

## Chapter 1 Introduction

### 1.1 Background

Fluorescein is a widely used tracer in the water industry because it is easy to detect by either absorbance or fluorescence techniques. Unfortunately fluorescein also has the reputation of being a non-conservative tracer and this limits its use to qualitative rather than quantitative analysis. However if this non-conservative behaviour is better understood, fluorescein tracer studies could be extended to generate quantitative data. The purpose of this research is to understand this non-conservative behaviour.

### 1.2 Reasons for non-conservative behaviour

There are a number of reasons for fluorescein's apparent non-conservative behaviour. One reason is that the different ionic forms of fluorescein have markedly different absorbance and fluorescent characteristics (Martin and Lindqvist, 1975), and as the largest change in fluorescein absorptivity occurs in the pH range typical of environmental waters (from pH 6 to 7) it is essential to account for this effect when performing fluorescein determinations.

There is little agreement on the fluorescein ionisation constant values and a single ionisation constant is often reported (Smart and Laidlaw, 1977, Wolfbeis *et al.*, 1983, Grotte, Mattox and Brubaker, 1985, Kasnavia, 1997, and Kasnavia, Vu and Sabatini, 1999). This is contrary to the evidence that shows fluorescein has a minimum of six ionic forms in water (Zanker and Peter, 1958, Lindqvist, 1960, Chen, Nakamura and Tamura, 1979, Sjöback, Nygren and Kubista, 1995, and Klonis and Sawyer, 1996) and significant interpretation errors can occur if the single ionisation constant is adopted.

Accurate ionisation constants are needed to predict the ionic form of fluorescein. The simplest ionic model uses three  $pK_{a}$ s (Klonis and Sawyer, 1996) and a range of values have been reported for these three constants;  $pK_{a1}$  values have been reported from 2.08 (Sjöback *et al.*, 1995) to 2.25 (Klonis and Sawyer, 1996);  $pK_{a2}$  values have been reported from 4.23 (Klonis and Sawyer, 1996) to 4.4 (Lindqvist, 1960);  $pK_{a3}$  values have been reported from 6.31 (Klonis and Sawyer, 1996) to 6.7 (Lindqvist, 1960). This suggests that the  $pK_{a}$  determination method should be examined to find the cause of the variance.

Another reason why fluorescein may not be recovered during tracer studies is that it degrades in the presence of light (Feuerstein and Selleck, 1963, and Smart and Laidlaw, 1977). However, while fluorescein does degrade quickly in the presence of strong light, this is generally not a factor in groundwater studies, in tests that take place in enclosed vessels, or at night. Heat has also been suggested to degrade fluorescein (Leonhardt, Gordon and Livingston, 1971) so it would be useful to know whether sample refrigeration is needed, as this would complicate fluorescein tests in hot climates.

### 1.3 Analytical issues affecting fluorescein determinations

Questions have been raised about the fluorescein absorptivity values by Boets *et al.* (1992) who compared their absorptivity value of  $8.7 \times 10^4$  with other published values of  $7.4 \times 10^4$  (Larsen and Johansson, 1989),  $8.4 \times 10^4$  (Hammond, 1979),  $8.9 \times 10^4$  (Delori, Castany and Webb, 1978, and Melhado *et al.*, 1982) and  $1.6 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$  (Grotte *et al.*, 1985). These inconsistent values may be the result of using an absorbance test to quantify a highly fluorescent compound and the range of these absorptivity values suggests that it is essential to determine values specific for the analytical instrument used in each study.

A number of studies have assumed that fluorescein obeys Beer's law, e.g. Adelman and Oster (1956), Delori *et al.* (1978) and Diehl (1989) but this assumption may not be justified because fluorescein is a strongly fluorescent compound. Fluorescence effects have been reported to cause deviations from Beer's law (Braude, Fawcett and Timmons, 1950) as well as Lambert's law (Moran and Stonehill, 1957) and it is known that fluorescence interferes with certain types of spectrophotometers (Gibson and Keegan, 1938) therefore there is reason to believe that fluorescein solutions will not obey Beer's law.

### 1.4 Study objectives

This study focuses on the absorbance method for quantifying fluorescein and deals with the problems outlined in Sections 1.2 and 1.3. It develops and tests a new ionisation constant determination method that simultaneously identifies absorptivity values and corrects for activity effects. It compares the influence of heat and sunlight on fluorescein samples, and also tests whether fluorescein obeys Beer's law. The  $\text{pK}_a$ s and absorptivity factors found in this study are then used in a laboratory trial to determine whether fluorescein can behave in a conservative manner.