The conservative behaviour of fluorescein

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Abstract

Failure to account for fluorescein absorbance changes with pH may be responsible for some of the apparent non-conservative behaviour of this easily detectable tracer compound. While it is possible to calculate an accurate absorptivity value for fluorescein at every pH, this calculation is not necessary if the sample pH is increased above pH 9 before measuring the absorbance. Intense sunlight degrades fluorescein quickly but even hot samples are stable if kept in the dark.

Introduction

Tracers are selected for their ability to be transported through a water system in a manner similar to the item of interest. A good tracer will be a stable substance, show no reactivity with the system components, and must have reliably quantitative detection at low concentrations (Behrens, 1986). These characteristics stress the conservative nature of a good tracer because tracer mass recovery is viewed as confirmation of a properly conducted field trial and necessary to obtain accurate measurement (Feuerstein and Selleck, 1963). Tracer recovery is also important because conservative elution patterns can be tested using different mathematical models to quantify the flow character, and the total amount of recovered tracer is used as a validity test for this approach (Levenspiel, 1972).

The organic compound fluorescein is easy to detect and can be measured using either its strong fluorescence or highly absorptive character (Klonis and Sawyer, 1996). Other advantages of fluorescein include its low sorption tendency (Behrens, 1986) and its relatively low temperature exponent (Feuerstein and Selleck, 1963). The disadvantages of fluorescein are its photochemical instability, pH sensitivity (Lindqvist, 1960), and it has been suggested that fluorescein solutions are unstable when heated (Leonhardt, et al., 1971).

The absorbance sensitivity of fluorescein to pH is a result of changes in the ionic form of the fluorescein molecule, with each ionic species having its own characteristic absorbance (Zanker and Peter, 1958). There are large differences between the absorptivities of the different ionic species (Lindqvist, 1960) so accurate dissociation constants must be used in order to predict these absorbance changes. However there was little agreement between the published fluorescein pK_a values until recently when it was shown that a number of these values do agree once activity corrections are applied (Smith and Pretorius, 2002).

Fluorescein's reputation of being a non-conservative tracer (Feuerstein and Selleck, 1963, and Smart and Laidlaw, 1977) limits its use to qualitative rather than quantitative studies. This paper examines the influence of pH, light, and heat on fluorescein measurements, to demonstrate that fluorescein can be used for quantitative studies. Two experiments are described. The first uses the previously determined pK_a and absorptivity values of the different fluorescein species (Smith and Pretorius, 2002) in a series of elution experiments to test the quantity of fluorescein recovered, and a second experiment compares the degradation effects of light and heat.

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Received 24 October 2001; accepted in revised form 22 July 2002.

Materials and methods

Chemical quality was certified to meet ACS (American Chemical Society) specifications and supplied by either Fluka or JT Baker. Distilled water was used throughout. Standard grade Fluka fluorescein was used without further purification. The fluorescein moisture content was measured by calculating the mass lost after overnight drying at 105°C under vacuum. This moisture correction was applied to all fluorescein mass measurements.

Absorbance readings were made using a Turner Model 350 spectrophotometer at the fluorescein, high-pH absorbance maximum, which was 492 nm on this instrument. Samples were collected and analysed in new, polished, 13 × 100 mm borosilicate glass tubes.

A Corning Model pH-30 meter was used and pH calibrations were performed before, during, and after each test session. Calibrations were performed in a water bath at 25°C (the titration temperature) at pH 4.00 and pH 7.00 with calibration solutions prepared using "pHydrion" buffer capsules supplied by Micro Essential Laboratories.

Elution tests

This experiment used previously published fluorescein pK_a and absorptivity values (Smith and Pretorius, 2002) to determine the amount of fluorescein recovered from a test column. The test apparatus is shown in Fig. 1 and comprised a constant pressure feed, connected to a column filled with non-epoxy coated, aquarium gravel. Solution flowed from the feed container, through the gravel column, into a tracer-sensor, and then into a sample collector. The tracer-sensor was connected to a data-logging system to ensure regular measurements. Data-logged signals were converted to the equivalent absorbance and the total recovered fluorescein was calculated at the end of each run.

The tracer-sensor was constructed specifically for this project. A light source and light detector faced each other at either end of the sensor tube (Fig. 1). A blue light-emitting diode (LED) served as a light source, while a light-dependent resistor was used as the

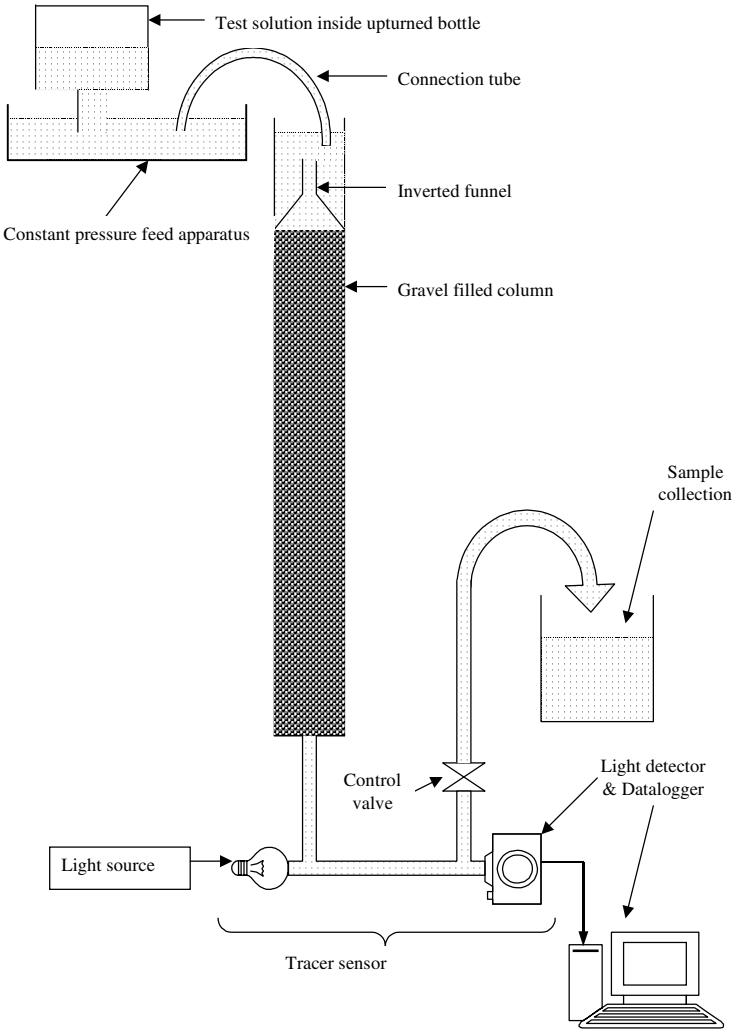

Figure 1
Schematic of elution test apparatus. The column was 1.5 m tall and had an internal diameter of 37 mm.

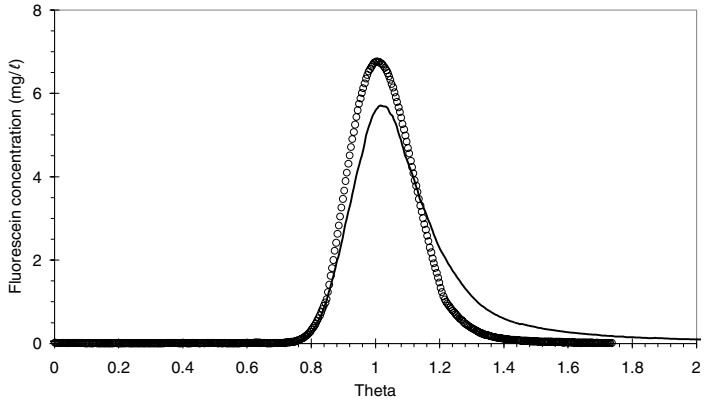

Figure 2
Two elution curves showing how reducing the pH retards the fluorescein elution profile. ○ pH 7.79 and — pH 5.12 elution profile.

detector. The detector was connected to a digital multimeter, which communicated directly with a computer. As the LED was expected to produce a broad spectrum of wavelengths within the blue range, it was calibrated using fluorescein solutions at a similar pH to the test solutions.

Quantities of buffered (5 mM sodium acetate (NaAc) and 5 mM KH_2PO_4) test solutions were prepared and adjusted to pH 8, pH 7, pH 6, and pH 5, using KOH or HCl. The flow rate was adjusted to 25 mℓ/min before starting each test run. In addition to the sensor information, samples were collected for flow rate and pH tests, and the solution temperature was monitored regularly. The test was stopped once the sensor reading approached its pre-test levels. Once the test run was complete the samples were retested using the tracer sensor and then measured using the spectrophotometer. A small quantity of solid KOH was then added to the sample and it was retested at the higher pH.

Sensor readings (MΩ) were converted to absorbance readings using calibrated conversion factors and these absorbance readings were then converted to fluorescein concentrations using the pK_a and absorptivity values. The total mass of fluorescein recovered was expressed as a fraction of the injected mass.

Light and heat degradation effects

Fluorescein was added to a bulk buffer solution (5 mM NaAc and 5 mM KH_2PO_4) to give a fluorescein concentration of 5 mg/ℓ, and this solution was split into two halves. To test the impact of redox potential, sodium sulphide was added to one half of the solution to a final concentration of 2.9mM. After standing overnight in airtight bottles these two solutions were split again, with one half of each being adjusted to pH 11.2 and the other half to pH 5.2. The four test solutions were pH tested, subdivided into three lots and poured into Ziploc® bags. These bags were sealed after removing any air bubbles.

During each experimental run two bags were kept in the dark; one at room temperature and the other in a water bath at more than 60°C; while a third bag was placed in full sun. Samples were taken from the bags at intervals and tested for transmittance. The test was performed in triplicate.

Results and discussion

Elution tests

Figure 2 shows the influence of pH on the movement of fluorescein. Two different pH elution profiles are shown overlaid on a standardised flow axis so that one theta unit is the volume required to fill the elution column. The retarded fluorescein elution profile of the pH 5.12 test is due to the increased association of the fluorescein with the column gravel (the stationary phase), which in turn is probably due to the increased proportion of the less soluble fluorescein neutral species at this pH.

The fraction of fluorescein recovered after each elution test is shown in Table 1. The percentage recovery tends to increase with each repetition of the test. This increase was due to residual fluorescein carrying over into the next column run which suggests that more time is required to completely purge fluorescein from the test apparatus, and also that longer recovery times would yield more consistent results. When this carry-over effect was eliminated by standardising the signal baseline of each run the standard deviation of the recoveries within

each pH trial was about 3%, which demonstrates that the elution test method is reproducible. The absence of any apparent correlation between the elution trial pH and the percentage recovery suggests that fluorescein is not binding irreversibly to the gravel bed as this sort of binding would be expected to increase with decreasing pH.

TABLE 1
Percentage fluorescein recovered in elution tests

Test	1	2	3	4	5	6	7	8	Average
pH 5.1	94	91	95	104	101	105	110	113	101.6
pH 6.2	87	87	96	104	103	99	105	105	98.4
pH 7.1	92	98	105	104	109	114			103.9
pH 7.8	94	98	90	93	88	91			92.3

If the tracer sensor and spectrophotometer readings have been correlated correctly and the absorbance/concentration determination methods are correct, then it would be reasonable to expect average recoveries of 100%. Although the pH 5.1, pH 6.2 and pH 7.2 trials show average recoveries of close to 100%, the pH 7.8 trials show an average recovery of 92.5%. There are at least two reasons for this lower recovery:

- While the sensor and spectrophotometer readings do correlate quite well within the experimental concentration range, fluorescein does not conform to Beer's law in this spectrophotometer. This Beer's law deviation underestimates the fluorescein concentration and becomes more pronounced as the absorbance increases so the highest and sharpest elution peaks of the high pH trials will be most likely to show reduced recoveries.
- The tracer sensor used in these tests is an experimental device and may be sensitive to unrecognised variables. This sensor was the third constructed during this investigation. Earlier designs suffered from changes in absorbance as the sensor construction materials deformed, transparent plastics whitened (on prolonged water contact), construction glues became opaque, and the light-dependent resistor corroded. These problems were eliminated in the third design.

While recognising these potential inaccuracies, the average fluorescein recovery in these trials was 99.2%, showing that fluorescein did behave conservatively. In future tracer studies it will be vital to identify the cause of any deviation from complete recovery as this investigation demonstrates that conservative behaviour of fluorescein is possible if one correctly evaluates the system parameters.

The fluorescein recoveries listed in Table 1 were calculated by converting elution absorbance measurements into fluorescein concentrations using the pK_a values reported earlier (Smith and Pretorius, 2002) in conjunction with the solution pH and absorptivity values of the different ionic species. Temperature and activity corrections were also required to allow accurate interpretation of the absorbance measurements and the complexity of this calculation and absence of equivalent buffering makes it impractical for field use. However, when a small quantity of solid KOH was added to the elution sample to raise its pH, the absorbance increased. This absorbance change and its consistency can be seen in Fig. 3 and suggests that no absorptivity calculations will be needed if the pH of the sample is increased above pH 9 before measuring the sample absorbance. The gradients of these different relationships are shown next to each data set in Fig. 3. In Table 2 these gradient values are compared to the predicted absorbance increase calculated using the activity corrected pK_a values. The small differences between the observed and predicted absorbance values confirm that the pK_a values of Smith and Pretorius (2002) are accurate.

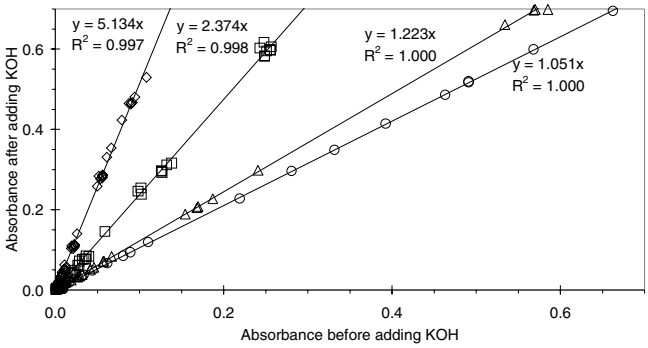

Figure 3
Fluorescein absorbance changes produced by adding KOH to increase the sample pH. ◇ pH 5.10, □ pH 6.17, △ pH 7.09 and ○ pH 7.79 data are shown along with the — fitted equations.

TABLE 2
Absorbance change due to adding KOH

Test pH	Observed absorbance increase	Predicted absorbance increase	Predicted/ observed error (%)
5.10	5.134	5.036	-2.1
6.17	2.374	2.377	0.8
7.09	1.223	1.235	0.9
7.79	1.051	1.049	-0.2

Light and heat degradation effects

The only buffered fluorescein solutions that showed a drop in absorbance during the test period were those exposed to sunlight. The average change in absorbance for the pH 11.2 (A), pH 5.2 (B), and pH 11.2 + Na_2S (C) solutions is shown in Fig. 4. The pH 5.2 + Na_2S (D) solution results are not shown because a precipitate formed during the test and interfered with the absorbance readings.

Figure 4 shows that the heated and room temperature fluorescein controls were stable throughout the 2h test period. The stability of these solutions compared with the instability of the sunlight-exposed solutions shows that it is more important to protect a fluorescein solution from bright light than from heat. The stability of these controls confirms the observations of Diehl and Horchak-Morris (1987) and Lindqvist (1960) that fluorescein solutions are stable if kept in the dark but contradicts the observation of Leonhardt et al. (1971) that hot aqueous fluorescein solutions are unstable.

Solutions exposed to sunlight showed a rapid absorbance decrease (Fig. 4). The average absorbance reduction for the 2h

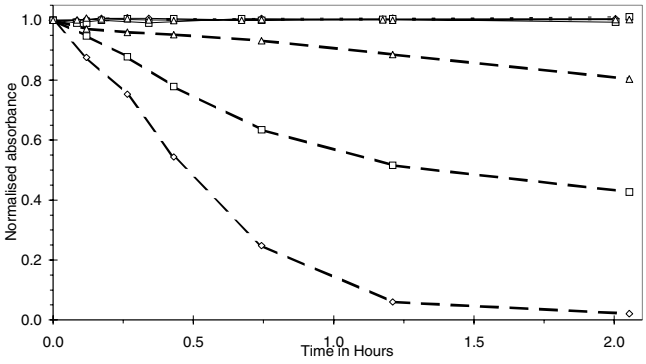

Figure 4

Absorbance changes produced by high temperature (>60°C) or sunlight on buffered fluorescein samples. —◇— Control @ pH 11.2, —□— Control @ pH 5.25, —△— Control @ pH 11.2 with Na_2S, ---◇--- Hot @ pH 11.2, ---□--- Hot @ pH 5.25 , ---△--- Hot @ pH 11.2 with Na_2S, – –◇– –Sun @ pH 11.2, – –□– – Sun @ pH 5.25, – –△– – Sun @ pH 11.2 with Na_2S.

exposure was 98% for Solution A, 57% for Solution B and 20% for Solution C. This confirms that fluorescein degrades quickly in bright sunlight and corroborates the observations made by Feuerstein and Selleck (1963) and Smart and Laidlaw (1977). The addition of a small quantity of Na_2S greatly reduced the rate of absorbance decline (Solution C) compared to the same solution without Na_2S (Solution A), and the pH 5.2 solution (Solution B) showed less photodegradation than the pH 11.2 solution (Solution A). This indicates that the photodegradation rate is not solely dependent on the light intensity and that both pH and redox potential influence the degradation rate.

The decay rate constants of $-1.96 \cdot h^{-1}$, $-0.47 \cdot h^{-1}$ and $-0.10 \cdot h^{-1}$ for Solutions A, B and C confirm that the solution composition has a substantial impact on the photodegradation rate. A $-0.256 \cdot h^{-1}$ photodegradation coefficient for fluorescein has been reported for a 3d period of bright sunlight; however, the nighttime hours were included in this calculation (Feuerstein and Selleck, 1963). This $-0.256 \cdot h^{-1}$ decay coefficient is almost eight times lower than the $-1.96 \cdot h^{-1}$ rate constant found in this study so this difference cannot be explained simply by assuming that the nighttime period halved the light exposure. One reason for this difference is that Feuerstein and Selleck (1963) used distilled water rather than the buffered high-pH solution used in this experiment so although their solution pH was not reported it might be expected to be closer to neutrality. This explanation appears correct because the Solution B (pH 5.2) photodegradation rate was $-0.47 \cdot h^{-1}$, which is close to the doubled $-0.256 \cdot h^{-1}$ value.

The practical implications of this experiment are that while reducing the pH and adding Na_2S might improve the absorbance stability of fluorescein samples, these benefits are not as important as protecting the solution from bright light. Further, the results show that it will not be possible to accurately compensate for the effects of light exposure unless the other rate-influencing variables have been quantified.

The high photodegradation rate of fluorescein does appear to compromise its use as a tracer but this same characteristic would make it especially useful in situations where a more visibly persistent tracer would be undesirable, e.g. provoking public concern. However, this photo-labile nature should not be a problem for tracer studies performed inside reactor vessels or at night.

The stability of the control and heated fluorescein samples (Fig. 4) also suggests that degradation was not the cause of the lower than expected fluorescein recoveries in the pH 7.8 elution trial because the elution trials took place in laboratory ambient light at room temperature.

Conclusions

Fluorescein behaved conservatively during elution trials when the elution absorbance levels were interpreted using the pK_a and absorptivity values of fluorescein. Furthermore, while this study shows that it is possible to accurately predict the fluorescein absorptivity value at different pH values, if the fluorescein solution pH is increased (above pH 9) before the calibration and sample testing is performed, it will only be necessary to calculate the dianion species absorptivity. In addition, a wide range of fluorescein calibration concentrations should be tested because Beer's law cannot be assumed to apply under all test conditions.

When using fluorescein as a tracer the test area and samples must be protected from bright light. The simplest way to do this is to perform tests at night and to store samples in the dark. While this fluorescein characteristic may appear to be a problem this high photodegradation rate may be an advantage in circumstances where visible tracer persistence is a problem.

Fluorescein is easy to detect and relies on cost-effective and widely available analytical instruments. With reasonable precautions fluorescein behaves conservatively. This suggests that fluorescein warrants re-evaluation as a quantitative tracer.

Acknowledgements

We thank Kelly Brayton for helpful discussion and critical review of the manuscript and Tex Hunter for providing the Turner 350 spectrophotometer. This work is in partial fulfilment of an M.Sc. degree (Smith, 2001).

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