Application Note: Fluorescein

INTRODUCTION

Fluorescein was the first fluorescent dye used for water tracing work[1] and is still used for qualitative (visual) studies of underground contamination of wells. In recent years, Rhodamine WT has almost completely replaced fluorescein for flow measurements[2] and circulation, dispersion, and plume studies[3]. Nonetheless, fluorescein has a role in such studies, and can be used for masking, hydraulic model studies, and underground water studies.

ADVANTAGES

Fluorescein has the following advantages over other tracer dyes:

Its low sorption rate is far better than Rhodamine B, and comparable to Rhodamine WT.

It has a temperature coefficient of only –0.36% per degree C, about one-eighth of the temperature coefficient of rhodamine dyes[2,4].

It emits a brilliant green fluorescence, which gives an excellent visual or photographic contrast against the backgrounds normally encountered in water transport studies. Therefore it is easy to visualize the progress of an experiment.

It is more aesthetic than the red dyes. This is psychologically important, especially in ocean areas subject to the blooms of certain dinoflagellates, called “red tides.” Less public resistance will be encountered using a dye that does not resemble red tide[5].

DISADVANTAGES

Fluorescein has been replaced by other dyes, principally Rhodamine WT, for the following reasons:

It is rapidly destroyed by sunlight. Reference 4 reports that a 50% loss occurred in three hours of sunlight exposure, with dye being held in an Erlenmeyer flask. Other tests in an flat, uncovered Pyrex dish showed an almost complete destruction in two hours[6].

Many naturally occurring fluorescent materials have similar characteristics and thus interfere with measurement. When carefully chosen optical filters are used, the situation is better than that reported in Reference 4, but higher concentrations are required to overcome the effect of higher and more variable “blank” fluorescence.

Fluorescein is more pH-sensitive than rhodamine dyes. Fluorescence drops very sharply at pH values below 5.5. For optimum results, pH should be between 6 and 10.

MASKING TECHNIQUES

In river, harbor, and ocean tests, fluorescein can be used to mask the objectionable color of the rhodamine dyes. Tests show that Fluorescein is an effective mask, subject to the following conditions[6]:

The concentration of fluorescein should be at least five times that of the active ingredients in the Rhodamine B or Rhodamine WT concentrate.

Where the receiving water is shallow, clear, and in full sunlight, the dyes must be dispersed quite rapidly. With slow dispersion, the photosensitive fluorescein will be destroyed before the masking effect is complete.

Masking is subjective. Lower (hence less costly) amounts of fluorescein may be effective, depending on water clarity, bottom color, wave action, etc. Small scale addition of the mixed dyes to the receiving water should be made in advance of a large scale test. This test should be made on a bright sunny day, if possible.
Note that fluorescein is not the ingredient measured. The optical filter and light source in the fluorometer read only rhodamine dye[7].

HYDRAULIC Model Studies

Fluorescein may be used in hydraulic model studies in exactly the same way that Rhodamine WT is used (See Refs. 2 and 3 for details).

The major advantage of using fluorescein is its visibility; the green color can be seen as the test proceeds. The major disadvantage is fluorescein's light sensitivity. It can be destroyed by light entering the test area, both from windows and from indoor lights, especially fluorescent ones.

Containers used for dye destruction tests must be transparent to light at shorter wavelengths. Clear borosilicate glass baking pans are handy, since they transmit light at shorter wavelengths than window glass or the glass envelopes of fluorescent lamps.

Test samples must be at low concentrations (around 0.2 PPM) so that the fluorescein in the bottom of the pan is not protected from the incident light by absorption of the fluorescein in the top of the pan.

In certain cases, deliberate destruction of the fluorescein by sunlight may be a convenience instead of a problem. Hydraulic models often recycle water. With the very stable Rhodamine WT, the concentration of dye in the entire system will build up over a sequence of several tests, requiring replacement of the water. If a shallow holding tank can be placed outdoors, the degradation of fluorescein by sunlight may eliminate the need to replace the water.

Underground Water Studies

Fluorescein can be used quantitatively for underground tests, subject to limitations imposed by the higher background of naturally occurring fluorescent materials.

An advantage of fluorescein in underground studies is its light sensitivity. Should it reach an open receiving body of water, the color will be less of a problem because it will disappear rapidly in the sunlight.

Filter and Light Source Selection

Using fluorescein, the following light sources and filters are recommended (referenced part numbers are specific to Turner Designs products):

	10-AU-005
Optical Kit	10-086 (Lamp and all filters are included in this kit.)
Light Source	10-089 Blue Lamp
Reference	10-063
Excitation	10-105
Emission	10-109R-C

We have found that background fluorescence can be very high in natural systems with the fluorescein setup. In most cases, this background should be adequately suppressed using the 10-AU fluorometer. If, however, background cannot be suppressed, a mask (attenuator) may be added to the excitation filter holder to reduce its diameter and the amount of light scatter. Attenuation by a factor of 5 can be obtained with the 10-318R Attenuator Plate.

Fluorescein, known as "Acid Yellow 73", "Acid Yellow T", "DNC Yellow 7", etc., can be obtained from the following sources (addresses checked and confirmed June 1996):

Pylam Products Company, Inc.
1001 Stewart Avenue
Garden City, NY 11530
516/222-1750

Tricon Colors, Inc.
16 Leliarts Lane
Elmwood Park, NJ 07407
201/794-3800

Lissamine FF

The properties of uses of Lissamine FF are reported in Reference 9. Its spectral characteristics are similar to those of fluorescein, but it does not decompose as rapidly in sunlight. Use the fluorescein filters detailed above with Lissamine FF. Pylam Products (address shown above) offers Lissamine FF as “Brilliant Acid Yellow 8G” or “Brilliant Sulphoflavine FFA”.

References

1) Dole, R. B., *Use of Fluorescein in the Study of Underground Waters*, USGS Water Supply Paper 160, 73-85 (1906).

2) *A Practical Guide to Flow Measurement*, monograph by Turner Designs, 845 W. Maude Avenue, Sunnyvale, CA 94086.

3) *Circulation, Dispersion, and Plume Studies*, monograph by Turner Designs, 845 W. Maude Avenue, Sunnyvale, CA 94086.

4) (0047) Feuerstein, D.L., Sellick, R.E., *Fluorescent Tracers for Dispersion Measurements*, Journal of Sanitary Engineering, ASCE 89 (SA4), 1-21 (1963).

5) (0031) Murakami, Ken, Water Quality Section, Water Quality Control Division, Public Works Research Institute, 5-41-7, Shimo, Kita-Ku, Tokyo, 115, personal communication.

6) Turner Designs Laboratory Tests conducted July 23, 1975.

7) “Filter Selection Guide” for Turner Designs Fluorometers, by Turner Designs, 845 W. Maude Avenue, Sunnyvale, CA 94086.

8) (0413) Smart, P.L., Laidlaw, I.M.S., An *Evaluation of Some Fluorescent Dyes for Water Tracing*, Water Resources Research, 13 (1), 15-33 (1977).