source and has two detectors placed at different distances from the source (Friedman et al., 1980). The difference in radiant flux detected by the two detectors allows direct determination of the absorption coefficient.

Researchers worldwide have measured the absorption properties of water in different sub-regions of the UV-VIS-NIR spectrum, for example, in the wavelength range of 200 – 700 nm (Hass and Davison, 1977), 380 – 700 nm (Morel and Prieur, 1977), 418.6 – 640.3 nm (Querry et al., 1978), 440 – 700 nm (Tam and Patel, 1979), 200 - 800 nm (Smith and Baker 1981; Hodgkinson et al., 1998), 400 – 800 nm (Friedman et al., 1980), 400 – 750 nm (Gallegos and Correll, 1990; Kirk and Oliver, 1995; Ferrari et al., 1996; Sydor et al., 1998), 350 – 750 nm (Cinque et al., 2000), 700 – 2500 nm (Curcio and Petty, 1951), 1121.7 – 1124.2 nm (Bozóki et al., 1999), and 2500 – 4000 nm (Bunkin et al., 2000; Dolenko et al., 2000).

However, “traditional” methods of measuring the optical absorbance of water samples cannot be employed when the samples are turbid, such as in situations where significant amounts of suspend material exist. This, of course, forms the rationale for the uses of an optical-fibre based technique. Optical fibres, and their use as optical sensors are introduced in the following chapter.
Commonwealth of Australia

Copyright Act 1968

Notice for paragraph 49 (7A) (c) of the Copyright Act 1968

Warning

This material has been provided to you under section 49 of the Copyright Act 1968 (the Act) for the purposes of research or study. The contents of the material may be subject to copyright protection under the Act.

Further dealings by you with this material may be a copyright infringement. To determine whether such a communication would be an infringement, it is necessary to have regard to the criteria set out in Division 3 of Part III of the Act.

For further information contact the Copyright Coordinator on ext. 34272 or at copyright@csu.edu.au
Chapter 3

Optical Fibre Sensing

3.1. Optical Fibre Theory

3.1.1. Internal Reflection

Optical fibres are circular dielectric waveguides, made of fused silica or plastics that are capable of transmitting radiation for considerable distances. They have a very significant application in the realm of communication (Palais, 1988). A general schematic diagram of an optical fibre is given in Figure 3.1. The central fibre core surrounded by concentric cladding, and a plastic protective coating, called the fibre jacket. The jacket protects the cladding from damage and actually gives the fibre-increased flexibility. Typical optical fibres have cores ranging from 10 to 1000 μm in diameter.

The fibre core and cladding is made of material (silica or plastic) of very similar physical characteristics (e.g. flexibility, coefficient of thermal expansion) but the refractive index of
the cladding is smaller than the refractive index of the core. The difference in refractive indices is such that light, when directed (or coupled) into the core of an optical fibre, is trapped and propagates along the fibre core via total internal reflections at the core-cladding interface. This is illustrated in Figure 3.2.

![Fibre Structure Diagram](image-url)

**Figure 3.1: Basic optical fibre structure.**

![Light Path Diagram](image-url)

**Figure 3.2: Path of a light beam travelling from medium with refractive index, \(n_0\), through an optical fibre undergoes total internal reflection, when \(n_1 > n_2\).**
The light is coupled into the fibre with incident angle, $\theta_i$, whereby it is refracted into the fibre core at an angle of $\theta_i$, according to Snell’s law of refraction,

$$\frac{\sin \theta_i}{\sin \theta_c} = \frac{n_i}{n_o} \quad (3.1)$$

where $n_o$ and $n_i$ are respectively the refractive index of the originating medium (typically air $n_o \approx 1.0$) and the fibre core. Total internal reflection at the core-cladding interface will occur at a critical angle, $\theta_c$, given by,

$$\sin \theta_c = \frac{n_o}{n_i} \quad (3.2)$$

![Figure 3.3: An optical fibre will only receive light rays incident within the maximum acceptance angle, $\theta$.](image)

The Numerical Aperture (NA) of an optical fibre, depicted in Figure 3.3, is defined mathematically by

$$NA = n_o \sin \theta \quad (3.3)$$

where, again, $n_o$ is the refractive index of medium surrounded the fibre (e.g. for air, $n_o = 1$), and $\theta$ is the maximum acceptance angle of radiation which can be trapped with the fibre by total internal reflection (TIR). For a typical fused-silica optical fibre, $\theta$ is about $10^\circ$ (Rogers, 1997). As depicted in Figure 3.3, the acceptance angle subtends half the collection cone for the fibre. Light rays incident at angles outside this cone will violate the condition for total internal reflection at the core-cladding interface, and hence will not propagate along the fibre. A fibre that has a low NA will also have a small acceptance angle. For long distance communication, fibres that have a low numerical aperture, which is typically from 0.1 to 0.3,
are generally used. On the other hand, fibres that have a higher numerical aperture, typically ranging from 0.4 to 0.5, are employed for short-path propagation of radiation (Palais, 1988).

From Figures 3.2 and 3.3 the mathematical relationship between numerical aperture and critical angle is

\[ \text{NA} = n_o \sin \theta = n_1 \cos \theta_c \]  

(3.4)

Rewriting Equation 3.2, such that \( \cos \theta_c = \frac{\sqrt{n_1^2 - n_2^2}}{n_1} \), and combining this with Equation 3.4 yields the numerical aperture of the optical fibre as

\[ \text{NA} = n_o \sin \theta = \frac{\sqrt{n_1^2 - n_2^2}}{n_1} \]  

(3.5)

Theoretically, all radiation incident at the core-cladding interface at angles greater than the critical angle will be confined within the fibre core by total internal reflection. However, not all of the radiation will propagate along the fibre. In fact, only certain ray directions, referred to as modes, are permitted and these depend on the fibre geometry and nature of the core and cladding.

Modes have two broad classifications: radiation modes and guided modes. Radiation modes carry energy out of the core, hence the light energy is rapidly dissipated. Guided modes are trapped in the core and propagate the radiation (its information and power) along the fibre. Multimode fibres allow many simultaneous guided modes, each with their own different velocity, which can be further decomposed into orthogonal, linearly-polarised components. Singlemode fibres, on the other hand support only one mode which consists of two orthogonal polarisation modes.

The existence of the modes can be understood by using an analogy with cavity resonance depicted in Figure 3.4. The wavefronts of propagating radiation associated with rays A and B in Figure 3.4 will constructively interfere when the additional path difference between them \((L)\) is equal to an integral number of wavelengths. Alternatively, the condition is where the phase shift \((\Delta \phi)\) for a complete round trip is equal to an integer multiple of \(2\pi\) radians, or

\[ \Delta \phi = m (2\pi) \]  

(3.6)
where $m$ is an integer. This resonance condition must still be fulfilled to obtain a stable interference pattern. Equation 3.6 can be satisfied for several distinct angles, $\theta$, and the waves travelling at these angles constitute the modes of the waveguide (Palais, 1988). Waves whose ray angles do not satisfy Equation 3.6 will diminish rapidly due to destructive interference.

![Diagram of one cycle of the zigzag light path of a propagating mode in an optical fibre.](image)

By solving the Maxwell’s equation for a hollow metallic waveguide, an electrical equivalent of an optical fibre, only a discrete number of transverse electric (TE) modes and transverse magnetic (TM) modes of electromagnetic radiation are found (Keiser, 2000). The ability of a waveguide to support modes of propagating radiation is expressed using the waveguide parameter $V$ (Verbet), or V-number (Snyder and Love, 1983). The V-number is a normalised frequency and is defined as

$$V = \frac{2 \pi a}{\lambda} \left( n_1^2 - n_2^2 \right)^{1/2} = \frac{2 \pi a}{\lambda} \text{NA}$$ \hspace{1cm} (3.7)$$

where $a$ and $\lambda$ are the core radius of optical fibre and free-space wavelength of the propagating radiation, respectively. The waveguide parameter is a dimensionless quantity that determines how many modes can exist in a fibre. For example, in a multimode fibre, $V$ is large ($V > 2.405$). The total number of allowed modes ($M$) in a fibre can be estimated from Equation 3.7 using

$$M \approx \frac{V^2}{2} = \frac{1}{2} \left( \frac{2 \pi a}{\lambda} \right)^2 \left( n_1^2 - n_2^2 \right)$$ \hspace{1cm} (3.8)$$
Chapter 3: Optical Fibre Sensing

For any given mode, the proportion of the power transmitted in the cladding relative to the total power associated with the mode, can be estimated by (Gloge, 1971; Keiser, 2000)

\[
\frac{P_{\text{clad}}}{P} \approx \frac{4}{3\sqrt{M}}
\]  
(3.9)

where \( P \) is the total optical power in the fibre. Since, from Equation 3.8, \( M \) is proportional to \( V^2 \), the relative amount of power travelling in the cladding decreases as \( V \) increases. At the cut-off value for the \( V \)-number associated with a given mode, that mode becomes radiative with all the optical power of the mode existing in the cladding.

When radiation is coupled into a fibre, the modes are excited to varying degrees depending on the transmitting conditions including input cone angle, spot size and axial centralisation. Furthermore, energy can be coupled from guided to radiation modes by phenomena such as microbending and twisting of the fibre, all of which will increase the attenuation of the fibre.

3.1.2. Fibre Construction

The material composition of the core and the nature of the core-cladding interface give rise to two main types of optical fibre: step-index fibre and graded-index fibre. These two types of optical fibre are illustrated in Figure 3.5 (Keiser, 2000). Step-index fibres have a core whose refractive index is uniform throughout and a sudden change (or step) down in refractive index at the cladding boundary. Graded-index fibres have a core refractive index which decreases as a function of the radial distance from the centre of the fibre. Both the step- and graded-index fibres can be divided into singlemode and multimode classes.
As discussed earlier, the core of the step-index fibres, of radius (a) and refractive index (n₁), is surrounded by cladding of lower refractive index (n₂). This can be written as

\[ n_2 = n_1 (1 - \Delta) \]  \hspace{1cm} (3.10)

where \( \Delta \) is a parameter known as the core-cladding index difference or simply the index difference, which is always positive. Values of \( n_2 \) are chosen such that \( \Delta \) is nominally 0.01. Typical values of \( \Delta \) for singlemode fibres range 0.2 to 1 \%, and for multimode fibres range from 1.0 to 3.0 \% (Keiser, 2000). Step-index optical fibres generally comprise a silica glass core and a glass cladding, a silica glass core and a plastic cladding or a plastic core and a plastic cladding (Palais, 1988). In the designing of graded-index (GRIN) fibres, the refractive
index profile of the core generally follows the power law relationship expressed as (Keiser, 2000),

\[
n(r) = \begin{cases} 
  n_1 \left[ 1 - 2\Delta \left( \frac{r}{a} \right)^{\alpha} \right]^{1/2} & \text{for } 0 \leq r \leq a \\
  n_1 \left( 1 - 2\Delta \right)^{1/2} & \text{for } r \geq a 
\end{cases}
\]  

(3.11)

where \( \alpha \) is a dimensionless parameter describing the refractive index profile, which has a typical value of 2 (Palais, 1988), and \( \Delta \) is the dimensionless index difference. For the graded-index fibre, solving the last Equation 3.11 for the usual case in which \( n_1 \approx n_2 \), the scale factor \( \Delta \) becomes

\[
\Delta = \frac{n_2^2 - n_1^2}{2n_1^2} \approx \frac{n_1 - n_2}{n_1}
\]

(3.12)

which is an approximation that reduces to Equation 3.10 for the step-index fibre.

The majority of optical fibres are made of glass consisting of either silica (SiO\(_2\)) or a silicate. The attenuation loss in an all-glass fibre is generally lower than in a plastic-cladded silica or an all-plastic fibre. Silica used in fibres has a refractive index of 1.458 at 850 nm (Keiser, 2000). In order to produce two similar materials that have somewhat different indices of refraction for the core and cladding, either fluorine or various oxides (referred to as dopants), such as B\(_2\)O\(_3\), GeO\(_2\), or P\(_2\)O\(_5\), are added to the silica (Ainslie, 1991). The addition of fluorine or B\(_2\)O\(_3\) decreases the refractive index, whereas doping the silica with GeO\(_2\), or P\(_2\)O\(_5\) increases it.

Plastic (polymer) optical fibres (POF) are often used as a high-bandwidth graded-index fibre link for short-distance applications because of its ductility and large core diameter (1mm) (Garito and Wang, 1998). The core of these fibres is either poly-methyl-methacrylate (PMMA) or a per-fluorinated polymer (PFP) (Ishigure et al., 1997). A summary of some properties of PMMA and PFP plastic optical fibres is given in Table 3.1 (Keiser, 2000). Although they exhibit significantly greater optical signal attenuations than glass fibres, they are tough and durable. The refractive index distribution as well as numerical aperture of graded-index POF is controlled by dopants such as diphenyl sulfide (DPS), benzyl benzoate (BEN), and benzyl n-butyl phthalate (BBP).
Table 3.1: Sample characteristics of PMMA and PFP polymer optical fibres (Extracted from Keiser, 2000)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PMMA POF</th>
<th>PFP POF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core diameter</td>
<td>0.4 mm</td>
<td>0.125 – 0.30 mm</td>
</tr>
<tr>
<td>Cladding diameter</td>
<td>1.0 mm</td>
<td>0.25 – 0.60 mm</td>
</tr>
<tr>
<td>Numerical aperture</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>Attenuation</td>
<td>150 dB/km at 650 nm</td>
<td>60 – 80 dB/km at 650 – 1300 nm</td>
</tr>
<tr>
<td>Bandwidth</td>
<td>2.5 Gb/s over 100 m</td>
<td>2.5 Gb/s over 300 m</td>
</tr>
</tbody>
</table>

3.2. Optical Fibre Sensors

The term sensor is generally used to describe an array of devices that may be employed for the detection and or quantitative determination of almost any physical or chemical and, more recently, biological quantity. One of the most convenient aspects of optical fibres employed as part of a sensor is the fact that the modulated signal can be transmitted from the sensing region without recourse to electrical connection (Culshaw, 1988; López-Higuera, 1998). Although optical fibres are fragile, they are small in size, low in weight and easy to manipulate. Optical fibre sensors (also called optrodes) can readily be used in a distributed array with a single remote monitoring station. Other desirable characteristics of optical fibre sensors include their dielectric properties, their immunity from electromagnetic interference and environmental noise, resistance to corrosion due to water or a wide range of commonly-encountered chemicals (for example organic solvents), often a superior sensitivity compared to equivalent electrical sensing techniques, their low cost and the ease of installation afforded by their flexibility geometry (for example Giallorenzi et al., 1982; Lines, 1984; Palais, 1988; McStay et al., 1995).

Optical fibre sensors range from the very simple to the very complex. Simple break-point sensors such as the temperature sensor depicted in Figure 3.6, simply utilise the optical path between two fibres which are placed in end-on contact (or butt-coupled) (Rogers, 1999). The exiting light level depends upon the alignment of the two fibre ends, and their alignment is controlled by the external temperature via the bending of bi-metallic strips.
As illustrated by the break-point sensor, common to any optical fibre sensor is a light source, a link of optical fibre and an optical detector. These are shown in Figure 3.7 (Roef, 1987). Depending on the type of sensor, singlemode laser, multimode laser, LEDs and continuous light sources are employed as a source. An optical fibre link carries light to a modulating region in which an external parameter is caused to interact with this light and modify some optical property of the incident radiation. This property, which is modified includes its intensity, its optical phase, its optical frequency, its state of polarisation or its wavelength distribution.

Optical fibre sensors are generally classified as intrinsic sensors and extrinsic sensors (Roef, 1987; Grattan and Ning, 1998). For an intrinsic optical fibre sensor, the measured parameter directly alters some physical features of the fibre and the sensing action takes place within
the fibre itself. The radiation does not leave the fibre. In an extrinsic sensor the radiation leaves the fibre, interacts with an optical sensing element or the medium being observed, and eventually returns to a detector via the same fibre or different collection fibre. A generalised schematic diagram of intrinsic and extrinsic sensors is given in Figure 3.8.

![Diagram of intrinsic and extrinsic sensors](image)

**Figure 3.8: Schematic comparison between (a) intrinsic, and (b) extrinsic sensor methods (Extracted from Grattan and Ning, 1998).**

### 3.2.1. Intrinsic Sensors

Intrinsic sensors use the fibre properties as the sensing element and are also known as active sensors. In interferometric sensors, changes in the phase of radiation propagating through an optical fibre immersed in an appropriate perturbing environment can be determined by using two fibres; one acting as the sensing fibre beam and the other as a reference fibre. Light from an original single fibre can be split into sensing and reference fibres and then recombined, post-interaction, using Y-splitters or directional couplers. (for example Priest *et al.*, 1997; Chtcherbakov *et al.*, 1998; Kaddu *et al.*, 1999).

An optical-fibre Mach-Zehnder interferometer is the most common intrinsic interferometer sensor used and a schematic of the fibre assembly is depicted in Figure 3.9 (Kersten, 1988; Rogers, 1997). Radiation is coupled from an appropriate monochromatic source such as a
laser or laser diode into one of the fibres of the directional coupler (DC1). The fibres are single mode. The sensing fibre is subject to an alteration of the length and/or refractive index and the reference fibre is held in an environment whereby it is insulated from the same perturbations. The radiation from the two fibres are recombined and brought to interference in the second directional coupler (DC2). Changes in phase of the sensing fibre compared to the phase of the reference fibres produce modulation of the signal detected at the photodetector.

![Diagram of an optical fibre sensing system](image)

Figure 3.9: The Diagram of intrinsic Mach-Zehnder fibre optic sensor. The light is split by directional coupler [DC (1)], and after passing through both arms, it is recombined by DC (2). The resultant interference pattern can be observed at either one or both of the DC (2) outputs. (Extracted from Kersten, 1988; Rogers, 1997).

The phase change of radiation $\varphi$, travelling a distance $L$, in an optical fibre (e.g. Lee and Taylor, 1995), is expressed by

$$\varphi = \frac{2\pi n L}{\lambda} \quad (3.13)$$

where $n$ = the refractive index of optical fibre, and $\lambda$ = the wavelength of the light wave. A phase change in the sensing fibre, resulting from either a change in fibre length or refractive index, is expressed by

$$\Delta \varphi = \frac{\delta \varphi}{\delta L} \Delta L + \frac{\delta \varphi}{\delta n} \Delta n \quad (3.14)$$

Using Equation 3.13, Equation 3.14 becomes (Yoshino et al., 1982)
\[ d\phi = \frac{2\pi}{\lambda} (n \, dL + L \, dn) \]

or

\[ d\phi = \frac{2\pi n L}{\lambda} \left( \frac{1}{L} \, dL + \frac{1}{n} \, dn \right) \quad (3.15) \]

If, for example, the sensing fibre is subjected to a change in temperature, \( \Delta T \), a change in the length of the sensing fibre because of thermal expansion or contraction, and the change of the refraction index because of the temperature dependence of refractive index, will occur. Accordingly, Equation 3.15 can be rewritten (Giallorenzi et al., 1982; Jackson and Jones, 1989; Jones, 1997)

\[ \frac{d\phi}{dT} = \frac{2\pi}{\lambda} \left[ n \, \frac{dL}{dT} + L \, \frac{dn}{dT} \right] \quad (3.16) \]

or

\[ \frac{1}{L} \frac{d\phi}{dT} = \frac{2\pi}{\lambda} \left[ n \, \frac{dL}{dT} + \frac{dn}{dT} \right] \quad (3.17) \]

For a fused silica fibre \((n = 1.456)\) and a He-Ne laser wavelength of 632.9 nm, the temperature-coefficients of length and refractive index (Hocker, 1979; Priest et al., 1997) are respectively

\[ \frac{1}{L} \frac{dL}{dT} = 0.5 \times 10^{-5} \cdot \degree C^{-1}, \]

and

\[ \frac{1}{n} \frac{dn}{dT} = 6.9 \times 10^{-6} \cdot \degree C^{-1}. \]

Therefore, Equation 3.17 gives

\[ \frac{1}{L} \frac{d\phi}{dT} = 107 \text{ radians/} \degree \text{C m}^{-1} \quad (3.18) \]

Because 1 fringe is equal to \(2\pi\) radians, the value of 107 radians \((\degree \text{C m})^{-1}\) corresponds to 17 interference fringes per \(\degree \text{C}\) per metre of fibre, and various researchers have experimentally verified this theoretical value (for example Hocker, 1979; Peterson and Yariv, 1966).

Optical fibre Mach-Zehnder interferometers have been used in applications ranging from measuring gas pressure and temperature (Hocker, 1979; Haruna et al., 1985; Beheim et al.,
1987; Okamoto and Yamaguchi, 1988; Iwamoto and Kamata, 1990), the velocity of moving gases and liquids (Woolsey et al., 1991; Lamb and Woolsey, 1995, Meggitt et al., 1990) and the presence of acoustic waves, magnetic fields, and stress and strain in solids (Grattan and Ning, 1998).

Figure 3.10: Schematic diagram of the set-up white-light intrinsic FFPI sensor (Extracted from Kaddu et al., 1999).

Optical fibre Fabry-Pérot interferometers (FFPI), an example of which is depicted in Figure 3.10, have similarly been employed in the sensing of temperature, strain, and ultrasonic pressure in composite materials (Yoshino et al., 1982; Lee and Taylor, 1995; DeMerchant et al., 2000). An example of the complexity of such optical apparatus is that of Kaddu et al. (1999) who used multiplexed fibre-optical sensors for measuring the temperature and strain.

As depicted in Figure 3.10, light from a multimode laser diode, of centre wavelength 810 nm and coherence length about 150 µm, is launched into the interferometer through a 2 x 2 directional coupler (DC1). The two reflections from the fibre Fabry-Pérot mirrors enter the second (receiver) interferometer via another directional coupler (DC2). Light launched from
the fibre end is collimated by a lens (L) and, following reflection from a mirror (M), returns via this lens into the same fibre.

The mirror (M) is mounted on a translation stage driven by a computerised precision driver with a 25 mm operating range and a 50 nm minimum displacement step. Scanning the receiver interferometer varied the optical path differences between the two interferometers and the fringe pattern produced at the photodetector are digitised and recorded on-line. The index-matching liquid is generally used for eliminating unwanted reflections. The temperature of 20 mm of the sensing fibre was varied using a miniature oven and monitored by a semiconductor transducer having 0.1 °C precision (Kaddu et al., 1999).

![Graph](image)

**Figure 3.11:** The shift in the centre of the central fringe of the white light interferometry pattern against temperature for a Fabry-Perot interferometer sensor of approximately 20 mm Length (Extracted from Kaddu et al., 1999).

Kaddu et al. (1999) found that the displacement of the central fringe increased linearly with temperature over a range of 50 °C, as depicted in Figure 3.11, with a temperature coefficient of 223 ± 3 nm K⁻¹.
3.2.2. Extrinsic Sensors

An optical fibre-based extrinsic sensor uses the optical fibre simply to carry radiation to a sensing element, collect, and send the perturbed radiation to a photodetector. Extrinsic sensors are also referred to as passive sensors (Jones, 1990). The performance of the extrinsic sensor is independent of the optical fibre(s) and depends only on the properties of the sensing element (Smith, 1988; and Ning et al., 1995). Consequently, extrinsic sensors are used in a much greater range of applications than intrinsic sensors. Optical spectroscopic techniques related to absorption, transmission or reflection of radiation from a sample are particularly amenable to incorporation with optical fibres.

![Diagram of extrinsic optical-fibre absorption sensor](image)

**Figure 3.12:** (a) An extrinsic optical-fibre absorption sensor. Radiation is launched into the transparent immobilised reagent and absorbed by it. The reflected light is coupled and transmitted by the same or a different fibre to a detector. (b) An optical fibre pH sensor based on the use of a dye-indicator (Extracted from Roef, 1987).

A schematic diagram of a simple extrinsic optical-fibre absorption sensor is shown in Figure 3.12 (a). Radiation is guided by an optical fibre or fibre bundle to an appropriate "reactant" whose absorption characteristics changes in response to variations in concentration. The same fibre(s) or another optical fibre or fibre bundle is then used to carry the perturbed radiation to optical detectors. An optical fibre pH sensor is depicted in Figure 3.12 (b)
Chapter 3: Optical Fibre Sensing

(Peterson et al., 1980; Roef, 1987). This sensor relies on the use of a dye indicator (phenol red or phenolsulfon-phtalein). In the pH range of interest, phenol red behaves like a weak acid and has two tautomeric forms of different light absorbing characteristics: green absorbing (base form) and blue absorbing (acid form).

The probe itself is constructed by a hydrophilic gel structure of polyacrylamide microspheres covalently bound to the dye phenol red for providing a fixed concentration, and containing also smaller polystyrene microspheres for light scattering. These are packed in an envelope of cellulose hydrogen-ion-permeable dyalisis tubing, at the end of a pair of large-NA plastic fibres of 0.15 mm diameter. The radiation scattered by the polystyrene microspheres on entering the probe is absorbed by the dye and returned to the other optical fibre for transmission to the measuring apparatus. As the pH of the solution changes, the heights of the absorption peaks alter in proportion to the changing relative concentrations of the acid and base forms of the dye. Therefore, the pH of the dye solution can be measured by measuring the amplitude of one of the absorption peaks of the dye solution, while the other absorption peak is used as a reference signal. This particular probe was originally designed for measuring tissue and blood pH (range 7.0 to 7.4) and yields a measurement accuracy of 0.01 pH unit (Roef, 1987).

However, in the context of optical absorption spectroscopy, care must be exercised when applying Beer's Law in calculating absorption properties as there is some divergence of the light beam emerging from optical fibres and the collection fibre is unable to gather all the transmitted or reflected light. These effects tend to complicate the sensing of the transmitted/reflected radiation. For example, in a system consisting of an illumination fibre with radius \( r_1 \) and collecting fibre of radius \( r_2 \) (\( r_2 < r_1 \)), and a distance \( z \) in an absorbing medium between the illumination and detection fibre, the collected light intensity can be expressed to a first order approximation using (Smith, 1988)

\[
I(z) = I(0) \left( \frac{r_2}{r_1 + z \tan \phi} \right)^2 e^{-\alpha z} \tag{3.19}
\]

where \( \phi \) is the divergence angle of the light emerging from the end of the input fibre, and \( \alpha \) is the absorption coefficient.
In another example illustrating the utility of multiplexed sensors, Dybko et al. (1998) designed a multiparameter fibre optic absorption probe appropriate for on-line monitoring of drinking water, where the three major parameters measured were pH, temperature and the concentration of calcium ions (a cause of water "hardness"). Light emitting diodes were used as the probing sources with wavelengths 560 nm for the calcium sensor, 630 nm for pH sensor and 650 nm for temperature sensor. Multimode fibre optic bundles were employed to guide the perturbed radiation to the respective photosensors.

A considerable number of optical-fibre sensors are based on detecting fluorescence or luminescence intensity. For example, one form of pH sensor employs immobilised fluoresceinamine, the fluorescence characteristics of which varies with pH and/or metal ion concentration. The fluoresceinamine can be immobilised on controlled-pore glass and on cellulose (Saari and Seitz, 1982). However, the existence of fluoresceinamine does not influence the pH. Saari and Seitz (1982) demonstrated that the fluorescence from the immobilised fluoresceinamine probe increases significantly with changes in pH of 3 to 6.

In another example of an optical fibre pH sensor based on fluorescence intensity, fluoresceinamine is incorporated into an acrylamide-methylenebis (acrylamide) copolymer which is attached covalently to the polished end of the fibre via thermal- or photo-polymerisation (Munkholm et al., 1986). This fibre optic pH sensor provides an instantaneous and reversible measurement over the pH range of 4.0 – 8.0 with signal-to-noise ratios typically 275/1.

In a final example, Coumarin-4 is a laser dye which can be employed as a humidity indicator since photoluminescence in coumarin-4 dye is dependent on humidity (Takahashi et al., 1998). In order to incorporate coumarin-4 into an optical fibre sensor, the dye is doped into a sol-gel silica matrix coating which is deposited as a thin film on a fibre (Takahashi et al., 1998).

Merchant et al. (1997) employed an optical fibre sensor, designed for the continuous detection of fluorescent emissions from liquids, for measuring the total toxicity of an aqueous sample containing colonies of micro organisms (bacteria, algae or similar) exposed
to a solution containing fluorescein diacetate (FDA) (C₂₉H₁₆O₂⁻ Sigma F7378). The apparatus comprised an optical fibre to carry excitation radiation from the radiation source to the sample and, in conjunction with some collimating optics, an optical fibre to collect the excitation radiation and carry it to a photomultiplier. By optically measuring the fluorescein levels using the optical fibre sensor, it was possible to estimate colony size and hence continuously quantify the toxicity of the aquatic system.

An example of an extrinsic Fabry-Pérot interferometric (EFPI) sensor that employs a cavity formed by the fibre end and a membrane located beyond the fibre end is depicted in Figure 3.13 (Lee and Taylor, 1995). The reflectance from the interferometer alters in response to a motion of the membrane that affects the length of the external cavity. Typical separation between the fibre end and the membrane is of the order of 1 μm. Due to its short cavity lengths, these sensors generally employ multimode fibres and a low-coherence light-emitting diode as the light source. Sensors of this type have been used to measure temperature, pressure, and refractive index of liquid.

![Figure 3.13: Extrinsic FFPI sensor with a cavity formed by the air gap between the fibre end and a membrane (Extracted from Lee and Taylor, 1995).](image)

The EFPI sensor is one of the most widely-used fibre optic sensors. The EFPI is an interferometric sensor in which the detected intensity is modulated by the parameter under measurement. The simplest configuration of an EFPI is depicted in Figure 3.14 (Bhatia et al., 1996), and consists of a singlemode laser diode illuminating a Fabry-Pérot cavity via a fused biconical tapered coupler. The cavity is formed between an input singlemode fibre and
a reflecting singlemode or multimode optical fibre. A hollow core silica fibre is used for aligning the input fibre and the reflecting fibre.

Figure 3.14: The simplest configuration of the extrinsic Fabry-Pérot interferometric (EFPI) sensing system (Extracted from Bhatia et al., 1996).

In Figure 3.14, \( R_1 \) is the reference reflection, which is independent of the applied perturbation, while \( R_2 \) is the sensing reflection depending on the length of the cavity, \( d \), which itself is modulated by the applied perturbation. These two reflections interfere (provided \( 2d < L_c \), where \( L_c \) is the laser diode's coherence length) and the intensity \( I \) at the detector varies as a function of the cavity length (Bhatia et al., 1996):

\[
I = I_o \cos \left( \frac{4\pi d}{\lambda} \right) \tag{3.20}
\]

where \( I_o \) is the maximum value of the output intensity and \( \lambda \) is the laser diode wavelength.

When the change of the cavity length (\( \Delta d \)) owing to the applied perturbation is measured, the strain may be calculated by means of (\( \Delta d / L \)), where \( L \) is the gauge length of the sensor. Gauge length is defined as the distance between the points on the reflecting and input fibres that are bonded to the hollow core tube.
Extrinsic Fabry-Pérot interferometric sensors have been employed in applications ranging from measuring fatigue loading on F-15 aircraft wings (Murphy et al., 1991), detection of crack formation and propagation in civil structures, and measuring electric fields (Priest et al., 1997a). In this latter example, Priest et al. (1997a) were able to measure electric fields in the range 135 – 650 V/cm, in the vicinity of a Van de Graaff generator. This particular device was based on a pressure sensor whereby the reflective surface flexes in response to electrostatic charging. This sensor is depicted in Figure 3.15.

![Singlemode fibre with cleaved end and reflected surface](image)

*Figure 3.15: Principle of the extrinsic optical FFPI for electric-field measurement (Extracted from Priest et al., 1997a).*

Although EFPI sensors have a potential for measuring displacements in order of $10^{-10}$ m, limitations of EFPI sensors include the fact that the change in output intensity is nonlinear in response to the perturbing parameter, and the system is unable to detect a change in direction of the local strain or any other perturbation that occurs at the maxima or minima of the sinusoidal transfer function curve.

In recent years many methods have been proposed to overcome the differential nature of such interferometers, including the dual wavelength technique (Wang et al., 1995) and white light tandem interferometry (Chen et al., 1991). The stabilisation of the centre wavelengths of the two optical sources for dual wavelength schemes is very critical and even small drifts can cause great errors in the output. On the other hand, tandem interferometry relies on the use of scanning or reference interferometer to demodulate the information transmitted by the sensing interferometer. The reference interferometer should be extremely stable and therefore needs to be shielded from changes in ambient conditions.
3.3. Evanescent Sensors

One particular type of extrinsic optical fibre sensor, which is central to the subject of this thesis, is based on the optical absorption of the evanescent field at the core-cladding interface of an optical fibre. This group of sensors are generally referred to as Fibre-optic based Evanescent Field Absorption (FEFA) sensors (Paul and Kychakoff, 1987; Katz et al., 1991; Messica et al., 1996).

3.3.1. The Evanescent Field

The general process of total internal reflection of radiation along the internal core-cladding interface of an optical fibre has been previously described (Section 3.1.1). At the point of reflection at the core-cladding boundary, depicted in Figure 3.16, optical interference between the incident and reflected rays produces a standing wave. Although all the power is reflected from the core-cladding interface back into the core, the electric field associated with the standing wave penetrates some distance into the cladding, diminishing with distance from the boundary. This field, which carries no energy away from the interface (Stewart et al., 1991; Dakin et al., 1997) is termed the evanescent field.

![Evanescent Field Diagram](image)

Figure 3.16: An evanescent field is generated from total internal reflection. An oscillatory standing wave is set up in the medium of higher refractive index, \(n_1\) (fibre core) and decays exponentially into the medium of lower refractive index, \(n_2\) (fibre cladding) (Extracted from Smith, 1988).
The evanescent field decays exponentially in z direction according to the expression (DeGrandpre and Burgess, 1988)

\[ E = E_0 e^{-\beta z} \]  

(3.21)

where \( z \) is the distance normal to the core interface, \( \beta \) is the attenuation factor or attenuation coefficient (Palais, 1988; DeGrandpre and Burgess, 1988; and Bürck et al., 1997), which is expressed as:

\[ \beta = k_c \sqrt{(n_i^3 \sin^2 \theta - n_f^2)} \]  

(3.22)

and \( k_c \) is the free-space propagation factor \((2\pi/\lambda)\). At the critical angle for internal reflection \((\sin \theta_c = n_2/n_1)\), \( \beta = 0 \) and the evanescent field penetrates into the cladding without attenuation. If \( \theta \) increases beyond \( \theta_c \), \( \beta \) increases and the evanescent field decays more strongly with penetration distance into the cladding. The depth of penetration, \( d_p \), is defined as the distance over which the evanescent field is reduced to 1/e of its interface-value, and can be calculated from the solution of Maxwell’s equations (Harrick, 1967) at the interface given by

\[ d_p = \frac{\lambda}{2\pi (n_i^2 \sin^2 \theta - n_f^2)^{1/2}} \]  

(3.23)

where \( \theta \) is the angle of incidence at the interface \((\theta > \theta_c)\), and \( \lambda \) is the free-space wavelength of the radiation. Equation 3.23 can be rewritten in terms of the critical angle, \( \theta_c \), to yield

\[ d_p = \frac{\lambda}{2\pi n_i (\sin^2 \theta - \sin^2 \theta_c)^{1/2}} \]  

(3.24)

which, in terms of numerical aperture (NA) becomes

\[ d_p = \frac{\lambda}{2\pi (NA^2 - n_i^2 \cos^2 \theta)^{1/2}} \]  

(3.25)

In a typical case, where the core refractive index of an optical fibre, \( n_1 \), has a value of about 1.50, and the environment under investigation is aqueous with \( n_2 \) approximately 1.33, the depth of penetration ranges from approximately \( \lambda/5 \) at an incident angle of close to 90°, to approximately \( \lambda \) for an incident angle within 1° of the critical angle (Smith, 1988; Harmer and Scheggi, 1989). While Equation 3.24 suggests that the depth of penetration is infinite at the critical angle, virtually no radiation is incident at the critical angle because of light leakage and scattering resulting from the "coarseness" of the interface surface.
3.3.2. Fibre-Optic Evanescent Field Absorption (FEFA) Sensors

A sensor based on evanescent field absorption relies on the interaction of a target substance with the evanescent field adjacent to the fibre core. A generalised schematic diagram of the evanescent-field optical fibre sensor is given in Figure 3.17. Removing the cladding from a portion of an optical fibre permits the evanescent field to interact with the substances within which the fibre is immersed. This interaction, which involves the process of optical absorption, will result in attenuation of the intensity of the radiation travelling down the fibre core (Gupta and Singh, 1994; Stewart et al., 1997). The alteration of this intensity may be measured and associated with the concentration of the material in contact with the fibre core surface.

![Diagram of fibre-optic sensor with cladding removed](image)

**Figure 3.17: Basic configuration of fibre-optic based evanescent field absorption (FEFA) sensor.**

The resulting evanescent field absorption spectra are actually attenuated total (internal) reflection spectra, which resemble those of conventional absorption spectra although they are complex functions of absorption and refractive index. Light attenuation in the evanescent field can be approximated using the equation of DeGrandpre and Burgess (1988a and 1990):

\[
\log \left( \frac{I_o}{I} \right) = \varepsilon_c L_c + \log \left( \frac{NA_o^2}{NA^2} \right)
\]  

(3.26)

where \(I_o\) and \(I\) is the transmitted light intensity with the sensor in air or pure water and analyte solution, respectively, \(\varepsilon_c\) is the effective absorptivity, \(L\) is the length of the sensor.
Chapter 3: Optical Fibre Sensing

fibre, \( c \) is the molar analyte concentration, and \( NA_o \) and \( NA \) are respectively the numerical aperture of the optical fibre sensor in air and the analyte solution, which are determined by Equation 3.27 (DeGrandpre and Burgess, 1990; Bürck et al., 1992):

\[
NA_o = \sqrt{n_1^2 - n_2^2} \quad \text{and} \quad NA = \sqrt{n_1^2 - n_m^2}
\] (3.27)

In Equation 3.27, \( n_1 \) and \( n_2 \) are the refractive indexes of fibre core and cladding, respectively, and \( n_m \) is the refractive index of the external matrix (cladding plus solvent).

The first term of Equation 3.26, is similar to Beer's law (Skoog and Leary, 1992; Payne and Hale, 1993). However, the effective absorptivity, which is defined as \( \varepsilon_e = \eta_m \varepsilon_m \), is dependent on \( \eta_m \), where \( \eta_m \) is the ratio of light intensity in the evanescent field to the total light intensity transported in the fibre core, and \( \varepsilon_m \) is the molar absorptivity of the analyte species. Since \( \eta_m \) is strongly influenced by refractive index changes in the surrounding analyte solution, \( \varepsilon_e \) cannot be considered as constant, especially at higher analyte concentrations (Bürck et al., 1994).

The second term of Equation 3.26 details the influences of refractive index due to variations in the numerical aperture of the optical fibre sensor caused by analyte diffusion into the cladding of the unmodified sections of the fibre and leads to shifts in the absorbance baseline. However, this particular effect of refractive index changes in the polymer cladding can only be found at relatively high analyte concentrations.

DeGrandpre and Burgess (1988) found that the power distribution is inversely proportional to the normalised frequency, \( V \) (introduced earlier in Equation 3.7), and follows:

\[
\eta_m = \frac{k}{V}
\] (3.28)

where, for the effective absorptivity, \( \eta_m \) can be written as \( P_m/P_s \), where \( P_m \) is the evanescent field intensity in the surrounding medium and \( P_s \) is the total light intensity in the fibre core; and \( k \) is proportionality constant.

Because of their smaller core diameter, the fraction of power propagating in the cladding is higher for singlemode fibres. However, in practise issues relating to mechanical strength precludes the use single-mode fibres in evanescent-wave sensing. Multimode optical fibres
are more widely employed in evanescent-field for sensing. Optical fibres are typically fabricated in order that the electromagnetic radiation travels mostly in the core and the cladding is sufficiently thick so that the evanescent field does not interact with the outside world. However, by appropriately modifying the cladding in a length of fibre by removal (discussed earlier) or by doping it with a sol-gel, or by modifying the fibre core geometry through tapering, it is possible to significantly increase the sensitivity of evanescent-field absorption to a surrounding environment (MacCraith, 1993; Blue and Stewart, 1995; Mignani et al., 1997; Falciati et al., 1997 and 1998).

The relative transmittance function of the light launched into a FEFA sensor has been modelled by Messica et al., (1994 and 1996) using three-dimensional model for a evanescent-wave spectroscopy system, which involves the different launching condition, input and output Fresnel transmission, multiple Fresnel transmission, and bulk and evanescent-wave absorption. If \( I_0 \) is the intensity transmitted by the unclad FEFA sensor in the absence of absorbing fluid, the transmitted intensity in the presence of an absorbing fluid is then given by (Gupta and Singh, 1994)

\[
I = I_0 e^{-\gamma L}
\]  
(3.29)

where \( L \) is the length of the sensing part of the fibre, and \( \gamma \) is the evanescent-absorption coefficient of the bulk absorbing fluid or media surround the core. The sensitivity of an optical fibre evanescent field absorption sensor therefore depends on \( L \) and \( \gamma \). As in Equation 3.30, \( \gamma \) depends on physical fibre parameters such as the fibre core diameter, the refractive indices of fibre core and cladding (or medium what surrounds the core), the bulk absorption coefficient of surrounding medium, and the nature of the light source (Ruddy et al., 1990; Gupta and Singh, 1994).

\[
\gamma = \frac{\alpha c n_2 \lambda_0 \cos \theta \cot \theta}{2\pi d n_1^2 \cos^2 \theta_c \sqrt{\sin^2 \theta - \sin^2 \theta_c}}
\]  
(3.30)

where, as in Section 2.2.1, \( \alpha \) and \( c \) are the absorptivity and the concentration of absorbing medium, respectively. While as in Section 3.3.1, \( \lambda_0 \) is the free-space wavelength of the radiation, and \( d \) is the diameter of optical fibre core, \( \theta \) and \( \theta_c \) are the angle of incidence at the interface and critical angle, respectively, \( n_1 \) and \( n_2 \) are the refractive index of fibre core and absorbing medium surrounding the core, respectively. Furthermore, by using Equations
3.30 and 3.29, Equation 2.10 (Section 2.2.1) can be modified for calculating the FEFA absorbance (D) of absorbing medium surround the fibre core:

\[
D = \frac{\alpha c L \lambda_2 \cos \theta \cot \theta}{2\pi d n_1^2 \cos^2 \theta_c \sqrt{\sin^2 \theta_c - \sin^2 \theta}}
\]  

(3.31)

Numerous researchers have modified the difference in refractive index between core and cladding by using porous glass coatings fabricated by the sol-gel process (Mukherjee, 1980; Sakka and Kamiya, 1982; Zusman et al., 1990). The use of sol-gel-derived thin films, particularly in an evanescent wave configuration, offers many advantages for sensor optimisation in terms of sensitivity, response time and signal to noise ratio via control of sol-gel film properties such as coating length, thickness and porosity (Mac Craith, 1993). Changing the geometrical form of the optical fibre, for example by tapering the fibre core is another technique used to enhance the performance of FEFA sensors (Gupta and Singh, 1994; Mignani et al., 1997; Falciai et al., 1997 and 1998).

Polymer claddings on silica fibres such as siloxane polymers (RMesio)n are generally used because of their low refractive index (n ≈ 1.44), and good transmittance characteristics from the ultraviolet (UV) to near-infrared (NIR) wavelengths. Furthermore, since many non-polar organic solvents readily swell siloxane polymer, these fibres can often be used as an evanescent field sensor with the cladding intact since an absorber can be carried by a solvent into the polymer where it can interact with the evanescent field. Unlike exposed-core FEFA sensors, siloxane-clad FEFA sensors are more durable and resistant to chemical degradation and fouling, and because of their superior mechanical strength, can be more easily coiled into a compact sensor.

FEFA sensing using multimode optical fibres has been used for a range of applications including the detection of organic gases in water (Tai et al., 1987; Culshaw et al., 1992; Jin et al., 1995; Taga et al., 1994; and Bunimovich et al., 1995), in other liquids (Harrick, 1967; Paul and Kychakoff, 1987; Simhony et al., 1988; B"urck et al., 1994; Kastner et al., 1996; Sensfelder et al., 1996; Mignani et al., 1997; Schwotzer et al., 1997; Falciai et al., (1997 and 1998); and Mizaikoff et al., 1999), and the presence of solid-phase substances (Woolsey et al., 1996; Vukovic, 1997; Irawan, 2000). By way of illustrating the many and varied
methods available for optimising the sensitivity of FEFA apparatus to suit a particular measurand, a number of specific examples are discussed in the following paragraphs.

Numerous workers have investigated FEFA sensors coated with siloxane polymers, which concentrate organic substances from aqueous solutions or the gas phase. The use of polydimethylsiloxane-coated silica fibres for evanescent-wave absorption sensors have been developed for the detection of vapour-phase organic compounds (Ronot-Trioli et al., 1995; Schwotzer et al., 1997), and liquid hydrocarbons (Tobiška et al., 1998; Bürck et al., 1992 and 1994, Sensfelder et al. 1996).

In their liquid hydrocarbon experiments, Tobiška et al. (1998) employed a straight polymer optical fibre (core and cladding diameters of 400 μm and 500 μm, respectively) with a 10-cm length of exposed core. They also used a "bent" fibre (diameter of core and cladding 200 μm and 380 μm, respectively) wound on a mandrel (diameter 15 or 25 mm) to give effective exposed lengths of 0.2 - 8.0 m.

Evanescent wave absorption features in pure water have been studied by Simhony et al. (1988) using silver halide optical fibres capable of transmitting mid infrared radiation. The apparatus depicted in Figure 3.18 comprises an optical fibre of 0.9 mm diameter placed on a microscope slide. Thin barriers of epoxy cement were placed at different intervals along the fibre to create a series of individual compartments, each of length, L. The incident angle θ and the total length L = ΣL of the absorbing liquid (in this case, water) could be varied. The assembled fibre cell was placed in the sample chamber of a FTIR spectrometer ( Nicolet model 5DX or 5ZDX). Two Φ1 ZnSe lenses, of 25 mm focal length, focused the FTIR beam onto and out of the fibre end faces. The system was aligned by adjusting the position of the lenses and the cell until maximum energy throughput was obtained. The IR absorption spectrum of water from 800 to 4400 cm⁻¹, for various fibre immersion lengths are depicted in Figure 3.19. Figure 3.19 illustrates that increasing the fibre immersion length increases the overall strength of the absorption.
Visible-wavelength FEFA sensing of an aqueous dye solution (methylene blue) was performed by Ruddy et al. (1990) in a concentration range $3 \times 10^{-8}$ to $5 \times 10^{-6}$ M using a multimode fused silica fibre. A schematic diagram of the optical system is illustrated in Figure 3.20. Ruddy et al. (1990) used the output of a 50 W tungsten halogen source, with enhanced blue output, coupled to a $f/4$ monochromator with a 1-mm-diameter exit port. An annular beam mask was used for collimating and spatially filtering the emerging beam that
was subsequently launched into 200 or 600 μm plastic-clad silica fibres of numerical aperture 0.4 using a 0.65 NA objective lens. A Hamamatsu R1546 photomultiplier was employed to detect the transmitted optical power.

![Diagram of optical system and optical mask](image)

**Figure 3.20:** Schematic diagram of the optical system and the optical mask used by Ruddy *et al.*, 1990.

A typical evanescent-wave spectrum of aqueous methylene blue dye recorded by Ruddy *et al.* (1990) is depicted in Figure 3.21. The values of absorbance were obtained in the case of aqueous dye solutions by referencing each spectrum against the spectrum attained with deionised water alone in the sample chamber. The spectrum shows two obvious peaks; one at about 664 nm corresponding to the monomer form of the molecule, and the second at approximately 609 nm is attributable to dimer formation (Ruddy *et al.*, 1990).
Figure 3.21: The evanescent absorbance spectrum of methylene blue (5 x 10⁻⁷ M) measured using 22 cm of unclad 600 μm fibre (Extracted from Ruddy et al., 1990).

Figure 3.22: Variation of evanescent absorbance with unclad fibre length for constant methylene blue dye concentration at wavelength 664 nm (Extracted from Ruddy et al., 1990).
Figure 3.22 shows the linear relationship between the evanescent absorbance at a wavelength 664 nm and length of exposed fibre core immersed in the sample chamber containing a fixed concentration of methylene blue solution.

This linear relationship was also observed in methylene blue solutions by Villarruel et al. (1987), and in numerous other fluids, including rhodamine-6G in a non-polar organic solvent (Paul and Kychakoff, 1987), water using silver halide optical fibres (Simhony et al., 1988), toluene using different polymer-cladded fibres (Schwotzer et al., 1997), and solutions of cobalt-chloride hexahydrate salt in 2-propanol 0.05 mol/L (Mignani et al., 1997).

The use of FEFA techniques compared to traditional light beam absorption techniques for measuring the optical absorption of a liquid offers numerous advantages and disadvantages. In the following chapters, these two techniques will be compared in the context of measuring the colour, via optical absorption, of water containing suspended material. A detailed insight into the working of the FEFA techniques will also be obtained through the development of a theoretical “skip-length” model and the model subsequently used to conduct a parametric investigation of the experimental apparatus.
Chapter 4

Measurement of Chlorophyll Pigments in Turbid Water Using the Direct-Absorption Technique

4.1. Introduction

In this Chapter, the materials, optical apparatus and procedures used for measuring chlorophyll-related pigments in turbid water by a traditional direct-absorption technique are described. This includes preparation of standard samples of chlorophyll-related pigments and sediment for subsequent suspension in water. The absorbance spectra acquired are then presented and their characteristics discussed.

4.2. Preparation of Standard Samples

4.2.1. Chlorophyll Pigments

In order to ascertain the sensitivity of the direct-absorption apparatus for measuring chlorophyll-related pigments in turbid water, a protocol for preparing and then measuring standard samples of chlorophyll-related pigments was developed.
Chlorophyll-related pigments for the use as the standards were extracted from plant samples by rupturing the cells using ultrasound sonication (Simon and Helliwell, 1998). A sample of "domestic" spinach (Figure 4.1 (a)) was cut into very small pieces (2 – 4 mm²) (Figure 4.1 (b)), placed in a 100 mL ultrasound sonication vessel and covered with 90% (v/v) acetone (V₁) (Figure 4.1 (c)). Due to ultrasonic heating of the liquid, the sonication vessel was kept cool by placing it in a beaker containing ice. The sample was sonicated for 10 minutes using a 25-mm tipped probe (Sonifier 450, Branson Ultrasonics, Dandury, CT, USA) (Figure 4.1 (d)).

Figure 4.1: Photograph of (a) the "domestic" spinach, (b) small pieces of cutting spinach, (c) small pieces of cutting spinach placed in a 100 mL ultrasound sonication vessel containing 90% (v/v) acetone, (d) Sonifier 450, and (e) a sample of chlorophyll-related pigments extracted from the "domestic" spinach.
Chapter 4: Measurement of Chlorophyll Pigments in Turbid Water Using the Direct-Absorption Technique

The output controller of the Sonifer cell disruptor (Model 450) was set for continuous output at approximately 19 W/cm². The sonicated sample was then filtered using a 47 mm Whatman GF/F glass-fibre filter, and the volume of the clear extract was recorded (V₂) (Figure 4.1 (e)). Approximately 3 mL of the extract was placed in a 1-cm path-length cuvette (L) for measurement of optical absorbance at 664 nm (664ₚ) and at 750 nm (750ₚ), respectively using the Novaspec II (Pharmacia Biotech) laboratory spectrophotometer, as depicted in Figure 4.2. The sample was then acidified with 0.1 mL of 0.1N hydrochloric acid (HCl) and the optical absorbance re-measured at 665 nm (665ₚ) and at 750 nm (750ₚ), respectively.

![Photograph of the Novaspec II (Pharmacia Biotech) laboratory spectrophotometer used to verify the chlorophyll concentration in the standard samples.](image)

The measured value of absorbance at 664 nm before acidification (664ₚ) was checked to ensure a value between 0.1 and 1.0. Where the absorbance value was greater than 1.0, the extracted sample (i.e. 1 mL) was diluted with 90% (v/v) acetone prior to measurement. Where employed, this subsequent modification was included in calculation of chlorophyll-related pigment concentration (Equation 4.1) from the absorption measurement.
Chapter 4: Measurement of Chlorophyll Pigments in Turbid Water Using the Direct-Absorption Technique

The acidification procedure, described above, corrects for the existence of another pigment associated with the natural degradation of chlorophyll-a: phaeophytin-a. Phaeophytin-a has an optical absorption peak at 654 nm (Tett et al., 1975); close to that of chlorophyll-a (Moss, 1967; Tietjen, 1968; Boto and Bunt, 1978; Marker, 1972). Its presence and concentration in a sample of chlorophyll-a can be accounted for by acidification of the sample and subsequent absorption measurement at 665 nm as acidification converts all chlorophyll-a present into phaeophytin-a. The concentration of chlorophyll-related pigments (chlorophyll-a) in the standard is then calculated from measured absorbance values using the following equation (Lorenzen, 1967; Daemen, 1986; APHA, 1992):

\[
\text{Chlorophyll-a (mg/m}^3\text{)} = \frac{26.7[(664_b - 750_b) - (665_a - 750_a)] \times V_i}{V_2 \times L} \quad (4.1)
\]

where

- \(664_b, 750_b\), \(665_a\), \(750_a\) = optical densities of 90\% (v/v) acetone extract before and after acidification (0.1 N HCl), respectively
- \(V_1\) = volume of 90\% (v/v) acetone used for extraction (L)
- \(V_2\) = volume of extracted sample (m\(^3\))
- \(L\) = light path length/width of cuvette (cm), and
- 26.7 = the absorbance correction, and equals: \(a \times K\), where
  - \(a\) = absorbance coefficient for chlorophyll-a at 664 nm = 11,
  - \(K\) = ratio expressing correction for acidification = 2.43.

In the following direct absorption measurements, a single prepared standard sample was used as the stock solution. The concentration of chlorophyll-related pigments in this standard was measured to be 116 mg/L.

4.2.2. Suspended Sediments

The preparation of sediment for suspension in the chlorophyll samples was completed following Shelton (1998). The sediment sample was gathered from the banks of the Murrumbidgee River near Wagga Wagga, New South Wales, Australia, followed by oven-drying at a temperature of 80 °C for 3 days. A sub-sample was then crushed using a mortar
and pestle, and then fired in a muffle furnace (Ceramic Engineering) for 5 hours at 500 °C to dispose of all the organic content of the sediment. The ashed sediment, hitherto referred to as clay had a Munsell colour of 10YR 7/6 dry (Birren, 1969; Davies-Colley and Close, 1990).

The clay samples were then sieved to a grain size no larger than 120 μm. This particle size was used on the basis that it is the largest sized particle likely to be in suspension in the Murrumbidgee River during normal flow conditions, which is typically of about 0.1 m s\(^{-1}\) during bank-full discharge conditions (Shelton, 1998).

A photograph of a samples, of prepared clay is given in Figure 4.3.

![Figure 4.3: Photograph of prepared clay for suspension in chlorophyll-containing water samples.](image)

### 4.3. Optical Instrumentation

The complete optical apparatus used for the direct absorption measurements is depicted in Figure 4.4. The apparatus comprises a regulated light source, collimating optics, a sample cell, optical fibre cable and a spectrometer. All optical measurements were conducted in a dark room.
4.3.1. Regulated Light Source

The regulated white light source comprised an OSRAM quartz-tungsten-halogen bulb (250 W, 24 V) mounted in a Series Q Lamp Housing (ORIEL Model 60000), complete with an integral condensing/collimating Lens Assembly as shown in Figure 4.5. The light source was driven by Constant Current Power Supply (Oriel Model 68830), with a maximum output of 360 W.

Figure 4.4: Schematic diagram of the complete apparatus for measuring chlorophyll-related pigments in water containing suspended sediment (SS) using the direct optical absorption technique.

Figure 4.5: Series Q lamp housing with integrating collimating optics condenser and rear reflector (Oriel Corporation, 1990).
4.3.2. Sample Cell

White light from the regulated light source was collimated and directed into a sample cell consisting of a ¾-cross section of PVC (polyvinylchloride) pipe. The pipe measured 40 mm diameter and was cut to varying lengths to facilitate measurements of absorption related to varying cell lengths (L). A fused-silica glass microscope slide, 1.2 mm thick, was glued to each end of the PVC pipe using Araldite Epoxy (Selleys Chemical Company). A schematic diagram of a sample cell is given in Figure 4.6. Sample cells of five different lengths were constructed; 3 cm, 5 cm, 8 cm, 10 cm and 15 cm. Three of these are depicted in the photograph of Figure 4.7.

Figure 4.6: Schematic diagram of a sample cell.

Figure 4.7: Photograph of three of the sample cells used. Lengths are: (a) 5 cm, (b) 10 cm, and (c) 15 cm.
4.3.3. Spectrometer

The Ocean Optics SD1000 spectrometer (Ocean Optics Inc., Dunedin, Florida, USA) is a low cost, versatile and portable spectrometer initially constructed for measuring ocean pH (Ocean Optics, 1996). On exiting the sample cell, the attenuated white light was directed into a single 200-μm core-diameter glass optical fibre cable via a 10-mm focal-length lens.

The optical fibre formed the input slit of the spectrometer and was connected to the box housing the spectrometer grating via a SMA 905 connector. The internal diffraction grating was ruled to 600 lines/mm and blazed at a wavelength of 500 nm. Light reflected from the grating was directed onto a linear 1024-element charged-couple-device (CCD) array. The CCD array, in conjunction with the input fibre and grating, recorded spectral information in the wavelength range of 390 – 890 nm with a spectral resolution of 1.4 nm. A schematic diagram of the spectrometer is given in Figure 4.8 and a photograph is given in Figure 4.9. The SD1000 was connected to a lap-top Toshiba computer running the software Spectra-Solve™ Junior, a spectra collection and analysis package specifically designed for Ocean Optics Optical Fibre Spectrometers as depicted in Figure 4.9.

Absorbance spectra were recorded using the following integral sequence of measurements:

i. Selection of integration time (50 ms)

ii. Measurement of dark current spectrum (DARK(λ))

iii. Measurement of radiation spectra using sample cell filled with clear distilled water (REFERENCE(λ))

iv. Dropwise addition of chlorophyll "standard" and sediment to water in the sample cell and subsequent measurement of radiation spectrum (SAMPLE(λ))

v. Calculation of absorbance spectrum using:

\[
Abs(\lambda) = -\log_{10} \left[ \frac{\text{SAMPLE}(\lambda) - \text{DARK}(\lambda)}{\text{REFERENCE}(\lambda) - \text{DARK}(\lambda)} \right] \quad (4.2)
\]
Figure 4.8: Schematic diagram of Ocean Optics SD1000 spectrometer unit.

Figure 4.9: Photograph of Ocean Optics SD1000 spectrometer and a lap-top Toshiba computer running the software Spectra-Solve™ Junior.
4.3.4. Spectrometer Performance – the Maximum Absorbance Limit

Using the spectrometer to measure pigment absorption in water containing suspended sediment will most likely involve very low light levels at the detector due to significant scattering. It was therefore important to ascertain the degree to which the instrument’s sensitivity to the introduction of pigment or suspended sediment in a sample would be affected by its low-light detection limit. In order to test the performance of the spectrometer for measuring absorbance at low light levels, a measurement of the maximum absorbance limit of the instrument was completed.

Clean distilled water was introduced into one of the sample cells depicted in Figure 4.7 (Section 4.3.2), and Reference and Dark spectra were recorded using the apparatus in Figure 4.4. The optical path was then completely blocked between the sample cell and the spectrometer using a piece of dark cardboard. The absorbance spectrum was then recorded. An absorbance spectrum of dark cardboard sample was then calculated using Equation 4.2.

The calculated absorbance spectrum is given in Figure 4.10. The absorbance is approximately 2 at 360 nm and increases to a maximum of 8.5 at 650 nm. This profile is actually the maximum absorbance limit of the instrument. It results from a combination of the spectral profile of the white light source and the spectral response of the silicon array in the spectrometer when recording Reference (\(\lambda\)). For example at low wavelengths (<450 nm) the white light source produces only 70% of the luminous intensity at 600 nm, while the quantum efficiency of the silicon array is only 50% of the quantum efficiency at 600 nm (Newport, 2000). A raw digital spectrum (measured in Reference mode and recorded as digital number - DN), illustrating this combined effect of source efficiency and detector sensitivity, is given in Figure 4.11. This spectrum was acquired using the white light source directly coupled to the input fibre of the spectrometer. The digital number recorded by spectrometer progressively decreases with decreasing wavelength below 650 nm, and is close to zero at wavelengths below 400 nm.
Figure 4.10: Absorbance spectrum corresponding to the maximum measurement limit of the Ocean Optics SD1000 spectrometer.

Figure 4.11: Raw digital spectrum of Ocean Optics SD1000 spectrometer coupled directly to the white light source, illustrating the compounding effects of source efficiency and spectrometer sensitivity as a function of wavelength.
4.4. Results

4.4.1. Absorbance of Chlorophyll-Related Pigments in Clear Water

Absorption spectra (relative to clear distilled water) were measured for five concentrations of chlorophyll-related pigments in clear distilled water (0.166 mg/L, 0.332 mg/L, 0.498 mg/L, 0.664 mg/L and 0.830 mg/L) and five different sample cell lengths (3 cm, 5 cm, 8 cm, 10 cm and 15 cm). These concentrations were determined by using Equation 4.3.

\[
c = \frac{V_{\text{chl}} C_{\text{chl}}}{V_w}
\]

(4.3)

where

\[V_{\text{chl}} \text{ (mL)}\] = volume of chlorophyll-related pigment,

\[C_{\text{chl}} \text{ (mg/L)}\] = concentration of chlorophyll-related pigment measured in Equation 4.1, and

\[V_w \text{ (mL)}\] = volume of distilled water.

A sample for the absorption spectra for three different sample cell lengths are depicted in Figure 4.12.
Figure 4.12: Absorption spectra of chlorophyll-related pigments in clear distilled water for three different sample cell lengths: (a) 3 cm, (b) 8 cm, and (c) 15 cm.
Chapter 4: Measurement of Chlorophyll Pigments in Turbid Water Using the Direct-Absorption Technique

The absorbance spectra of Figure 4.12 (a) to (c) are characterised by a number of prominent features. The peak at approximately 670 nm is attributable to chlorophyll-a and this has been observed by numerous researchers (for example, Davies-Colley et al. 1986, Ganf et al. 1989; Mittenzwey et al. 1992; Ferrari et al., 1996; Shooter et al., 1998; Graham and Mitchell, 1999; Umesu et al., 1999). Chlorophyll-a is also responsible for the absorption peak at a wavelength of approximately 430 nm (for example, Geider and Osborne, 1987; Ganf et al., 1989; Ferrari et al., 1996; Graham and Mitchell, 1999). Furthermore, the chlorophyll-a absorption is stronger in the blue (430 nm) than in the red (670 nm) (Hoge and Swift, 1987).

The absorption spectra of Figure 4.12, in particular for the higher pigment concentrations, features a small shoulder in the wavelength range of 600 - 630 nm which is attributable to chlorophyll-c (Lorenzen, 1967; Bricaud et al., 1983; Graham and Mitchell, 1999), although another small prominent peak at approximately 627 nm may be attributable to phycobilin (Ó hEocha, 1963; Davies-Colley et al., 1986). The broad, inclined shoulder between 450 nm and 490 nm is mainly caused by carotenoid absorption (Paerl et al., 1983; Faust and Norris, 1985). Broad-band absorption also appears in the wavelength range 490 nm to 560 nm and results from carotenoid fucoxanthin (Davies-Colley et al., 1986; Smith and Alberete, 1994; Zeebe et al., 1996).

The low light levels associated with the short-wavelength absorption by the pigments are evident in the spectra corresponding to the longest sample cell [15 cm, Figure 4.12 (c)]. The maximum measurement limit of the ocean optic SD1000 spectrometer, as depicted earlier in Figure 4.10 has distorted the short-wavelength (<450 nm) portions of the absorbance spectra.

In all cases increasing the concentration of chlorophyll-related pigments in the sample cells resulted in an increase in absorbance at the two peak wavelengths. However, closer examination Figure 4.12 (a) – (c), and the other absorbance spectrum recorded but not included in Figure 4.12, reveals that the peak wavelength associated with the blue absorption feature (400 – 470 nm) also shifts with increasing pigment concentration. This peak
absorbance wavelength (hitherto referred to as BLUE) as a function of pigment concentration is plotted in Figure 4.13.

![Graph showing the relationship between chlorophyll-a concentration and peak absorbance wavelength for different sample cell lengths.](image)

**Figure 4.13:** Apparent shift of wavelength of BLUE absorption peak with increasing chlorophyll-a concentration for three sample cell lengths, produced by "clipping" of the absorbance profile by the maximum measurement limit of the spectrometer.

As depicted in Figure 4.13, for sample cell length of 3 cm the chlorophyll-a blue peak is approximately 413 nm for a pigment concentration of 0.166 mg/L, and is shifted to longer wavelength of approximately 420 nm at a pigment concentration of 0.830 mg/L. Furthermore, for a sample cell length of 15 cm the blue peak shifts from 414 nm to approximately 460 nm for the same pigment concentration range.

The shift of blue absorption peak is again a result of the maximum measurement limit of the ocean optic SD1000 spectrometer. In Figure 4.10 the shape of the maximum absorbance limit profile results in the "clipping" of the BLUE peak when the absorbance is greater than 2. Since the clipping occurs at the low-wavelength side of the BLUE peak, this produces an apparent shift in the position of the peak to higher wavelengths.
In contrast, the longer-wavelength absorbance peaks of chlorophyll-a at approximately 670 nm (hitherto referred to as RED) are not shifted by increasing pigment concentration (Figure 4.14) (Ganf et al., 1989; Ferrari et al., 1996). The maximum absorbance limit profile of spectrometer (Figure 4.10) is flat for wavelengths greater than 600 nm, hence no clipping would occur, and the absorbance values encountered in these current measurements do not exceed the maximum absorbance limit of instrument.

\[ \text{Peak Absorbance Wavelength (nm)} \]

\[ \text{Chlorophyll-a Concentration (mg/L)} \]

**Figure 4.14:** Relationship between apparent wavelength of RED absorption peak and chlorophyll-a concentration for sample cell length of 3 cm. No "clipping" of the absorbance profile occurs at this wavelength.

Not withstanding this discussion of the influence of the maximum absorbance limit on the absorption profiles, another possible reason for the shift in the BLUE absorbance peak is the solvent used in the preparation of chlorophyll sample. Under some conditions, researchers have observed that the solvent within which the chlorophyll is dissolved during extraction may shift the absorption peak to larger wavelengths (Marker, 1972, Bricaud et al., 1983; Davies-Colley et al., 1986). In the case of acetone, subsequent mixing with water (as conducted in this work with the dropwise addition of the standard to the sample cell containing water) can shift the BLUE absorption peak to higher wavelengths by tens of nm.
and this is generally associated with electron transition induced by changing polarity of the solvent (Skoog and Leary, 1992).

In order to observe whether this process contributes to the observed shifts in our BLUE peak, the following simple experiment was conducted. The absorbance spectrum of 0.830 mg/L chlorophyll-related pigment in clear distilled water was recorded with the addition of greater amounts of clear 90% (v/v) acetone to the 8 cm sample cell as depicted in Figure 4.15. The BLUE peak absorbance wavelength is plotted as a function of acetone added in Figure 4.16. While there is an expected decrease in the magnitudes of the absorption peaks with addition of acetone (Figure 4.15), there does not appear to be a significant effect of acetone concentration on the wavelength of the BLUE peak (Figure 4.16). As a consequence of influence of the absorbance limit on the chlorophyll-a BLUE peak, the chlorophyll-a RED absorption peak only was chosen for subsequent investigations of the direct absorption technique, and the FEFA technique described later in Chapter 5.

![Figure 4.15: Absorption spectra of 0.83 mg/L chlorophyll-related pigments in clear distilled water with further addition of acetone-90% (v/v) solvent using sample cell length of 8 cm.](image-url)
The influence of chlorophyll-related pigment concentration and sample cell length on the measured absorbance at 670 nm is depicted in Figure 4.17 and Figure 4.18, respectively. As shown in Figure 4.17 the absorbance at 670 nm increases linearly with the concentration of chlorophyll-related pigment in clear distilled water. Furthermore, as depicted in Figure 4.18, at any given concentration of chlorophyll-related pigment the absorbance at 670 nm increases linearly with increasing the sample cell length. However, the graph shows the non-zero absorbance for various sample cell lengths. This is most probably due to the light scattering of chlorophyll-related pigment in distilled water.

The measured absorbance plotted in Figures 4.17 and 4.18 support Beer’s law as described in Section 2.2.1, where the absorbance is directly proportional to the concentration of the absorbing species and sample cell length (Skoog and Leary, 1992).
Figure 4.17: Relationship between absorbance at 670 nm and concentration of chlorophyll-related pigment in water for various sample cell lengths (L).

Figure 4.18: Relationship between absorbance at 670 nm and sample cell lengths for various chlorophyll-related pigment concentration levels in clear distilled water.
4.4.2. Absorbance of Chlorophyll-Related Pigments in Turbid Water

Absorption spectra (relative to clear distilled water) were also measured for the same five concentrations of chlorophyll-related pigments and sample cell lengths used in Section 4.4.1 but with the further addition of five concentrations of clay as suspended sediment (2 g/L, 4 g/L, 6 g/L, 8 g/L and 10 g/L). Representative examples of the absorbance profiles are provided in Figure 4.19. Each spectrum was acquired immediately after stirring the clay in the sample cell to ensure that all the added sediment was in suspension.
Figure 4.19: Selected absorbance spectra of 0.830 mg/L chlorophyll-related pigments in water containing various concentrations of CLAY for different sample cell lengths: (a) 3 cm, (b) 8 cm, and (c) 15 cm.
In Figure 4.19 (a) the 670 nm absorption peak of chlorophyll-a pigment in distilled water containing the suspended sediment is observable at low concentrations of clay and using the shortest sample cell (3 cm). However, for the 3-cm sample cell the introduction of suspended sediment in concentrations greater than 6 g/L, or for the longer sample cells introduction of only 2 g/L suspended sediment, attenuates the light beam such that no pigment absorption features are observable at all. At this point the absorbance profile corresponds to the maximum absorbance limit profile of the spectrometer as described earlier in Section 4.3.4.

The relationship between absorbance at 670 nm, and clay concentration and cell absorption lengths are plotted in Figures 4.20. Note here that the absorbance value for zero suspended sediment is consistent with the absorbance values in Figures 4.17 and 4.18.

![Graph showing relationship between absorbance and clay concentration](image)

**Figure 4.20: Relationship between absorbance at 670 nm of 0.830 mg/L chlorophyll-related pigments in clear distilled water and varying suspended clay concentrations.**

Figure 4.20 highlights the significant limitation of scattering by suspended sediment on optical absorption measurements. The maximum measurement limit of the instrument precludes the measurement of any pigment profiles in cell lengths larger than the shortest
used in this work, and for suspended sediment concentrations greater than approximately 2 g/L.

However, it is interesting to note that at low suspended sediment concentrations, where the pigment absorption features are observable, the absorption peaks are simply superimposed on the broadband absorption associated with scattering by the suspended sediment. This is particularly evident when, in conjunction with the shortest sample cell, fractional concentrations of clay (0 – 2 g/L) are introduced into the water containing chlorophyll-related pigments (Figure 4.21).

![Figure 4.21: Representative absorbance spectra of 0.83 mg/L chlorophyll-related pigments in water containing 0 – 2 g/L of clay. Sample cell length is 3 cm.](image)

It can be observed in Figure 4.21 that the BLUE absorbance peak is again shifted towards the longer wavelength with increasing sediment concentration, but the RED absorbance peak does not appear to shift. As encountered earlier with high chlorophyll concentrations, at higher clay concentrations, the maximum absorbance limit of the instrument distorts the absorbance profiles in the vicinity of BLUE peaks.
Quibell (1991), Munday and Alföldi (1979), Sydor (1980), Chen et al. (1992) and Han (1997) observed that addition of sediment to pure algae cultures produced a shift in the peak reflectance wavelength from 550 nm to a broad sediment reflectance peak at approximately 600 – 650 nm. Similar observations were also recorded by Goodin et al. (1993). In our absorption measurements this would be equivalent to a shift in the wavelength of the absorption minima at approximately 550 nm. Reference to Figure 4.21 shows only a broad absorption minima at this wavelength and there is not sufficient radiometric resolution (i.e. in absorbance axis) to discern any effect of this nature. However, it should never the less be recognised that the wavelength of the maximum RED absorbance peak, when plotted against clay concentration in Figure 4.22 is not shifted as the suspended sediment concentration is increased.

Having now demonstrated the characteristics of absorption spectra for chlorophyll-related pigments in clear and turbid water acquired using a traditional direct absorption technique, the following chapter will describe the features of the FEFA technique.
Measurement of Chlorophyll Pigments in Turbid Water Using FEFA Technique

5.1. Introduction

In this Chapter, the experiments performed in Chapter 4 will be repeated using the Fibre-optic Evanescent Field Absorption (FEFA) technique. The regulated light source and spectrometer are the same as described in Chapter 4, as are the protocols for preparing standard samples of chlorophyll-related pigments and sediment.

Given the FEFA technique relies on the interaction between the liquid media and the optical fibre rather than just the characteristics of the sample cell itself, a different cell to those described in Chapter 4 was required. Consequently this Chapter commences with a description of the sample cell as well as an outline of the methods used to prepare suitable optical fibres. The absorption spectra acquired are presented and their characteristics discussed. Finally, a discussion of the key differences between the direct absorption and FEFA technique are presented.
5.2. Optical Fibre Preparation and Sample Cell

The evaluation of the FEFA Technique was carried out using a pure core silica (PCS) optical fibre. The basic components of the PCS are depicted in Figure 5.1. The core, which is made of pure silica, is surrounded by bonded hard polymer cladding and protected by a tefzel® buffer coating (Newport, 2000). The PCS optical fibre has a core diameter of 600 μm and a numerical aperture of 0.37.

![Diagram of pure core silica (PCS) step-index optical fibre.]

For FEFA measurements, the PCS optical fibre was prepared as follows. Approximately 3 mm of the tefzel® buffer coating and cladding of both ends of the PCS optical fibre was stripped using a scalpel. Both ends of the fibre (about 60 cm in length) were cleaved with a fibre scribe (Newport, Model F-CL1), and ground and polished using three progressively-finer grades of sand paper on glass plate dipped in water.

To establish the FEFA region of the fibre, the cladding of the middle-portion of that fibre, for certain lengths, was stripped by burning with a butane pencil torch (Blue Blazer, China) with maximum flame temperature of approximately 1316 °C. The exposed core was subsequently cleaned with isopropyl alcohol using lint-free tissue paper (Kimwipes, Kimberly-Clark Australia Pty. Limited) to remove any residual carbonisation that was
produced by combustion of dust or debris on the cladding. A schematic diagram of the prepared optical fibre is depicted in Figure 5.2.

![Schematic diagram of optical fibre](image)

Figure 5.2: Length of prepared PCS optical fibre for evaluation of the FEFA technique. FIL is the fibre interaction length.

Seven different lengths of exposed fibre core were used in the subsequent FEFA evaluation; 2 cm, 4 cm, 6 cm, 8 cm, 10 cm, 12 cm and 14 cm. These are subsequently referred to as fibre interaction length (FIL). The prepared fibres were inserted into a sample cell. The cell was constructed from perspex measuring 4 cm (width) x 4 cm (depth) x 16 cm (length). A schematic diagram and a photograph of sample cell are depicted in Figures 5.3 and 5.4, respectively.

![Schematic diagram of sample cell](image)

Figure 5.3: Schematic diagram of “perpex” sample cell.
5.3. Optical Instrumentation

The complete apparatus used for evaluating the FEFA technique is depicted in Figure 5.5. The regulated white light source and the fibre optic cable and SD1000 spectrometer combination are the same as that described in Chapter 4 (Sections 4.3.1 and 4.3.3). The collimated beam from the regulated white light source was directed into a precision optical fibre coupler (Newport, Model F-915), which contained one end of the selected fibre used for the FEFA evaluation. The output-end of the selected fibre (downstream of the sample cell) was butt-coupled to the input fibre cable of the SD1000 spectrometer using a precision optical fibre positioner (Newport, Model FP-1).

Absorbance spectra were recorded using the same sequence of measurements described in Section 4.3.3. As in Chapter 4, all measurements were conducted in a dark room.
5.4. Results

5.4.1. Absorbance of Chlorophyll-Related Pigments in Clear Distilled Water

Fives times higher concentration of chlorophyll-related pigments, prepared following the procedure described in Section 4.2.1, were used in the FEFA evaluation, namely: 0.83 mg/L, 1.66 mg/L, 2.49 mg/L, 3.32 mg/L and 4.15 mg/L. These concentration levels were determined using Equation 4.3 (Section 4.4.1), and applied for each of the seven fibre interaction lengths (FIL). The results for three of the seven fibre interaction lengths are depicted in Figure 5.6.
Chapter 5: Measurement Chlorophyll Pigments in Turbid Water Using FEFA Technique

(a)

(b)
A number of prominent spectral features are observed in the FEFA spectra depicted in Figure 5.6 (a) to (c). These are similar with those illustrated in Figure 4.12 (a) to (c) acquired using direct absorption technique (Section 4.4.1). Again, the strongest absorbance of chlorophyll-related pigments in water occurs at a wavelength of approximately 430 nm, which is attributable to chlorophyll-a blue pigment (Geider and Osborne, 1987; Ganf et al., 1989; Ferrari et al., 1996; Graham and Mitchell, 1999). The other prominent absorption peak occurs at a wavelength of approximately 670 nm, which is attributable to chlorophyll-a red pigment (Davies-Colley et al. 1986, Ganf et al. 1989; Mittenzwey et al. 1992; Ferrari et al., 1996; Graham and Mitchell, 1999; Umetsu et al., 1999).
The absorption spectra of Figure 5.6, in particular for the higher pigment concentrations, features a small shoulder in the wavelength range of 600 - 630 nm which, like the direct absorption spectra of Figure 4.12, is attributable to chlorophyll-c (Bricaud et al., 1983 and Lorenzen, 1967). The other small prominent peak at approximately 627 nm is also observable, as is the broad, inclined shoulder between 450 nm and 490 nm.

The prominent peak in the FEFA spectra for wavelengths less than 400 nm is observed in all concentrations of pigment and for all FIL. This feature, like that described earlier in Section 4.3.4 (Spectrometer Performance – the Maximum Absorbance Limit) is an artefact of the spectrometer. The source of this artefact becomes evident when, as depicted in Figure 5.7, the absorption measurement sequence is followed on a sample of clear distilled water only.

![Graph](image)

**Figure 5.7:** FEFA spectrum obtained by using the same sample of clear distilled water for both reference and sample measurements.

The absorbance spectra of Figure 5.7 should be a horizontal line of "zero" absorbance. However, the resulting profile occurs because of a small error in the A/D conversion of the "sample measurement" component of the absorption protocol (Step iv in Section 4.3.3) (Dr. Mark Aizengendler, Lastek Pty. Ltd, Adelaide, Personal Communication, November 12,
2001). This error is not observable in the earlier direct absorption measurements (Section 4.3.4) as the absorbance values are at least one order of magnitude higher. Fortunately, in these, and later, FEFA measurements, the absorbance values at wavelengths below 400 nm are not applicable to our overall investigations.

In Figure 5.6, increasing the concentration of chlorophyll-related pigments in the sample cell resulted in an increase in absorbance at the two peak wavelengths, referred to in the earlier direct absorption measurement as BLUE and RED absorption peaks. One important difference between the FEFA spectra of Figure 5.6 and the earlier direct absorption spectra of Figure 4.12 is that there is no significant change in centre wavelength of the BLUE absorption peak with increasing pigment concentration. This is clearly evident in contrasting Figure 5.8 against Figure 4.13.

![Graph showing relationship between wavelength of BLUE absorption peak and chlorophyll-a concentration for three FIL: 2 cm, 8 cm and 14 cm.]

In these measurements, the FEFA absorbance values are below the maximum limit envelope of Figure 4.10 and the BLUE absorption peak is not “clipped”. This is clearly illustrated in
Figure 5.8 for BLUE absorption peak, and also in Figure 5.9 for RED absorption peak, which, again, is not affected by the maximum absorbance limit of the instrument.

Since the central wavelength of both peaks is invariant to pigment concentration, either could be used to investigate the effect of pigment concentration and fibre interaction length (FIL) on the FEFA spectra. However, to maintain consistency with the earlier direct absorption measurements, only the RED absorption peak will be discussed.

![Graph showing relationship between wavelength of RED absorption peak and chlorophyll-a concentration for three FILs: 2 cm, 8 cm, and 14 cm.](image)

**Figure 5.9: Relationship between wavelength of RED absorption peak and chlorophyll-a concentration for three FILs: 2 cm, 8 cm, and 14 cm.**

The influence of chlorophyll-related pigment concentration and FIL on the measured absorbance at a wavelength of 670 nm is depicted in Figure 5.10 and Figure 5.11, respectively. As shown in Figure 5.10, the absorbance at 670 nm increases with the concentration of chlorophyll-related pigment in clear distilled water. For the lowest FIL of 2 cm in Figure 5.10, a distinctly linear relationship is observed between the absorbance and concentration of pigment. This linearity has also been observed with rhodamine-6G in a nonpolar organic solvent (Paul and Kychakoff, 1987), methylene blue in water (Villarruel et al., 1987) and hydrocarbons in water (Sensfelder et al., 1996).
Figure 5.10: Relationship between absorbance at 670 nm (RED peak) and concentration of chlorophyll-related pigment in clear distilled water using the FEFA technique for three fibre interaction lengths (FIL).

Figure 5.11: Relationship between absorbance at 670 nm and FIL using the FEFA technique for three chlorophyll-related pigment concentration levels in clear distilled water.
Furthermore, as depicted in Figure 5.11, at any given concentration of chlorophyll-related pigment the absorbance at 670 nm increases linearly with the fibre interaction length (FIL). The linear relationship between FEFA absorbance and FIL has also been observed in other pigments by Simhony et al. (1988) and Ruddy et al. (1990). Linear trends in concentration and FIL support Beer’s law as described in Section 2.3.1. However, the absorbance-concentration profiles are not linear for larger FIL (8 and 14 cm). Such deviations from linearity have also been observed by Ruddy et al. (1990) for the methylene blue dye.

At high concentrations, the average distance between the species responsible for absorption is reduced to the point where each affects the charge distribution of its neighbours. As a result of this interaction, the species’ ability to absorb a given wavelength of radiation can be altered because of the dependence between the extent of interaction and the species concentration. Therefore, this phenomenon causes deviations from the linear relationship between absorbance and concentration as clearly shown in Figure 5.10 for longer FIL (i.e. 8 cm and 14 cm). Furthermore, a similar consequence is sometimes encountered in solutions containing low concentrations of absorbing species but high concentrations of other species, particularly electrolytes (Skoog and Leary, 1992).

The non-linearity relationship between measured FEFA absorbance and concentration as depicted in Figure 5.10 for 8 cm and 14 cm FIL implies a square root dependence of FEFA absorbance on concentration. This is consistent with the Debye and Hückel approximation (Hiemenz, 1986; Hunter, 1996) for ion-ion interactions, where the Debye-Hückel parameter (which has the unit of length\(^{-1}\)) is proportional with the square root of concentration. Furthermore, as reported by Ruddy et al., 1990 that the square root dependence of absorbance on concentration is attributed to surface adsorption due to electrostatic interactions between the polar unclad silica fibre core surface and the ionic solution.

The deviation from Beer’s law in multimode optical fibre evanescent field sensor has also been discussed by Payne and Hale (1993) in terms of the nature of the guided radiation itself. The FEFA technique (using multimode fibres) is based on the interaction between the evanescent field and the absorbing material surrounding the fibre, and this depends critically on the nature of the guided modes travelling along the fibre (Section 3.2.1). Each mode has
a different evanescent field and experiences a different attenuation in the external medium. This is in contrast with the direct absorption technique where Beer’s law describes the attenuation of a beam of light as it passes directly through a solution of an absorbing species. The influence of the guided radiation on the sensitivity of the FEFA technique to an absorbing media will be discussed in further detail in Chapter 6.

5.4.2. Absorbance of Chlorophyll-Related Pigments in Turbid Water

Absorption spectra (relative to clear distilled water) were also measured using the FEFA technique for the same five concentrations of chlorophyll-related pigments and fibre interaction lengths (FIL) used in Section 5.4.1, but, with the addition of five concentrations of clay as suspended sediment (2 g/L, 4 g/L, 6 g/L, 8 g/L and 10 g/L) as used for the direct absorption technique (Section 4.4.2). Representative examples of the resulting FEFA spectra for three fibre interaction lengths (FIL) are given in Figure 5.12.
Figure 5.12: Selected FEFA spectra of 4.15 mg/L chlorophyll-related pigments in water containing various concentrations of CLAY for three different FIL: (a) 2 cm, (b) 8 cm, and (b) 14 cm.
Figure 5.12 illustrates that the absorption peaks of chlorophyll-related pigments in clear distilled water are not significantly affected by addition of suspended sediments. This is further demonstrated in the graph of absorbance at 670 nm versus clay concentration depicted in Figure 5.13. This result is in stark contrast to those observed in the earlier traditional direct absorption measurement described in Section 4.4.2. In these earlier results, addition of 0.4 g/L clay produced a significant change in the pigment-related absorption (approximately 50% - refer to Figure 4.21) and 4 g/L was found to reduce the measured light signal to that associated with the maximum absorbance limit of instrument (Figure 4.19).

![Absorbance vs Clay Concentration Graph]

**Figure 5.13:** Relationship between absorbance at 670 nm (RED peak) of 4.15 mg/L chlorophyll-related pigments in clear distilled water and suspended sediment concentrations of CLAY.
Figure 5.14: Relationship between wavelength of BLUE absorbance peak and concentration of CLAY in clear distilled water containing 4.15 mg/L chlorophyll-related pigments for various FIL.

Figure 5.15: Relationship between wavelength of RED absorbance peak and concentration of CLAY in clear distilled water containing 4.15 mg/L chlorophyll-related pigments for various FIL.
Since the absorbance values in the presence of suspended sediment, are still considerably lower than the maximum limit absorbance of instrument, it is no surprise that the graphs of the centre wavelengths of both the RED and BLUE absorption peaks versus sediment concentration (Figures 5.14 and 5.15) show the centre wavelengths are not significantly influenced by varying concentrations of suspended sediment.

The influence of suspended clay sediment on the FEFA measurement is again depicted in Figure 5.16. Only this time the graph illustrates the relationship between absorbance and fibre interaction length. This graph, for different levels of suspended clay sediment clearly demonstrates the invariance of measured absorbance in response to the presence of suspended sediment.

![Graph showing absorbance vs fibre interaction length](image)

**Figure 5.16: Relationship between absorbance at 670 nm of 4.15 mg/L chlorophyll-related pigments in clear distilled water containing suspended sediment and fibre interaction length (FIL) using the FEFA technique.**

The key differences observed thus far between the direct-absorption and FEFA techniques are in relation to the effect of suspended sediment on the measured absorption spectra of
chlorophyll-related pigments in clear distilled water. However, the value of absorbance obtained by using the direct-absorption technique was approximately one order of magnitude higher than that achieved by using the FEFA technique for the same concentration levels of chlorophyll-related pigments in clear distilled water. This issue will also form the subject of the next Chapter.
Chapter 6

Investigating the Sensitivity of FEFA -
A Theoretical and Experimental Parametric Study

6.1. Introduction

In the previous two Chapters, the comparison of the traditional direct absorption and FEFA techniques has highlighted a key advantage of using the FEFA technique to measure absorbance spectra of dissolved pigments in the presence of suspended sediment. However, one potential limitation is that the sensitivity of FEFA is at least an order of magnitude smaller in a single-pass (i.e. straight optical path) apparatus of similar length to its direct absorption counterpart.

In this Chapter, an investigation of the factors that influence the sensitivity of the FEFA technique are reported. These factors, or physical parameters, include fibre interaction length (FIL), fibre core diameter (d) and light launch angle (into the fibre) (θL). The first step in this investigation is the construction of a theoretical model of the optical fibre used in the FEFA apparatus based on the “skip-length” theory. Theoretical predictions of this “skip-length model” based on Pedrotti-Pedrotti (1993) are then compared to actual measurements of
absorption completed using the FEFA apparatus described in Chapter 5, and varying the key parameters: FIL, d and $\theta_1$.

6.2. The Skip-Length Model

There are two theoretical models concerning light propagation in fibres, the ray model and the electromagnetic wave model. The ray model is more appropriate for multimode fibres that have a large diameter of core, whereas the electromagnetic wave model is more appropriate with singlemode fibres that have a smaller core diameter. As this work involves the use of multimode PCS optical fibre, where the core diameter is much larger than the wavelength of the light, an intuitive picture of the propagation mechanism in an ideal multimode step-index optical waveguide is most easily modelled by a simple ray (geometrical) optics representation (Keiser, 2000). The following section therefore describes a skip-length model, which is largely based on the ray model.

6.2.1. Depth of Penetration and Skip Length

The zigzag path of the light propagating in an optical fibre, resulting from total internal reflection, has been described earlier (Section 3.1.1).

![Figure 6.1: An electromagnetic wave undergoes reflection and refraction at the interface of two optical different materials ($n_1 > n_2$) in an optical fibre.](image)
Chapter 6: Investigating the Sensitivity of FEFA –
a Theoretical and Experimental Parametric Study

The boundary conditions at the core-cladding interface, redrawn in Figure 6.1, dictate that the tangential field components of the electromagnetic field must be continuous. If the electric field component of the refracted electromagnetic wave in the cladding is \( E_0 \), then the wavefront equation of the electric field in the second medium is

\[
E_2 = E_0 \exp\left[ i \frac{2\pi}{\lambda} (x \sin \theta_2 + z \cos \theta_2) \right]
\] (6.1)

where \( \lambda \) is the wavelength in the cladding medium. If the wavelength of the electromagnetic wave in free space is \( \lambda_0 \), then \( \lambda = \lambda_0 / n_2 \), and consequently Equation 6.1 becomes

\[
E_2 = E_0 \exp\left[ i \frac{2\pi n_2}{\lambda_0} (x \sin \theta_2 + z \cos \theta_2) \right]
\] (6.2)

By applying Snell’s law at the core-cladding interface, namely: \( n_1 \sin \theta_1 = n_2 \sin \theta_2 \), it can be shown that

\[
\cos \theta_2 = \sqrt{1 - \left( \frac{n_1}{n_2} \right)^2 \sin^2 \theta_1}
\] (6.3)

Hence, Equation 6.2 can be expressed as

\[
E_2 = E_0 \exp\left[ i \frac{2\pi}{\lambda_0} \left( x n_1 \sin \theta_1 + z \sqrt{n_2^2 - n_1^2 \sin^2 \theta_1} \right) \right]
\] (6.4)

In Equation 6.4, the term \( n_2^2 - n_2^2 \sin^2 \theta_1 \) is negative due to the condition for total internal reflection, where the refractive index of the cladding is less than the core (\( n_2 < n_1 \)). Therefore, Equation 6.4 can be rewritten as

\[
E_2 = E_0 \exp\left[ i \frac{2\pi}{\lambda_0} \left( x n_1 \sin \theta_1 + i z \sqrt{n_2^2 \sin^2 \theta_1 - n_2^2} \right) \right]
\] (6.5)

Since \( i^2 = -1 \), Equation 6.5 becomes

\[
E_2 = E_0 \exp\left[ i x \frac{2\pi}{\lambda_0} n_1 \sin \theta_1 \right] \exp\left[ -z \frac{2\pi}{\lambda_0} \sqrt{n_2^2 \sin^2 \theta_1 - n_2^2} \right]
\] (6.6)

The first exponential term of Equation 6.6 shows that the field propagates along the \( x \)-axis in the same direction and with the same phase as the wavefront in the waveguide. Meanwhile, the real negative exponential term shows that the electric field reduces exponentially in the \( z \)-
direction. This field is known as an evanescent field whose electric field amplitude falls off exponentially with distance from the interface (Harrick, 1967). In Equation 6.6, this is

$$E_z = E_o \exp \left[ -\frac{z}{d_p} \right] \quad (6.7)$$

where $d_p$ is the depth of evanescent-field penetration (as introduced in Section 3.3.1), which is defined as the distance over which the amplitude of field reduces to 1/e of its value at the core-cladding interface. Furthermore, from Equation 6.6, the penetration depth of evanescent field is given by

$$d_p = \frac{\lambda_o}{2\pi \sqrt{n_1^2 \sin^2 \theta_i - n_2^2}} \quad (6.8)$$

In terms of the FEFA apparatus described in Chapter 5, the light propagates in the PCS optical fibre and interacts with the water containing chlorophyll-related pigments and suspended sediment along the fibre interaction length. Equation 6.8 can be applied to this system as visualised in Figure 6.2. The refractive indices of air, optical fibre core and surrounding medium (no longer cladding but chlorophyll-related pigment in water) are $n_o$, $n_1$ and $n_2$ (or $n_m$) respectively. The light launched into the PCS optical fibre at launch angle ($\theta_l$), travels along the fibre of core diameter, d, via internal reflections or skips at the core-water interface. With each internal reflection or skip, the light penetrates into the liquid to its penetration depth, $d_p$ (Equation 6.8). The distance travelled along the fibre by the light during this penetration into the liquid is referred to as displacement, $\delta$ (discussed later in Section 6.2.2). The distance along the fibre travelled by the light between successive internal reflections, or skips, is the skip-length, $L_s$ as defined by Pedrotti and Pedrotti (1993).
At a single point of internal reflection, labelled “C” in Figure 6.2, Equation 6.8 can be rewritten in terms of the light launch angle, $\theta_L$, as

$$d_p = \frac{\lambda_o}{2\pi \sqrt{n_1^2 \sin^2 \theta_L - n_2^2}}, \quad \text{or}$$

$$d_p = \frac{\lambda_o}{2\pi \sqrt{n_1^2 (1 - \cos^2 \theta_L) - n_2^2}} \quad (6.9)$$

Since $\theta_r = 90 - \theta_o$, Equation 6.9 becomes

$$d_p = \frac{\lambda_o}{2\pi \sqrt{n_1^2 - n_2^2 - n_1^2 \sin^2 \theta_L}} \quad (6.10)$$

By applying Snell’s law at the point where the light first enters the fibre, labelled “A” in Figure 6.2, and $n_o = 1$ for air, the relationship between launching angle ($\theta_L$) and refracting angle ($\theta_r$) can be written as

$$\sin \theta_L = n_1 \sin \theta_r \quad (6.11)$$

Then, Equation 6.10 becomes
\[ d_p = \frac{\lambda_0}{2\pi \sqrt{n_1^2 - n_2^2 \sin^2 \theta_L}} \]  \hspace{1cm} (6.12)

The penetration depth (Equation 6.12) can also be expressed as

\[ d_p = \frac{\lambda_0}{2\pi \sqrt{NA^2 \cdot \sin^2 \theta_L}} \]  \hspace{1cm} (6.13)

where \( NA = \sqrt{n_1^2 - n_2^2} \) is the numerical aperture of optical fibre as described previously in Section 3.1.

Based on the definition of skip-length, and using Figure 6.2, the skip length \( L_s \) can be written as

\[
L_s = d \tan \theta_i \\
= d \sqrt{\sec^2 \theta_i - 1} \\
= d \sqrt{\frac{1}{\cos^2 \theta_i} - 1} \hspace{1cm} (6.14)
\]

Again, since \( \theta_i = 90 - \theta_L \), Equation 6.14 becomes

\[
L_s = d \sqrt{\frac{1}{\sin^2 \theta_L} - 1} \hspace{1cm} (6.15)
\]

Then, using Snell's law form Equation 6.11,

\[
L_s = d \sqrt{\frac{n_1^2}{\sin^2 \theta_L} - 1} \hspace{1cm} (6.16)
\]

Equation 6.16 demonstrates that the skip length is proportional to optical fibre core diameter, and depends also on the PCS fibre core refractive index and light launch angle.

**6.2.2. Displacement and the Effective Length of the Absorption Path in FEFA**

As mentioned earlier, the distance in the direction of propagation (x axis) travelled by the electromagnetic wave while it has penetrated into the fibre cladding (or in this case the water containing chlorophyll-related pigment), is defined as the displacement, \( \delta \). The displacement can therefore be considered to be the effective absorbing path of the electromagnetic wave by the liquid media with each internal reflection down the fibre.
Chapter 6: Investigating the Sensitivity of FEFA – a Theoretical and Experimental Parametric Study

In spite of the fact that electromagnetic theory, and total internal reflection in particular, was studied in great detail around the turn of the century, the phenomenon of displacement was only first discovered in 1947 and graphically demonstrated by Goos and Hänchen (Harrick, 1967). The displacement, which is a fraction of the wavelength of the propagating radiation, is proportional to the penetration depth, and is greater for radiation of polarisation parallel to the plane of reflection than it is for polarisation perpendicular to the plane of reflection.

Referring back to Figure 6.2, it can be seen that

\[ \tan \theta_r = \frac{d + d_p}{L_s} = \frac{d_2}{\delta/2} \]  

(6.17)

therefore the displacement can be formulated as

\[ \delta = \frac{2d_2 L_s}{d + d_p} \]  

(6.18)

Since the evanescent field penetration depth is much smaller than the optical fibre core diameter, \( d_p \) in the denominator of Equation 6.18 can be neglected. Equation 6.18 then becomes

\[ \delta = \frac{2d_p L_s}{d} = 2d_p \sqrt{\frac{n_i^2}{\sin^2 \theta_L}} \cdot 1 \]  

(6.19)

The total number of internal reflections (or skips), \( N \), in a given length of fibre core exposed to the liquid media (defined as fibre interaction length, \( FIL \), in Chapter 5) can be calculated from

\[ N = \frac{FIL}{L_s} \]  

(6.20)

and the total length of the absorption path, \( L_{abs} \), will be

\[ L_{abs} = N \delta = \frac{(FIL) \delta}{L_s} \]  

(6.21)

Furthermore, by combining Equations 6.13, 6.16 and 6.19 into Equation 6.21,

\[ L_{abs} = \frac{(FIL) \lambda_0}{\pi d \sqrt{n_i^2 - n_f^2 - \sin^2 \theta_L}} \]  

(6.22)
6.2.3. Calculating Absorbance Using the Skip-Length Model

As described earlier in Section 2.2.1 (Equation 2.10), the physical optical density or absorbance \( D \) of the liquid media is commonly defined as the logarithm to the base 10 of the ratio of the light intensity incident on the media \( (I_o) \) to the light intensity transmitted by the media \( (I) \). The light intensity transmitted therefore can be expressed as

\[
I = I_o e^{-D}
\]  
(6.23)

where \( D = \ln \frac{I_o}{I} \).

By definition (Equation 2.10), the relative absorbance of an absorbing species with respect to clear water can be written as

\[
DR = \log_{10} \frac{I_w}{I_s}
\]  
(6.24)

where \( I_w \) and \( I_s \) are respectively the light intensity transmitted by clear water and the absorbing species, and both can be expressed as

\[
I_w = I_o e^{-D_w} \quad \text{and} \quad I_s = I_o e^{-D_s}
\]  
(6.25)

Furthermore, by using Equation 6.25, Equation 6.24 becomes

\[
DR = \frac{D_s - D_w}{\ln(10)}
\]  
(6.26)

Following Beer's Law, described previously in Section 2.2.1, that physical absorbance is directly proportional to the path length \( (r) \) through the solution and the concentration of the absorbing species \( (c) \) (Skoog and Leary, 1992). This is shown mathematically by

\[
D = \alpha c r
\]  
(6.27)

where \( \alpha \) is a proportionality constant called the absorptivity (introduced in Equation 2.14, Section 2.2.1); and the product of absorptivity, \( \alpha \), and concentration, \( c \), is called the absorption coefficient, \( a \), (i.e., \( a = \alpha c \)). By replacing the path length \( (r) \) with the fibre absorption length, \( L_{abs} \) (Equation 6.22), Equation 6.27 becomes

\[
D = a L_{abs}
\]  
(6.28)

or
\[ D = \frac{a \,(FIL) \lambda_0}{\pi \, d \sqrt{NA^2 - \sin^2 \theta_L}} \quad (6.29) \]

As shown in Equation 3.27 (Section 3.3.2), the numerical aperture in Equation 6.29 can be calculated in terms of the PCS optical fibre used (NAo), the refractive index of the optical fibre core (n1), of the cladding (n2) and of liquid media (nm) surrounded the PCS fibre (DeGrandpre and Burgess, 1990). This is expressed mathematically as

\[ NA = \sqrt{n_1^2 - n_m^2} \quad (6.30) \]

As will be described in more detail in the Section 6.3.1, nm is a function of chlorophyll-pigment concentration and will be replaced by n(c). While, the absorption coefficient (a) will be replaced by the net absorption (A(c)) which depends on the two parameters (β and γ which will be determined later in Section 6.3.2) and concentration (c). This can be modelled by the linear relationship:

\[ A(c) = \beta + \gamma \, c \quad (6.31) \]

Where the underlying assumption is that there is a pigment-independent coefficient (β) such as would result from the water itself interacting with the guided radiation, and a pigment-dependent coefficient (γ) such as would result from the absorptivity of the pigment and the refractive index changes associated with the pigment and the water.

For distilled water, the net absorption (Equation 6.31) becomes \( A(0) = a_m = \beta \); and for absorbing species (i.e. chlorophyll-related pigment in distilled water), Equation (6.31) becomes \( A(c) = a_\alpha = \beta + \gamma \, c \).

The physical FEFA absorbance (D) in Equation 3.29, is then expressed by

\[ D = \frac{(\beta + \gamma \, c) \,(FIL) \lambda_0}{\pi \, d \sqrt{n_1^2 - n(c)^2 - \sin^2 \theta_L}} \quad (6.32) \]

Following Equation 6.26, the relative FEFA absorbance as a function of concentration \( c \), fibre interaction length (FIL), fibre core diameter (d) and light launch angle (\( \theta_L \)) is written as

\[ DR(c, \, FIL, \, d, \, \theta_L) = \frac{D(c, \, FIL, \, d, \, \theta_L) \cdot D(0, \, FIL, \, d, \, \theta_L)}{\ln(10)} \quad (6.33) \]
Chapter 6: Investigating the Sensitivity of FEFA –
a Theoretical and Experimental Parametric Study

Hence, incorporating Equation 6.32, the relative FEFA absorbance becomes

$$\text{DR}(c, \text{FIL}, d, \theta_L) = \frac{(\text{FIL})\lambda_0}{\pi d \ln(10)} \left[ \frac{(\beta + \gamma c)}{\sqrt{n_i^2 - n(c)^2} - \sin^2\theta_L} - \frac{\beta}{\sqrt{n_i^2 - n(0)^2} - \sin^2\theta_L} \right]$$

(6.34)

Equation 6.34 allows calculation of absorbance given the pigment-dependent and pigment-independent coefficients ($\gamma$ and $\beta$) of the liquid media, the liquid concentration ($c$), the fibre interaction length (FIL), free-space wavelength ($\lambda_0$), fibre core diameter ($d$), refractive indices of PCS fibre core ($n_i$) and liquid media ($n(c)$), and light launch angle, $\theta_L$.

6.3. Initialising the Skip-Length Model

The first step in using the skip-length model to calculate the absorbance in response to varying pigment concentration, fibre interaction length, optical fibre core diameter and light launch angle, is to determine the liquid refractive index as a function of concentration ($n(c)$) and net absorption parameters ($a_w$ and $a_a$). These initialisation data were acquired through simple experimental measurements described in the following sections.

6.3.1. Refractive Index of Distilled Water Containing Chlorophyll-Related Pigment

The refractive index of samples of distilled water containing the chlorophyll-related pigment was measured using an automatic temperature-compensating sugar/brix refractometer (ATC Model 23286, Japan). The hand-held refractometer, shown in Figure 6.3, is designed to measure %-Brix, a measure of sugar concentration (in mg sugar per g water). However, conversion of %-Brix to absolute refractive index is readily achieved through a standard conversion table (reproduced in Appendix 1).
The measured values of % Brix, and subsequent liquid refractive indices for the chlorophyll-related pigment concentrations used in this work are listed in Table 6.1. These data are also graphically depicted in Figure 6.4. The graph shows that the refractive index increases quadratically with increasing chlorophyll-a concentration, which is in keeping with general principles (for example, Meeten and North, 1995; Powitz and Balsamo Jr., 2001).

Table 6.1: Measured refractive index of chlorophyll-related pigments in clear distilled water using the ATC Refractometer

<table>
<thead>
<tr>
<th>Chl-a Concentration (mg/L)</th>
<th>% Brix</th>
<th>Obtained Refractive Index, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.332986</td>
</tr>
<tr>
<td>0.83</td>
<td>0.9</td>
<td>1.334276</td>
</tr>
<tr>
<td>1.66</td>
<td>2.0</td>
<td>1.335864</td>
</tr>
<tr>
<td>2.49</td>
<td>2.8</td>
<td>1.337028</td>
</tr>
<tr>
<td>3.32</td>
<td>3.4</td>
<td>1.337905</td>
</tr>
<tr>
<td>4.15</td>
<td>3.8</td>
<td>1.338492</td>
</tr>
</tbody>
</table>
By using a quadratic model and values of regression coefficients listed in Figure 6.4, the refractive index of absorbing species, as a function of the concentration, can be written as

\[ n(c) = 1.330144 + 0.156554 \times 10^4 \sqrt{0.314128 \times 10^9 + 0.638758 \times 10^{12} c} \]  

(6.35)

Equation 6.35 shows that when \( c = 0 \), the refractive index of water \( n(0) = 1.332919 \), and when \( c = 4.15 \times 10^3 \) kg/m\(^3\), the refractive index of chlorophyll-a pigment in water \( n(4.15 \times 10^3) = 1.338668 \).

### 6.3.2. Estimating Net Absorption Parameters \( a_w \) and \( a_o \)

The net absorption parameters \( a_w \) and \( a_o \) can be estimated from measuring the transmittance of the optical fibre as a function of light launch angle, \( \theta_L \). The light launch angle is defined as the angle between the direction of the incident light ray and the normal of the fibre tip (refer to Figure 6.2).
In this experiment, the FEFA was held at a fixed position and the light source rotated to provide varying angles of radiation input into the fibre. Modifications to the FEFA apparatus, depicted in the photograph of Figure 6.5, were made to the method of coupling the output light of laser diode into the PCS optical fibre of 1000 μm core diameter (Table 6.2).

![Figure 6.5: Additional modification to the FEFA apparatus to allow variation, for measuring the transmission of laser diode as a function of light launch angle (θ_L)](image)

Following Figure 6.5, a laser diode (Laser Diode Module, Model Number: 623-301, United Kingdom) of wavelength 670 nm (A) was used as the light source and was mounted on the rotating arm of a wavelength spectrometer (Griffin and George Ltd., London, Britain) (E). The light beam output of the laser diode was directed into the input end of the FEFA optical fibre (C). At a point coincident with the rotation axis of the wavelength spectrometer, the input end of the FEFA optical fibre (C) was mounted in a precision optical fibre positioner (Newport, Model FP-1) (B). This arrangement allowed the laser diode source to be rotated around the tip of the FEFA fibre, and the angle relative to the normal of the FEFA fibre to be accurately measured using a vernier scale (F) to within 1 minute of arc. The optical PCS fibre used in this initialising experiment had a core diameter of 1000 μm and a FIL of 0.1 m (D). For every change in the light launch angle, light power of laser diode emerging from the FEFA PCS optical fibre was measured using a digital photometer (Industrial Fiber Optics, USA) (G) and transmission calculated using
Chapter 6: Investigating the Sensitivity of FEFA –
a Theoretical and Experimental Parametric Study

\[ T(\theta_t)_{670nm} = \frac{P_{\text{liquid}}(\theta_t)}{P_{\text{air}}(\theta_t)} \]  \hspace{1cm} (6.36)

where \( P_{\text{liquid}} \) is the power emerging from the fibre when suspended in either clean distilled water or distilled water containing the chlorophyll-related pigments, and \( P_{\text{air}} \) is the power emerging from the fibre when suspended in air. For the experimental measurements of transmission versus light launch angle, clean distilled water (0 kg/m³) and 4.15 x 10⁻³ kg/m³ chlorophyll-related pigment in distilled water were used. The measured transmission versus light launch angle for both the clean distilled water and the water containing chlorophyll-related pigments is given in Figure 6.6.

![Light Transmission vs. Launch Angle](image)

**Figure 6.6:** Relationship between light transmission and launch angle for distilled water containing: 0 mg/L and 4.15 mg/L chlorophyll-related pigment.

Figure 6.6 shows that for both distilled water and water containing chlorophyll-related pigment, the light transmission decreases rapidly in the launch angle range from approximately 20° to 25°. This corresponds to the PCS fibre critical launching angle of approximately 22°, which corresponds to the critical for internal reflection at the core-water interface. Furthermore, the displacement between the two curves in Figure 6.6 implies that
the absorbance is higher for distilled water containing chlorophyll-related pigment than for pure distilled water itself.

6.3.2.1. Parameter $a_w$ for Distilled Water

As shown earlier in Equation 6.25, the output light intensity for the FEFA fibre immersed in water can be written as

$$I_o = I_o e^{-D_w}$$  \hspace{1cm} \text{(6.37)}

where $D_w$ is the absorbance due to the distilled water which is characterised by parameter $a_w$, and is expressed as

$$D_w = \frac{a_w (\text{FIL}) \lambda_0}{\pi d \sqrt{n_i^2 - n(0)^2} \cdot \sin^2 \theta_L}$$  \hspace{1cm} \text{(6.38)}

Using Equation 6.38, the input light intensity, $I_o$, in Equation 6.37 can be estimated from the zero launch angle output of the distilled water ($I_o(\theta_L = 0)$) as

$$I_o = \frac{I_o(\theta_L = 0)}{\exp \left[ -\frac{a_w (\text{FIL}) \lambda_0}{\pi d \sqrt{n_i^2 - n(0)^2} \cdot \sin^2 \theta_L} \right]}$$  \hspace{1cm} \text{(6.39)}

The value of $I_o(\theta_L = 0)$ is chosen at the first data point in Figure 6.6 for $\theta_L = 0$. Hence, the parameter $a_w$ is now chosen to fit the observed transmission as a function of light launch angle data (Figure 6.6) for water ($c = 0$) using the least square error function (Kreyszig, 1999) as follows:

$$E(a_w) = \sum_i \left[ \frac{I_o(\theta_L = 0) \exp \left[ -\frac{a_w (\text{FIL}) \lambda_0}{\pi d \sqrt{n_i^2 - n(0)^2} \cdot \sin^2 \theta_L} \right]}{\exp \left[ -\frac{a_w (\text{FIL}) \lambda_0}{\pi d \sqrt{n_i^2 - n(0)^2} \cdot \sin^2 \theta_L} \right]} \right]^2$$  \hspace{1cm} \text{(6.40)}

where the sum is taken over all sub-critical model launch angles.

Then, by solving $\frac{\partial E(a_w)}{\partial a_w} = 0$, the least square estimate for the bulk absorption parameter for distilled water can be determined as $a_w = 103661.07$. 


6.3.2.2. Parameter \( a_s \) for Water Containing Chlorophyll-Related Pigment

As described previously in Section 6.2.3, the net absorption parameter for distilled water containing chlorophyll-related pigment is characterised by \( a_s \). The parameter \( a_s \), which is determined in the same manner as for \( a_w \), is chosen to fit the observed transmission as a function of light launch angle data (Figure 6.6) for water containing chlorophyll-related pigment \((c = 4.15 \times 10^{-3} \text{ kg/m}^3)\). In this case, the least square error function (Kreyszig, 1999) as follows:

\[
E(a_s) = \sum_i \left[ I_i^{(ch)} - I_0 \exp \left\{ -\frac{a_s (\text{FIL}) \lambda_0}{\pi d \sqrt{n_1^2 - n(c)^2 - \sin^2 \theta_c}} \right\} \right]^2
\]

(6.41)

where \( I_0 \) is determined by Equation 6.39.

After solving for \( \frac{\partial E(a_s)}{\partial a_s} = 0 \), the parameter \( a_s \) is estimated as 102136.45.

The coefficients \( \beta \) and \( \gamma \) in the net absorption model (Equation 6.31) can now be estimated from \( a_w \) and \( a_s \), as follows:

\[
a_w = A(0) = \beta, \quad \text{and} \]

\[
a_s = A(c) = \beta + \gamma c
\]

(6.42)

Hence, solving for \( \beta \) and \( \gamma \):

\[
\beta = a_w, \quad \text{and}
\]

\[
\gamma = \frac{a_s - a_w}{c}
\]

(6.43)

(6.44)

From the parameter values estimated earlier for \( a_w \) and \( a_s \), and using Equations 6.42 and 6.44, and \( c = 4.15 \times 10^{-3} \text{ kg/m}^3 \), the values of the pigment-dependent and pigment-independent coefficients of the net absorption parameter are respectively: \( \gamma = -367378.48 \) and \( \beta = 103661.07 \).

The net absorption \((A(c))\) which is determined by coefficients \( \beta \) and \( \gamma \) will decrease when the chlorophyll concentration increases. This is caused by the dominant negative-coefficient \( \gamma \). Physically, this is most likely due to the fibre rejecting modes as the refractive index of the surrounding liquid increases with concentration of chlorophyll. Using Equations 3.7 and 3.8, for distilled water the fibre guides about 52,000 more modes than with distilled water.
containing chlorophyll-related pigment, where the refractive indices of fibre core, water and water containing chlorophyll are respectively 1.457, 1.333 and 1.338, the fibre core diameter and free-space wavelength are respectively 600 µm and 670 nm. As the refractive index of the liquid increases with increasing chlorophyll-related pigment concentration, the modes that will be progressively rejected will be the higher order ones, i.e. the ones that have a shorter skip-length. These higher order modes have greater penetration depth (since the launch angle is effectively higher). Thus, as the pigment concentration increases, one would expect the FEFA absorbance to increase (based on penetration depth remaining constant), except now the fibre rejects the higher-penetration-depth rays (higher order modes) and the remaining lower-penetration-depth rays have less absorbing path. This pushes the net effect in reverse, i.e. the FEFA absorbance decreases since the net absorption (A(c)) decreases.

6.4. Experimental Parametric Measurements and Comparison with Skip-Length Model

The experimental parametric study of the FEFA technique was completed by using pure core silica (PCS) optical fibres of four different core diameters including the 600 µm core diameter used earlier in Chapter 5. The preparation of these fibres, and the sample cell used to contain the chlorophyll-related pigment, are the same as that described earlier in Section 5.2. The four different PCS fibres, which all have a numerical aperture of 0.370, core refractive index of 1.457 (hence cladding refractive index of 1.409) and operating wavelength range of 500 – 1100 nm, are described in Table 6.2.

Seven different lengths of exposed fibre core (fibre interaction length, FIL) for each PCS optical fibre were used in the FEFA evaluation; 2 cm, 4 cm, 6 cm, 8 cm, 10 cm, 12 cm and 14 cm (and 16 cm for the 1000 µm core diameter).
Table 6.2: Multimode PCS optical fibres used in the parametric study (Extracted from Newport, 2000)

<table>
<thead>
<tr>
<th>Model (Newport)</th>
<th>Diameter of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Core (µm)</td>
</tr>
<tr>
<td>F-MBB</td>
<td>200 ± 4</td>
</tr>
<tr>
<td>F-MBC</td>
<td>400 ± 8</td>
</tr>
<tr>
<td>F-MBD</td>
<td>600 ± 10</td>
</tr>
<tr>
<td>F-MBE</td>
<td>1000 ± 15</td>
</tr>
</tbody>
</table>

The regulated white light source used and the fibre optic cable and SD1000 spectrometer combination are the same as that described earlier in Section 5.3 (Chapter 5). However, for investigating the relationship between absorbance and light launch angle, the apparatus was modified. This will be discussed in the relevant following section.

The observed influences of physical parameters including chlorophyll-related pigment concentration, fibre interaction length, PCS optical fibre core diameter and light launch angle on absorbance are presented and compared with the predictions of the skip-length model (Equation 6.34) in the following sections.

6.4.1. Chlorophyll-Related Pigment Concentration

Figure 6.7 is a graph of measured absorbance versus pigment concentration for the 600 µm core diameter fibre and a FIL of 0.1 m. The solid line is that produced from Equation 6.34 using values of input parameters summarised in Table 6.3, and using refractive index (n(c)) defined in Equation 6.35. The difference between the measured and predicted relative absorbance is quantified using the average root-mean-square error (RMSE), where

$$\text{RMSE} = \sqrt{\frac{\sum(n\text{ (experimental absorbance - theoretical absorbance)}^2}{n}} \quad (6.45)$$

In Figure 6.7 the RMSE is 0.007.
Table 6.3: Input parameters used to theoretically predict relative absorbance versus pigment concentration in Figure 6.6

<table>
<thead>
<tr>
<th>Input parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk parameter for water, $\gamma$</td>
<td>-367378.48</td>
<td>$\text{m}^2\text{kg}^{-1}$</td>
</tr>
<tr>
<td>Bulk parameter for chlorophyll, $\beta$</td>
<td>103661.07</td>
<td>$\text{m}^{-1}$</td>
</tr>
<tr>
<td>Fibre interaction length, FIL</td>
<td>0.100</td>
<td>$\text{m}$</td>
</tr>
<tr>
<td>Wavelength (free-space), $\lambda_{\infty}$</td>
<td>$6.70 \times 10^{-7}$</td>
<td>$\text{m}$</td>
</tr>
<tr>
<td>Diameter of PCS fibre, $d$</td>
<td>$6.00 \times 10^{-4}$</td>
<td>$\text{m}$</td>
</tr>
<tr>
<td>Core refractive index, $n_1$</td>
<td>1.457</td>
<td></td>
</tr>
<tr>
<td>Light launch angle, $\theta_l$</td>
<td>24.20</td>
<td>Degrees</td>
</tr>
</tbody>
</table>

Figure 6.7: Absorbance at 670 nm as a function of chlorophyll-a pigment concentration in clear distilled water. The points are experimental data and the line represents the calculated absorbance using Equation 6.34.

The trend of Figure 6.7 is that both calculated and measured absorbance increases with increasing pigment concentration. This is consistent with observations in other different
liquid media (i.e. Villarruel et al., 1987; Ruddy et al., 1990; Bornstein et al., 1991; Gupta et al., 1994; Schwotzer et al., 1997; Mignani et al., 1997). In these experimental data, and in the research of the previously cited authors (the data of Bornstein et al., 1991 are reproduced in Figure 6.8 for comparison) the observed dependence of the evanescent absorbance on pigment concentration deviated considerably from Beer-Lambert-type linear dependence. In fact the shape of the experimental data in Figure 6.7 is wholly consistent with the model predictions based on the fact that the net absorbance parameter ($A(c)$) in the model decreases with increasing pigment concentration. Previous authors have concentrated their explanation of the deviation from linearity of FEFA absorbance versus pigment concentration on surface adsorption caused by electrostatic interactions between the polar silica surface of the fibre and the ionic solution (e.g. Ruddy et al., 1990) and Debye-Hückel’s approximation of absorbance versus concentration. While these are nevertheless legitimate sources of such a deviation, in this work the skip-length model suggests fibre-mode-rejection to be another, if not the primary source, of this deviation.

![Figure 6.8: Absorbance as a function of acetone concentration in clear freon using attenuated total reflection spectroscopy with chalcogenide bi-tapered fibre (Data extracted from Bornstein et al., 1991).](image)
6.4.2. Optical Fibre Interaction Length

Table 6.4 summarises the input data used to calculate the absorbance as a function of fibre interaction length (FIL). Figure 6.9 depicts both the calculated and measured absorbance values for a range of fibre optic interaction lengths (0 – 0.15 m). The RMSE of Figure 6.9, is 0.007.

The theoretical relative absorbance calculated using Equation 6.34 and the measured absorbance both increase linearly with increasing PCS fibre interaction length as predicted by Beer's law. The increase of calculated absorbance with increasing FIL is attributable to the increase in the total number of internal reflections (Equation 6.20) occurring at the core-liquid interface of the optical fibre. The linear relationship between absorbance and FIL has also been observed by Simhony et al. (1988), Ruddy et al. (1990), Katz et al. (1994) and Messica et al. (1996). For comparison, the data of, Ruddy et al. (1990) are reproduced in Figure 6.10.

Table 6.4: Input parameters used to calculate relative absorbance versus fibre interaction length

<table>
<thead>
<tr>
<th>Input parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk parameter for water, $\gamma$</td>
<td>-367378.48</td>
<td>m$^2$kg$^{-1}$</td>
</tr>
<tr>
<td>Bulk parameter for chlorophyll, $\beta$</td>
<td>103661.07</td>
<td>m$^4$</td>
</tr>
<tr>
<td>Pigment concentration, $c$</td>
<td>4.15 x 10$^3$</td>
<td>kg.m$^{-3}$</td>
</tr>
<tr>
<td>Wavelength (free-space), $\lambda_0$</td>
<td>6.70 x 10$^{-7}$</td>
<td>m</td>
</tr>
<tr>
<td>Diameter of PCS fibre, $d$</td>
<td>6.00 x 10$^{-4}$</td>
<td>m</td>
</tr>
<tr>
<td>Core refractive index, $n_1$</td>
<td>1.457</td>
<td>-</td>
</tr>
<tr>
<td>Solvent refractive index, $n(c)$</td>
<td>1.338</td>
<td>-</td>
</tr>
<tr>
<td>Light launch angle, $\theta_l$</td>
<td>23.90</td>
<td>Degrees</td>
</tr>
</tbody>
</table>
Figure 6.9: Absorbance at 670 nm of chlorophyll-a pigment in clear distilled water as a function of fibre interaction length. The points are experimental data and the line represents the calculated relative absorbance using Equation 6.34.

Figure 6.10: Absorbance at 664 nm of methylene blue as a function of fibre interaction length. (Data extracted from Ruddy et al., 1990).
6.4.3. Optical Fibre Core Diameter

Table 6.5 summarises the model data used to calculate relative absorbance as a function of fibre core diameter (d). Figure 6.11 depicts both the calculated and measured absorbance for a range of fibre core diameters (0.2 – 1.0 mm). The RMSE of Figure 6.11 is 0.023.

Both the theoretical and measured values of relative absorbance show a similar relationship, where the absorbance increases significantly with a decrease in PCS optical fibre core diameter. The absorbance is predicted by Equation 6.34 to be inversely proportional to fibre core diameter. Following the skip-length model, as the fibre core diameter is reduced there is an increase in the number of internal reflections of the guided radiation propagating along a given length of optical fibre. This is depicted in Figure 6.12. Similar experimental observations have been reported by, Katz et al. (1991, 1991a), and Messica et al. (1996). For comparison, the results of Messica et al. (1996) are reproduced in Figure 6.13.

<table>
<thead>
<tr>
<th>Input parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk parameter for water, $\gamma$</td>
<td>-367378.48</td>
<td>m$^2$ kg$^{-1}$</td>
</tr>
<tr>
<td>Bulk parameter for chlorophyll, $\beta$</td>
<td>103661.07</td>
<td>m$^{-1}$</td>
</tr>
<tr>
<td>Pigment concentration, $c$</td>
<td>$4.15 \times 10^{-3}$</td>
<td>kg.m$^{-3}$</td>
</tr>
<tr>
<td>Fibre interaction length, FIL</td>
<td>0.100</td>
<td>m</td>
</tr>
<tr>
<td>Wavelength (free-space), $\lambda_0$</td>
<td>$6.70 \times 10^{-7}$</td>
<td>m</td>
</tr>
<tr>
<td>Core refractive index, $n_1$</td>
<td>1.457</td>
<td>-</td>
</tr>
<tr>
<td>Solvent refractive index, $n(c)$</td>
<td>1.338</td>
<td>-</td>
</tr>
<tr>
<td>Light launch angle, $\theta_l$</td>
<td>20.40</td>
<td>Degrees</td>
</tr>
</tbody>
</table>
Figure 6.11: Absorbance at 670 nm of 4.15 mg/L chlorophyll-a pigment in clear distilled water as a function of optical fibre (OF) core diameter. The points are experimental data and the line represents the absorbance calculated using Equation 6.34.

Figure 6.12: A propagating ray suffers a greater number of internal reflections for a given FIL in smaller-diameter fibre cores.
6.4.4. Light Launch Angle

In order to observe the effect of varying the angle at which light is launched into the FEFA fibre, the FEFA apparatus was again modified. Following Section 6.3.2 the light launch angle is defined as the angle between the direction of the incident light ray and the normal of the fibre tip. Similar to Section 6.3.2, modifications to the FEFA apparatus, depicted in the photograph of Figure 6.14, were made to the method of coupling the white-light from the source into the PCS optical fibre.

Following Figure 6.14, the white light source used in Chapters 4 and 5 was first directed into a short bundle of plastic optical fibre (A) (The Optometrics Group, Catalogue Number: 37-2507, 1993) mounted on the rotating arm (E) of the same wavelength spectrometer described in Section 6.3.2. The white light, on exiting the fibre bundle was then collimated using the 1.5 cm focal length lens (B). At a point coincident with the rotation axis of the wavelength spectrometer, the input end of the FEFA optical fibre (D) was again mounted in a precision optical fibre positioner (C) (Newport, Model FP-1). The angle of incidence, relative to the normal of the FEFA fibre could again be accurately measured using the integral vernier scale (F) to within 1 minute of arc.
For the experimental measurements of absorbance versus light launch angle, the $4.15 \times 10^{-3}$ kg/m$^3$ concentration of chlorophyll-related pigments in clear distilled water was used, with a 1000 μm core diameter PCS fibre and FIL of 0.1 m. At each launch angle the lateral position of the FEFA fibre tip was adjusted using the FP-1 positioner to provide maximum light output at the SD-1000 spectrometer. Each absorbance measurement was completed following the protocol described earlier (Section 4.3.3).

The measured absorbance, coincident with the RED absorption peak, is plotted as a function of light launch angle in Figure 6.15. The theoretical absorbance versus light launch angle, calculated using the skip-length model (Equation 6.34) and the parameters in Table 6.6, is also plotted in Figure 6.15 (BLUE line). The RMSE between the skip-length model calculations and the measured data of Figure 6.15, calculated over $0 \leq \theta_L \leq 22^\circ$, is approximately 0.011 (RMSE$_i$).
Table 6.6: Input parameters used to calculate relative absorbance versus light launch angle

<table>
<thead>
<tr>
<th>Input parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk parameter for water, $\gamma$</td>
<td>-367378.48</td>
<td>m$^2$ kg$^{-1}$</td>
</tr>
<tr>
<td>Bulk parameter for chlorophyll, $\beta$</td>
<td>103661.07</td>
<td>m$^{-1}$</td>
</tr>
<tr>
<td>Pigment concentration, $c$</td>
<td>$4.19 \times 10^{-3}$</td>
<td>kg.m$^{-3}$</td>
</tr>
<tr>
<td>Fibre interaction length, FIL</td>
<td>0.100</td>
<td>m</td>
</tr>
<tr>
<td>Wavelength (free-space), $\lambda_0$</td>
<td>$6.70 \times 10^{-7}$</td>
<td>m</td>
</tr>
<tr>
<td>Diameter of PCS fibre, $d$</td>
<td>$1.00 \times 10^{-3}$</td>
<td>m</td>
</tr>
<tr>
<td>Core refractive index, $n_1$</td>
<td>1.457</td>
<td>-</td>
</tr>
<tr>
<td>Solvent refractive index, $n(c)$</td>
<td>1.338</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 6.15: Absorbance at 670 nm of 4.15 mg/L chlorophyll-a pigment in clear distilled water as a function of light launch angle. The points are experimental data and the BLUE line represents the theoretical calculation from Equation 6.34, the BLACK line represents the modifying model of Equation 6.45.
Figure 6.15 shows that the measured absorbance increases with increasing light launch angle up to an angle of approximately 23°, and then becomes significantly smaller at angles higher than this value. The critical angle for total internal reflection at the core-cladding interface of the non-FEFA sections of the PCS fibre is 75°. This corresponds to a light launch angle of approximately 22°. However, the critical angle for total internal reflection at the core-liquid interface of the FEFA section of the PCS fibre (where the liquid refractive index is approximately 1.338) is approximately 67°. This corresponds to a light launch angle of about 35°. Therefore, although the section of the fibre whose core is directly immersed in liquid is capable of carrying rays at a higher launch angle, the critical angle is exceeded in the non-FEFA section of the fibre in which the light is originally launched.

The skip-length model predicts a vertical asymptote in the absorbance of chlorophyll-related pigment in distilled water at approximately 35°, while the measurements show a maximum at approximately 23°. This is because the model deals only with that section of the PCS fibre where the core is directly immersed in the liquid, and does not account for the different launch requirements of the unmodified section of the PCS fibre (that is, with the plastic rather than liquid cladding).

The maximum measured absorbance occurs at a light launch angle of approximately 23° which is close to the PCS fibre critical launching angle of approximately 22°. This mismatch is caused by the cylindrical geometry of the fibre itself (Gupta et al., 1994) as the fibre can guide both meridional rays (rays which cross the central axis of the fibre) and skew rays (rays which do not cross the central axis, but instead tend to follow a helical-type path along the fibre) (Stewart et al., 1991; Keiser, 2000).

An analysis of skew rays (Snyder et al., 1974; Stewart et al., 1991) shows that they are less sensitive to an external absorber than meridional rays, depending on the degree of skewness. However, they will change the light-acceptance ability of the fibre and power losses of light travelling along the fibre (Keiser, 2000). This is probably deduced from the fact that for a given angular position of the fibre axis, the skew ray strikes the core-fluid interface at a more glancing angle, reducing the penetration of evanescent-field into the absorbing media. However the number of reflections per unit length is somewhat greater for the skew ray
which partially compensates for the reduced field penetration and so, overall, the skew ray sensitivity is about 30-70% that of a meridional ray (Stewart et al., 1991). Therefore, an alternative approach is to use a singlemode fibre, or to use multimode fibre without any cladding.

Furthermore, the skip-length model is based on a single ray propagating along the fibre at a single angle of incidence corresponding to its particular launch angle. However, the white light launched into the PCS fibre in this apparatus is not perfectly collimated, and was estimated to have a divergence in excess of 12°. The difference in absolute values of measured and calculated relative absorbance, at light launch angles ranging from 0° to 22° in Figure 6.15, is most likely due to the imperfectly collimated beam of white light. In particular, for light launch angles close to 0°, the presence of impurities in the PCS fibre core may cause some scattering of these small-angle rays to greater angles (Gupta et al. 1994). By further modifying the skip-length model in Equation 6.34 to simulate a finite-width gaussian beam with a half angle of 6°, the absorbance at a light launch angle of 0° increases and the RMSE decreases to 0.005 (RMSE₂) as depicted in Figure 6.15 (BLACK line). In this case, the modified skip-length model becomes

\[
DR_{M}(c, FIL, d, \theta_{l}) = \int_{35}^{35} DR(c, FIL, d, \theta_{v}) \exp\left(-\frac{(\theta_{l} - \theta_{c})^2}{\theta_{*}^2}\right) d\theta_{l}
\]

(6.46)

where \(DR(c, FIL, d, \theta_{l})\) is Equation 6.34, and \(\theta_{c}\) and \(\theta_{v}\) are, respectively, the PCS fibre critical launching angle and the beam width angle.

The non-zero absorbance at a launch angle of 0° in Figure 6.15 was also observed by Gupta et al. (1994) who measured absorbance of methylene blue dye versus light launch angle. These results are reproduced in Figure 6.16.
Figure 6.16: Absorbance of methylene blue as a function of light launch angle. (Data extracted from Gupta et al., 1994).

Other workers have attempted to directly account for meridional rays, such as in the meridional ray model of Ruddy et al. (1990). Ruddy et al. (1990) developed an alternative expression for the evanescent absorption coefficient, γ, (equivalent to the optical density, D) based on the equation of Snyder and Love (1983)

\[
\gamma = N T
\]  

(6.47)

where \( N \) is the number of reflections per unit length of the fibre and \( T \) is the Fresnel transmission coefficient at the interface of a lossless core and a lossy cladding. Ruddy et al. (1990), Gupta et al. (1994), Gupta and Singh (1996) all showed that, in the case of a silica core in contact with an aqueous solution,

\[
\gamma = \frac{\alpha c n_e \lambda c \cos \theta \cot \theta}{2\pi d n_i^2 \cos^2 \theta_c \sqrt{\sin^2 \theta - \sin^2 \theta_c}}
\]  

(6.48)

where \( \theta \) and \( \theta_c \) are respectively the ray angle and critical angle at the core-liquid interface. For comparison, Equation 6.48, when rewritten using the nomenclature of the skip-length model, gives
\[
D = \frac{(\beta^* + \gamma^* c)(FIL)\lambda_o n(c) - \sin^2 \theta_L}{2\pi d (n_i^2 - n(c)^2) \sqrt{n_i^2 - n(c)^2 - \sin^2 \theta_L}}
\]

Equation 6.49 predicts the same relationships as the skip-length model (Equation 6.34) between relative FEFA absorbance and concentration of absorbing species, fibre interaction length and fibre core diameter. However, the launch angle dependence is different. The data and skip-length model calculations of Figure 6.15 are replotted in Figure 6.17 along with the calculated relative FEFA absorbance using the meridional-ray model of Gupta et al. (1994) (Equation 6.49). The input values used for the meridional-ray model were also taken from Table 6.6, except for the values of bulk absorption parameter \(\beta^*\) and \(\gamma^*\). By follow the same procedure as in Section 6.3.2 for initialising the meridional-ray model, the values of the concentration-independent and concentration-dependent coefficients of the net absorption parameter are estimated as \(\beta^* = 123928.18\) and \(\gamma^* = 801923.98\).

![Figure 6.17: Measured absorbance at 670 nm of 4.15 mg/L chlorophyll-a pigment in clear distilled water as a function of light launch angle. The points are experimental data and the lines represent the theoretical calculations of the skip-length model (—) and the meridional-ray model (——) of Gupta et al. (1994). The RMSE of the skip-length model is given by RMSE1 and the RMSE of the meridional-ray model is given by RMSE2.](image-url)
The meridional-ray model fails to predict the shape of the absorbance-light launch angle curve, and an increase in the RMSE from 0.011 to 0.014 over $0 \leq \theta_L \leq 22^\circ$ results. Like the skip-length model, the meridional-ray model also only deals with the fibre core-liquid cladding section of the fibre and does not account for the light exceeding the critical angle for the fibre core-plastic cladding interface in the section of the fibre where it is initially launched. Therefore the same deviation from calculated versus measured absorbance at angles in excess of $22^\circ$ also occurs. Furthermore, the meridional model predicts an absorbance of zero at a light launch angle of $0^\circ$.

6.5. Estimating the Sensitivity of FEFA to Suspended Sediment

Using the skip-length model, and in particular the penetration depth, $d_p$ (Equation 6.12), it is now possible to understand the reason for the observations of Section 5.4.2, where the evanescent-field absorption is sensitive to pigment concentration but insensitive to suspended sediment. Using Equation 6.12 with $\lambda_o = 670$ nm, a value of $n_i$ calculated using Equation 6.35 to correspond to a chlorophyll-related pigment concentration of $4.15 \times 10^3$ kg/m$^3$, and a light launch angle close to critical angle ($35^\circ$), the penetration depth achievable using the existing PCS fibre is approximately 1.77 $\mu$m.

If the clay particles used as suspended sediment are assumed to be spherical, and their average diameter, $d$, as a result of grinding and sifting is 120 $\mu$m (Section 4.2.2), the mass of individual clay particles is then

$$ m = \rho \frac{1}{6} \pi d^3 $$

(6.50)

where $\rho$ is the density of silica, taken to be $\rho = 2.33 \times 10^3$ g/L (CRC Handbook, 1971).

The total mass of clay introduced into the liquid is $M = N m$, where $N$ is the number of clay particles in the sample. Therefore $N$ is given by

$$ N = \frac{6 M}{\rho \pi d^3} $$

(6.51).
If, when introduced into a cubic volume \( V \) of liquid of side length of \( L \), the clay particles are evenly distributed throughout the liquid, then the number of particles per unit length in one dimension can be approximated by

\[
N_L = \frac{\sqrt[N]{N}}{L}
\]  

(6.52)

Hence, the distance between particles can be estimated as

\[
x = \frac{L}{\sqrt[N]{N}} = L \cdot d \sqrt{\frac{p\pi}{6M}}
\]  

(6.53)

Therefore, a suspended sediment concentration of 10 g/L, equates to \( M = 10 \) g of clay and \( L = 10 \) cm. The approximate distance, \( x \), between the centres of adjacent clay particles is therefore \( x = 0.61 \) mm. Allowing for a particle diameter of 120 \( \mu m \), this equates to an approximate free-space separation of 0.49 mm. This distance is approximately 277 times greater than the calculated achievable penetration depth of the optical radiation from the PCS fibre core into the liquid.

Following this simple comparison between the penetration depth of probing radiation and average free-space distance between clay particles, it is therefore possible to estimate the concentration of clay particles which could influence the evanescent absorption of any pigment in the water sample. Using Equation 6.53 in reverse, the average free-space distance between adjacent clay particles would be comparable to the penetration depth associated with the PCS fibre core (1.77 \( \mu m \)) when the concentration is approximately 1.20 kg/L. This is an enormous level of sediment loading in any river system, and is approximately two thousand-times higher than values reported in typical Australian inland rivers such as the Murray, Murrumbidgee and Latrobe Rivers (for example, Olive et al., 1994; Grayson et al., 1994; Lamb & O’Donnell, 1996).

In fact, the clay concentrations used in this work (for example in Section 5.4.2) is much greater than those found in the rivers world-wide, for example, 350 mg/L in the surface water of six northern Mississippi Reservoirs (Ritchie et al., 1976); 1500 mg/L in the Minas Basin, Bay of Fundy, Nova Scotia (Munday and Alfoldy, 1979); 141 mg/L in the nearshore zone at Holderness, United Kingdom (Curran et al., 1987); 200 mg/L in Moon Lake,
Mississippi (Ritchie et al., 1987); 700 mg/L in the Lake Chicot, Arkansas (Schiebe et al., 1992); 50 mg/L in the Tawa river, Central India (Choubey and Subramanian, 1992); 410 mg/L in Tarcoola-pond, Australia (Lamb and O'Donnel, 1996); and 500 mg/L in Yellow river, China (Sui et al., 2000).
Chapter 7

Summary and Conclusions

The alteration of "natural" water colour, such as caused by the presence of natural metallic ions, humus and peat materials, plankton and industrial waste, is an important indicator of water quality. Chlorophyll-related pigments are a major source of natural water colour.

The measurement of water colour can be objectively completed using spectrophotometric techniques to quantify the optical absorbance at pre-defined wavelengths. However, natural, fresh waters also contain quantities of suspended sediment, both organic and inorganic. Measuring water colour using spectrophotometric absorption techniques therefore requires that the water samples be filtered or centrifuged in order to remove the suspended material as their presence will cause scattering of the probing radiation and thereby confound the estimation of optical absorption by dissolved pigments alone. The requirements of sample pre-treatment and preparation preclude in-situ measurements and consequently such measurements are usually completed in the laboratory.
Chapter 7: Summary and Conclusions

This thesis has set out to achieve a number of objectives in the evaluation of an optical fibre technique as a possible method of measuring water colour in samples containing suspended material; a technique which would not require sample pre-treatment (filtering or centrifugation). The optical fibre technique, known as fibre-evanescent-field absorption (FEFA) relies on the pigmented sample absorbing radiation from the evanescent field of light propagating within the exposed core of a short length of optical fibre.

In this thesis, a comparison has been made between the single-pass optical absorption of radiation from a light source directed through a sample cell containing chlorophyll-related pigments, and the FEFA technique in an optical system of similar dimensions. In the traditional direct or bulk absorption experiment, a concentration of 2g/L of suspended clay particles in water containing chlorophyll-related pigments was found to have precluded the measurement of the chlorophyll-related absorption due to scattering of all the incident light (Chapter 4). However, by comparison the FEFA technique was found to be sensitive to absorption by the chlorophyll-related pigments in the water, yet insensitive to the presence of suspended clay particles up to and including the maximum introduced concentration of 10 g/L (Chapter 5). Simple calculations, based on the FEFA apparatus used (Chapter 6) subsequently demonstrated that the apparatus would remain insensitive to the introduction of suspended sediment up to a concentration of 1.2 kg/L. Such concentrations are of the order of 1000 times greater than that which would be found in typical freshwater bodies in Australia, or indeed worldwide. These observations support the feasibility of using the FEFA technique as the basis of monitoring water colour in turbid water.

The value of absorbance obtained from the direct absorption technique was approximately one order of magnitude higher than that achieved by using the FEFA technique for the same levels of chlorophyll-related pigments in water. In this work, the direct absorption technique was observed to follow Beer's Law where the absorbance increases linearly with increasing pigment concentration or optical path length through the sample. However, the FEFA technique, while showing a linear response in absorbance with increasing optical path length, or FIL, exhibited a significant deviation from linearity in response to increasing pigment concentration. In the past this has been attributed to liquid-fibre surface phenomena including surface adsorption of the pigment as a result of electrostatic interactions between polar silica surface and the ionic solution.
Chapter 7: Summary and Conclusions

A subsequent experimental investigation of the influence of physical fibre characteristics on the measured absorbance of the FEFA apparatus (Chapter 5) also demonstrated that the FEFA sensitivity increases linearly with increasing fibre interaction length (equivalent to optical path length in the direct absorption technique), inversely proportional with fibre diameter (that is, according to 1/d where d is the fibre diameter) and non-linearly with increasing light launch angle into the fibre.

In order to understand the reasons behind these parametric trends (chlorophyll concentration, FIL, diameter and light launch angle), a mathematical model of the FEFA apparatus was constructed based on the skip-length theory (Chapter 6). The model calculated the evanescent field absorbance resulting from a ray of radiation propagating along the fibre core via numerous internal reflections at the core-liquid interface. At each internal reflection location, the radiation penetrates into the liquid to its penetration depth (and this constitutes the evanescent field) as well as undergoes a small displacement in the along-fibre direction. The net absorbance results from the summation of the penetration-displacement events experienced by the radiation on each internal reflection as it propagates down the fibre. However, unlike similar models of other workers, this model was initially calibrated to account for internal fibre attenuation resulting from coupling the radiation into the fibre and immersing the fibre in clear water.

This enhanced skip-length model successfully reproduced the experimental observations of a linear increase in absorbance observed with increasing FIL, an inverse-proportional increase in absorbance with fibre diameter (that is, 1/d), and, importantly, the non-linear increase in absorbance observed with increasing chlorophyll concentration (Chapter 6). In this particular aspect, the model calculations suggested the departure from linearity of increasing absorbance with increasing pigment concentration resulted from an increase in refractive index of the liquid with increasing chlorophyll concentration, and subsequent rejection of higher-order modes of propagating radiation, that is those which have greater penetration depth, from the fibre.

A complex relationship between measured absorbance and increasing light launch angle was also observed in the experimental parametric study (Chapter 5). Neither the meridional ray model nor the simple skip-length model (where bulk fibre attenuation is not initially
calibrated in the calculations), as proposed by other workers, were able to reproduce these observations. However, the enhanced skip-length model adequately reproduced these trends (Chapter 6). The measured absorbance was also found to decrease with light launch angles exceeding the critical angle for internal reflection at the fibre core-cladding interface. The behaviour of the absorbance characteristic beyond the critical light launch angle is most likely related to the propagation of light directly through the cladding.

Notwithstanding the positive aspects of the FEFA technique as a potential means of measuring water colour in turbid samples, a number of potential limitations to the technique can also be identified in the context of field applications. While insensitive to suspended material, the FEFA technique is one order of magnitude less sensitive to the absorbing species— in this case chlorophyll, than the traditional direct absorption technique. However, the flexibility of optical fibres offers the ability to significantly increase the optical path length in the liquid media by winding them into coils. It must also be recognised that an optical fibre when stripped of its cladding (in this case polymer cladding) is generally less flexible and more susceptible to breaking when bent or wound into a coil. Further investigations into the structural integrity of exposed glass cores are required. Reducing the core diameter of the fibre does increase its sensitivity, although fibres of smaller diameter are more fragile, especially when wound into coils. Alternatives would also include the use of alternative plastic fibres or fibre coatings, for example sol-gel coatings. Regardless of these issues, the use of optical fibres as the basis of an instrument capable of measuring water colour in turbid samples offers numerous advantages over techniques currently in use.
References


References


References


Goodin D. G., Han L., Fraser R. N., Rundquist D. C., Stebbins W. A. and Schalles J. F., (1993), Analysis of suspended solids in water using remotely sensed high


References


References


References


References


References


### Appendix 1

**Relationship between %-Brix and Refractive Index**

<table>
<thead>
<tr>
<th>% Brix</th>
<th>0.0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.332986</td>
<td>1.333129</td>
<td>1.333272</td>
<td>1.333415</td>
<td>1.333558</td>
<td>1.333702</td>
<td>1.333845</td>
<td>1.333989</td>
<td>1.334132</td>
<td>1.334276</td>
</tr>
<tr>
<td>1</td>
<td>1.334420</td>
<td>1.334564</td>
<td>1.334708</td>
<td>1.334852</td>
<td>1.334996</td>
<td>1.335141</td>
<td>1.335285</td>
<td>1.335430</td>
<td>1.335574</td>
<td>1.335719</td>
</tr>
<tr>
<td>2</td>
<td>1.335864</td>
<td>1.336009</td>
<td>1.336154</td>
<td>1.336300</td>
<td>1.336445</td>
<td>1.336590</td>
<td>1.336736</td>
<td>1.336882</td>
<td>1.337028</td>
<td>1.337174</td>
</tr>
<tr>
<td>3</td>
<td>1.337320</td>
<td>1.337466</td>
<td>1.337612</td>
<td>1.337758</td>
<td>1.337905</td>
<td>1.338051</td>
<td>1.338198</td>
<td>1.338345</td>
<td>1.338492</td>
<td>1.338639</td>
</tr>
<tr>
<td>4</td>
<td>1.338786</td>
<td>1.338933</td>
<td>1.339081</td>
<td>1.339228</td>
<td>1.339376</td>
<td>1.339524</td>
<td>1.339671</td>
<td>1.339819</td>
<td>1.339967</td>
<td>1.340116</td>
</tr>
<tr>
<td>5</td>
<td>1.340264</td>
<td>1.340412</td>
<td>1.340561</td>
<td>1.340709</td>
<td>1.340858</td>
<td>1.341007</td>
<td>1.341156</td>
<td>1.341305</td>
<td>1.341454</td>
<td>1.341604</td>
</tr>
<tr>
<td>6</td>
<td>1.341753</td>
<td>1.341903</td>
<td>1.342052</td>
<td>1.342202</td>
<td>1.342352</td>
<td>1.342502</td>
<td>1.342652</td>
<td>1.342802</td>
<td>1.342952</td>
<td>1.343103</td>
</tr>
<tr>
<td>7</td>
<td>1.343253</td>
<td>1.343404</td>
<td>1.343555</td>
<td>1.343706</td>
<td>1.343857</td>
<td>1.344008</td>
<td>1.344159</td>
<td>1.344311</td>
<td>1.344462</td>
<td>1.344614</td>
</tr>
<tr>
<td>8</td>
<td>1.344765</td>
<td>1.344917</td>
<td>1.345069</td>
<td>1.345221</td>
<td>1.345373</td>
<td>1.345526</td>
<td>1.345678</td>
<td>1.345831</td>
<td>1.345983</td>
<td>1.346136</td>
</tr>
<tr>
<td>9</td>
<td>1.346289</td>
<td>1.346442</td>
<td>1.346595</td>
<td>1.346748</td>
<td>1.346892</td>
<td>1.347055</td>
<td>1.347209</td>
<td>1.347362</td>
<td>1.347516</td>
<td>1.347670</td>
</tr>
<tr>
<td>10</td>
<td>1.347824</td>
<td>1.347978</td>
<td>1.348133</td>
<td>1.348287</td>
<td>1.348442</td>
<td>1.348596</td>
<td>1.348751</td>
<td>1.348906</td>
<td>1.349061</td>
<td>1.349216</td>
</tr>
<tr>
<td>11</td>
<td>1.349371</td>
<td>1.349527</td>
<td>1.349682</td>
<td>1.349838</td>
<td>1.349993</td>
<td>1.350149</td>
<td>1.350305</td>
<td>1.350461</td>
<td>1.350617</td>
<td>1.350774</td>
</tr>
<tr>
<td>12</td>
<td>1.350930</td>
<td>1.351087</td>
<td>1.351243</td>
<td>1.351400</td>
<td>1.351557</td>
<td>1.351714</td>
<td>1.351871</td>
<td>1.352029</td>
<td>1.352186</td>
<td>1.352434</td>
</tr>
<tr>
<td>13</td>
<td>1.352501</td>
<td>1.352659</td>
<td>1.352817</td>
<td>1.352975</td>
<td>1.353133</td>
<td>1.353291</td>
<td>1.353449</td>
<td>1.353608</td>
<td>1.353767</td>
<td>1.353925</td>
</tr>
<tr>
<td>14</td>
<td>1.354084</td>
<td>1.354243</td>
<td>1.354402</td>
<td>1.354561</td>
<td>1.354721</td>
<td>1.354880</td>
<td>1.355040</td>
<td>1.355199</td>
<td>1.355359</td>
<td>1.355519</td>
</tr>
<tr>
<td>15</td>
<td>1.355679</td>
<td>1.355840</td>
<td>1.356000</td>
<td>1.356160</td>
<td>1.356321</td>
<td>1.356482</td>
<td>1.356642</td>
<td>1.356803</td>
<td>1.356964</td>
<td>1.357126</td>
</tr>
<tr>
<td>16</td>
<td>1.357287</td>
<td>1.357448</td>
<td>1.357610</td>
<td>1.357772</td>
<td>1.357933</td>
<td>1.358095</td>
<td>1.358257</td>
<td>1.358420</td>
<td>1.358582</td>
<td>1.358744</td>
</tr>
<tr>
<td>17</td>
<td>1.358907</td>
<td>1.359070</td>
<td>1.359232</td>
<td>1.359395</td>
<td>1.359558</td>
<td>1.359722</td>
<td>1.359885</td>
<td>1.360048</td>
<td>1.360212</td>
<td>1.360376</td>
</tr>
<tr>
<td>18</td>
<td>1.360539</td>
<td>1.360703</td>
<td>1.360867</td>
<td>1.361032</td>
<td>1.361196</td>
<td>1.361360</td>
<td>1.361525</td>
<td>1.361690</td>
<td>1.361854</td>
<td>1.362019</td>
</tr>
<tr>
<td>19</td>
<td>1.362185</td>
<td>1.362350</td>
<td>1.362515</td>
<td>1.362681</td>
<td>1.362846</td>
<td>1.363012</td>
<td>1.363178</td>
<td>1.363344</td>
<td>1.363510</td>
<td>1.363676</td>
</tr>
<tr>
<td>20</td>
<td>1.363842</td>
<td>1.364009</td>
<td>1.364176</td>
<td>1.364342</td>
<td>1.364509</td>
<td>1.364676</td>
<td>1.364843</td>
<td>1.365011</td>
<td>1.365178</td>
<td>1.365346</td>
</tr>
</tbody>
</table>
## Table (Continued)

<table>
<thead>
<tr>
<th>% Brix</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

### Appendix 1: Relation between %-Brix and Refractive Index

- **1.36551**: 0.00000
- **1.36719**: 0.00000
- **1.36880**: 0.00000
- **1.37060**: 0.00000
- **1.37232**: 0.00000
- **1.37406**: 0.00000
- **1.37581**: 0.00000
- **1.37759**: 0.00000
- **1.37935**: 0.00000
- **1.38114**: 0.00000
- **1.38295**: 0.00000
- **1.38475**: 0.00000
- **1.38660**: 0.00000
- **1.38845**: 0.00000
- **1.39032**: 0.00000
- **1.39220**: 0.00000
- **1.39409**: 0.00000
- **1.39600**: 0.00000
- **1.39792**: 0.00000
- **1.39986**: 0.00000
- **1.40181**: 0.00000
- **1.40378**: 0.00000
- **1.40576**: 0.00000
- **1.40776**: 0.00000
- **1.40977**: 0.00000
- **1.41180**: 0.00000
- **1.41383**: 0.00000
- **1.41591**: 0.00000
- **1.41799**: 0.00000
- **1.42008**: 0.00000
- **1.42217**: 0.00000
- **1.42423**: 0.00000
- **1.42646**: 0.00000
- **1.42862**: 0.00000
- **1.43080**: 0.00000
- **1.43299**: 0.00000
- **1.43520**: 0.00000
- **1.43742**: 0.00000
- **1.43969**: 0.00000
- **1.44192**: 0.00000

### Additional Data

- **0.01**: 0.00000
- **0.02**: 0.00000
- **0.03**: 0.00000
- **0.04**: 0.00000
- **0.05**: 0.00000
- **0.06**: 0.00000
- **0.07**: 0.00000
- **0.08**: 0.00000
- **0.09**: 0.00000

### Conversion Formulas

1. **% Brix = ((Refractive Index - 1) / 2.26)**
2. **Refractive Index = (2.26 * % Brix) + 1**
### Table (Continued)

<table>
<thead>
<tr>
<th>% Brix</th>
<th>0.0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
<td>1.444204</td>
<td>1.444432</td>
<td>1.444661</td>
<td>1.444890</td>
<td>1.445119</td>
<td>1.445348</td>
<td>1.445578</td>
<td>1.445807</td>
<td>1.446037</td>
<td>1.446267</td>
</tr>
<tr>
<td>62</td>
<td>1.446497</td>
<td>1.446727</td>
<td>1.446957</td>
<td>1.447188</td>
<td>1.447419</td>
<td>1.447650</td>
<td>1.447881</td>
<td>1.448112</td>
<td>1.448343</td>
<td>1.448575</td>
</tr>
<tr>
<td>63</td>
<td>1.448807</td>
<td>1.449037</td>
<td>1.449271</td>
<td>1.449503</td>
<td>1.449736</td>
<td>1.449968</td>
<td>1.450201</td>
<td>1.450434</td>
<td>1.450667</td>
<td>1.450900</td>
</tr>
<tr>
<td>64</td>
<td>1.451134</td>
<td>1.451367</td>
<td>1.451601</td>
<td>1.451835</td>
<td>1.452069</td>
<td>1.452304</td>
<td>1.452538</td>
<td>1.452773</td>
<td>1.453008</td>
<td>1.453243</td>
</tr>
<tr>
<td>65</td>
<td>1.453478</td>
<td>1.453713</td>
<td>1.453949</td>
<td>1.454184</td>
<td>1.454420</td>
<td>1.454656</td>
<td>1.454893</td>
<td>1.455129</td>
<td>1.455365</td>
<td>1.455602</td>
</tr>
<tr>
<td>66</td>
<td>1.455839</td>
<td>1.456076</td>
<td>1.456313</td>
<td>1.456551</td>
<td>1.456788</td>
<td>1.457026</td>
<td>1.457264</td>
<td>1.457502</td>
<td>1.457740</td>
<td>1.457979</td>
</tr>
<tr>
<td>67</td>
<td>1.458217</td>
<td>1.458456</td>
<td>1.458695</td>
<td>1.458934</td>
<td>1.459174</td>
<td>1.459413</td>
<td>1.459653</td>
<td>1.459893</td>
<td>1.460133</td>
<td>1.460373</td>
</tr>
<tr>
<td>68</td>
<td>1.460613</td>
<td>1.460854</td>
<td>1.461094</td>
<td>1.461335</td>
<td>1.461576</td>
<td>1.461817</td>
<td>1.462059</td>
<td>1.462300</td>
<td>1.462542</td>
<td>1.462784</td>
</tr>
<tr>
<td>69</td>
<td>1.463026</td>
<td>1.463268</td>
<td>1.463511</td>
<td>1.463753</td>
<td>1.463996</td>
<td>1.464239</td>
<td>1.464482</td>
<td>1.464725</td>
<td>1.464969</td>
<td>1.465212</td>
</tr>
<tr>
<td>70</td>
<td>1.465456</td>
<td>1.465706</td>
<td>1.465944</td>
<td>1.466188</td>
<td>1.466433</td>
<td>1.466678</td>
<td>1.466922</td>
<td>1.467167</td>
<td>1.467413</td>
<td>1.467658</td>
</tr>
<tr>
<td>71</td>
<td>1.467903</td>
<td>1.468149</td>
<td>1.468395</td>
<td>1.468641</td>
<td>1.468887</td>
<td>1.469134</td>
<td>1.469380</td>
<td>1.469627</td>
<td>1.469874</td>
<td>1.470121</td>
</tr>
<tr>
<td>72</td>
<td>1.470368</td>
<td>1.470616</td>
<td>1.470863</td>
<td>1.471111</td>
<td>1.471359</td>
<td>1.471607</td>
<td>1.471855</td>
<td>1.472104</td>
<td>1.472352</td>
<td>1.472601</td>
</tr>
<tr>
<td>73</td>
<td>1.472850</td>
<td>1.473099</td>
<td>1.473349</td>
<td>1.473598</td>
<td>1.473848</td>
<td>1.474098</td>
<td>1.474348</td>
<td>1.474598</td>
<td>1.474848</td>
<td>1.475099</td>
</tr>
<tr>
<td>74</td>
<td>1.475349</td>
<td>1.475590</td>
<td>1.475831</td>
<td>1.476083</td>
<td>1.476335</td>
<td>1.476586</td>
<td>1.476837</td>
<td>1.477090</td>
<td>1.477351</td>
<td>1.477614</td>
</tr>
<tr>
<td>75</td>
<td>1.477866</td>
<td>1.478119</td>
<td>1.478371</td>
<td>1.478624</td>
<td>1.478878</td>
<td>1.479131</td>
<td>1.479384</td>
<td>1.479638</td>
<td>1.479892</td>
<td>1.480146</td>
</tr>
<tr>
<td>76</td>
<td>1.480400</td>
<td>1.480654</td>
<td>1.480909</td>
<td>1.481163</td>
<td>1.481418</td>
<td>1.481673</td>
<td>1.481929</td>
<td>1.482184</td>
<td>1.482440</td>
<td>1.482695</td>
</tr>
<tr>
<td>77</td>
<td>1.482951</td>
<td>1.483207</td>
<td>1.483463</td>
<td>1.483720</td>
<td>1.483976</td>
<td>1.484233</td>
<td>1.484490</td>
<td>1.484747</td>
<td>1.485005</td>
<td>1.485262</td>
</tr>
<tr>
<td>78</td>
<td>1.485220</td>
<td>1.485577</td>
<td>1.485935</td>
<td>1.486294</td>
<td>1.486552</td>
<td>1.486810</td>
<td>1.487069</td>
<td>1.487328</td>
<td>1.487587</td>
<td>1.487846</td>
</tr>
<tr>
<td>79</td>
<td>1.488105</td>
<td>1.488365</td>
<td>1.488625</td>
<td>1.488884</td>
<td>1.489144</td>
<td>1.489405</td>
<td>1.489665</td>
<td>1.489926</td>
<td>1.490186</td>
<td>1.490447</td>
</tr>
<tr>
<td>80</td>
<td>1.490708</td>
<td>1.490970</td>
<td>1.491231</td>
<td>1.491493</td>
<td>1.491754</td>
<td>1.492016</td>
<td>1.492278</td>
<td>1.492541</td>
<td>1.492803</td>
<td>1.493066</td>
</tr>
<tr>
<td>81</td>
<td>1.493328</td>
<td>1.493591</td>
<td>1.493855</td>
<td>1.494118</td>
<td>1.494381</td>
<td>1.494645</td>
<td>1.494909</td>
<td>1.495173</td>
<td>1.495437</td>
<td>1.495701</td>
</tr>
<tr>
<td>82</td>
<td>1.495966</td>
<td>1.496230</td>
<td>1.496495</td>
<td>1.496760</td>
<td>1.497026</td>
<td>1.497291</td>
<td>1.497556</td>
<td>1.497822</td>
<td>1.498088</td>
<td>1.498354</td>
</tr>
<tr>
<td>83</td>
<td>1.498620</td>
<td>1.498887</td>
<td>1.499153</td>
<td>1.499420</td>
<td>1.499687</td>
<td>1.499954</td>
<td>1.500221</td>
<td>1.500488</td>
<td>1.500756</td>
<td>1.501024</td>
</tr>
<tr>
<td>84</td>
<td>1.501292</td>
<td>1.501560</td>
<td>1.501828</td>
<td>1.502096</td>
<td>1.502365</td>
<td>1.502634</td>
<td>1.502903</td>
<td>1.503172</td>
<td>1.503441</td>
<td>1.503711</td>
</tr>
<tr>
<td>85</td>
<td>1.503980</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>