Chapter 6 Summary and Conclusions

1. Precise values for the different pK_as of fluorescein were determined without sophisticated laboratory equipment. The pK_a determination method developed here builds on the simplified method of Clark and Cunliffe (1973) but is further refined by including corrections for activity and temperature effects.
2. Rather than attempting to tightly control the pK_a determination conditions, the approach has been to measure the conditions and compensate for any changes using well-established correction formulas. This approach gave precise and reproducible results.
3. Once activity effects were taken into account in the pK_a determination it made no difference whether the predominant solution cation was sodium or potassium, or the predominant anion was chloride, nitrate or sulphate.
4. The three thermodynamic pK_a values for fluorescein determined in this study are 2.22, 4.35 and 6.68.
5. When activity corrections were applied to the pK_a values of Diehl and Markusewski (1989), Klonis and Sawyer (1996) and Sjöback *et al.* (1995) the corrected values were similar to those of Lindqvist (1960) and those calculated in this investigation. This suggests that the differences between many of the reported pK_as may be due to a failure to correct for activity effects.
6. The temperature corrected absorptivity factors (product of the molar absorptivity and sample path length) were 19 Mol^{-1} for the cation, 1496 Mol^{-1} for the neutral, 9387 Mol^{-1} for the monoanion, and 48299 Mol^{-1} for the dianion species.
7. The large differences between previously published absorptivity values and the absorptivity factors calculated for this study's spectrophotometer highlights the need to determine absorptivity factors specific for the analytical equipment.
8. The grade of fluorescein used for tracer studies may contain significant quantities of impurities and this prevents the direct use of published absorptivity values.
9. The Beer's law assumption was not valid for the test spectrophotometer at high absorbance values.
10. It is more important to protect fluorescein samples from bright light than from heat.

11. The photodegradation rate of fluorescein cannot be predicted simply by incorporating a factor for light exposure intensity, because the water chemical composition has a substantial influence on the decomposition rate.
12. When sample fluorescein concentrations were calculated using this study's pK_a values the results were almost identical to the concentrations determined by increasing the sample pH and then measuring the absorbance. This similarity helps confirm the validity of both methods and also suggests that the simplest fluorescein determination method will be to increase the sample pH before measurement.
13. If the field spectrophotometer is calibrated with known concentrations of fluorescein above pH 9 and the field samples are adjusted to a similar pH then the complicated pH compensation calculations can be avoided, although this simpler approach will obviously not be possible if the field samples develop a precipitate or undergo some other absorbance change at elevated pH.
14. Data from investigations that show poor recoveries of fluorescein might be profitably re-evaluated to determine whether the analytical instrument absorptivity factors were appropriate, whether pH corrections should have been included, or whether the Beer's law assumption was valid. The conclusion that fluorescein is a non-conservative tracer is not warranted unless these factors have been taken into account.
15. The fact that fluorescein is easy to detect; that its photodecomposition can be eliminated by testing at night; and that its pH/absorbance variability can be avoided by increasing the sample pH; justify its re-evaluation as a quantitative tracer compound.