

This monograph is designed to aid you in field and laboratory studies with a fluorometer and fluorescent dyes. The fluorometric techniques described have major advantages over other available techniques.

Learn how to:

- **Calibrate flow meters on site**
- **Calibrate weirs and flumes in the field**
- **Correlate stream-level gauges with the flow rate**
- **Measure stream flow directly, without a weir, flume, or level gauge even under ice**
- **Measure canal, drainage ditch, and sewer flow with portable equipment**
- **Study sewer system infiltration, without cleaning**
- **Study time-of-travel in small streams or over hundreds of miles in large rivers**
- **Discover residence time and channeling in settling basins and chlorine contact chambers**

In addition to these studies, the fluorometer can be used to determine clarity,* chlorophyll,* and pheophytin.* It can also perform circulation, dispersion and plume studies.* While this monograph focuses primarily on field studies, the fluorometer is used for thousands of fluorometric analysis procedures in the laboratory.

Flow measurements will help you understand the theory, select the equipment, and solve the problem of flow measurement in the laboratory, or backpacking into the wilderness.

** Monographs are available from Turner Designs.*

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A. INTRODUCTION

The use of fluorescent dyes and a fluorometer yields a nearly perfect tracer system, with the following advantages:

LOW COST. \$.50 of dye will measure a flow rate of 100 cubic feet per second.

DIRECT MEASUREMENT. Readings may be made directly on a continuous-flow sample or on an individual sample, without processing.

LOW TRACER CONCENTRATION. A fluorometer can detect tracer concentrations as low as 0.01 parts per billion (ppb). Most studies yield concentrations between 1 and 10 ppb. Detection would be impossible without a fluorometer.

DURABLE AND COMPACT. The system is ideal for use in remote locations as all parts are durable and easily transportable.

ACCURATE. Fluorescent tracer techniques are so accurate that they are used to calibrate flow meters. Where flow meters, weirs and other techniques are impractical, a fluorometer continues to yield accurate measurements.

CONVENIENT. The low tracer concentration required makes handling the tracers much easier.

STABLE. Because of their stability, samples collected in the field may be returned to the laboratory for measuring.

The following example of a "typical" flow measurement will illustrate the practicality of a fluorometer in tracer studies.

To measure the rate of flow (discharge) of a small stream, inject a solution of dye into the stream at a steady rate of one milliliter per minute. Downstream, the dye has been diluted by a factor of ten million. Conclusion: the stream has a flow rate of ten million milliliters per minute, or 5.89 cubic feet per second.

As is evident from the example, it is only necessary to know the dilution factor, or the concentration downstream relative to that of the injected dye.

Knowing the exact concentration of the injected dye and downstream dye is unimportant. You may, however, calibrate your injector in cubic feet per second (cfs), gallons per minute (gpm), or acre feet per hour; these will be the measured flow rate units.

Continuous injection and determination of dilution is not the only method used, although it is easiest and more accurate, especially in natural streams. Sometimes it is more convenient to pour a small amount of dye, all at once, into the stream. The derivation of flow rate from the downstream measurements is less obvious, but this slug technique is still relatively simple. Its most common use is to determine time-of-travel. Introducing a slug of dye at point A and noting the time it takes to appear at point B obviously gives you time-of-travel. With this technique you can also find the rate of flow with just a few minutes more calculation.

The National Bureau of Standards (NBS) and the Environmental Protection Agency (EPA) have each included, in special publications, a discussion of chemical additive (dilution) techniques for measuring flow (30, 31). The NBS publication is a general discussion covering both the steady-state and slug methods. The EPA publication mentions the wide use and convenience of fluorescent dyes.

The slug technique has another advantage. If it is injected at the inlet of a contact chamber or settling basin, the profile of tracer concentration versus time at the exit will yield minimum, mean, and median retention times, and will detect channeling.

The examples in this monograph should illustrate, in relative detail, the theory, practice, equipment requirements, and possible problems in determining flow rate and time-of-travel in typical situations. It is impossible to cite all situations and literature pertaining to the subject. However, several detailed and readily available articles are cited, and reading is recommended where appropriate.

B. EXAMPLES

Twenty-four tests of dye technique in 15 different field situations have been summarized by Kilpatrick (1). Discharge could be accurately measured by meter or weir in these situations, although the probable accuracy of the latter methods was not included. Flow rates ranged from 1 to 3,000 cubic feet per second. The studies included both smooth and turbulent flow in various types of natural streams, canals (lined and unlined), concrete pipes, and even under ice.

The researchers concluded that the constant-rate injection (steady-state) technique compared favorably to other techniques. The largest discrepancy was 6.4%, but most were less than 2%.

The single-slug technique was very accurate in canals and other artificial conveyance structures, but was less accurate in natural streams. A portion of the inaccuracy in natural streams could be attributed to the manual sampling technique used in the study.

Kilpatrick, et al., lists a large number of applications where the dye method is more convenient and/or accurate than other techniques. The dye method is very convenient and low in cost, including labor costs (1).

Replogle, et al., demonstrates the accuracy of the single-slug technique in artificial structures. A laboratory flume was precisely calibrated at various flow rates with gravimetric techniques. The variation coefficient was 0.9%, and the maximum error was only 1.4% (2).

Morgan, et al., researches the steady-state dye-dilution method in a volumetrically-calibrated flume at six flows, ranging from 54 cfs to 198 cfs (32). With one exception, the correlation ranged from 1.3 to 2.6%. The correlation would probably have been better, but for two problems. First, the volumetric determination only gave the average flow over a 4.00-second time period, although it was evident that short-term fluctuations varied as much as 8-10%. This particular problem was largely, but not totally solved by averaging the readings of 20-30 dye samples taken during the 400-second run. Secondly, there were massive levels of micro air bubbles and

not enough dye was used. This will be discussed in detail in section G3 on Turbidity.

Kilpatrick reiterates the need for an in-place calibration technique for many of the permanent devices used for fluid discharge measurements (3). Removal of these instruments to the laboratory is inconvenient and usually expensive. In addition, the hydraulic and sediment conditions existing in the field may be impossible to duplicate in the calibration laboratory. Excellent correlations were found between the constant-rate dye dilution technique, and volumetric or other careful calibrations of an orifice, a spillway, a sharp-crested weir and a trapezoid flume. Discharge ranged from 0.5 to 400 cfs.

In addition, four parshall flumes, used to measure raw sewage, were tested. With two of the flumes, the measurements agreed within 3% of the ratings. Two others were discovered to be carrying more flow than is allowed in the standard ratings. As a result of the dye technique, the correct rating brought the inflow/outflow budget of the sewage plant into balance, correcting the considerable discrepancy.

Morgan, et al., uses the dye-dilution technique to validate the performance of a single-stage, mixed-flow, vertical turbine pump (32). The newly installed pump was operating to within 1.5% of its design point of 70,000 gpm at 93 feet TDH. Periodic testing of large pumps to determine the necessity of overhaul becomes a relatively simple project with the dye-dilution method.

C. FLOW RATE THEORY

1. Constant-Rate Injection

Typically, in constant-rate injection, dye is continuously injected upstream at a spot far enough from the measurement site that the dye has time to mix thoroughly, producing a uniform concentration across the section of stream at the measurement site. Under these conditions, the rate of flow is calculated as follows:

$$(1) \quad Q = q \frac{C}{c}$$

Where:

- Q is the discharge rate in the desired units, usually cubic feet per second.
- q is the rate at which the dye is injected (in the same units as Q).
- C is the concentration of the injected dye.
- c is the concentration of the dye at the point of measurement.

It is assumed that the rate of addition of the dye is negligible compared to the stream flow.

Because C/c is simply the dilution factor, the actual concentration of dye is not an element of the equation. Simply compare the stream sample with an accurate dilution of the injected sample.

The calculation can be broken down further (33):

$$(1a) \quad Q = q \frac{R_{st} D}{R_s}$$

Where:

- R_{st} is the instrument readout or recorder reading of the standard, with the blank value set to 0 (or subtracted).
- R_s is the instrument readout or recorder reading of the sample, with the blank value set to 0 (or subtracted).

- D is the dilution factor used in preparing the standard.

Because the calibration of the instrument is stable, accurate dilution may be made and measured on the instrument at any time before or after the study, in the laboratory or field. If you need to change the injected dye concentration in the field because it is too concentrated downstream, simply pour in water and mix. Remember, however, to save a sample of the new solution for accurate dilution to calibrate the fluorometer.

In large, slow-moving streams or in situations where it is impossible or impractical to sample at a location where the dye is uniformly dispersed, equation 1 is not valid. Such situations are relatively rare (generally large-scale studies), and Kilpatrick, et al., gives the more general equation and discusses a successful solution using special sampling techniques (1). In general, requirements for this type of study include multiple sampling devices, whose individual volumes are weighted by the velocity of the stream at the point of sampling.

Although in a vast majority of cases, the sample point is far enough downstream to ensure complete mixing, in those few cases of incomplete mixing, the error in the uncorrected measurement will be small. Estimate by sampling the cross section. If the concentration is constant over the majority of the section, but changes at the slow-moving edges, an approximate correction can be made. Estimate the cross sections involved, and measure (or estimate) the velocities. Weigh the sections in direct proportion to size and velocity. Even an approximate correction of a small error yields an accurate answer. For example, a 20% error in making a 5% correction yields an overall error of just 1%.

Another technique that is useful in cases of incomplete mixing is forcing uniform concentration at the site of measurement. Multiple injection sites are set up, on a bridge for example, and injection rates are adjusted until the downstream profile is uniform. Using this method:

$$(2) \quad Q = (q_1 + q_2 + \dots q_n) \frac{C}{C}$$

Where:

$(q_1+q_2+\dots+q_n)$ is the arithmetic sum of the flows of all the injectors.

There also may be cases in which the range of injection rate available is insufficient. For these cases, another technically correct approach is possible, although there is no formal report of its use. Keep all the injection rates the same and adjust the concentration of the dye in the individual reservoirs. Using this method:

$$(3) \quad Q = (C_1+C_2+\dots+C_n) \frac{q}{c}$$

It is possible to vary both the injection rate and the concentration without too much complication. Using this method:

$$(4) \quad Q = \frac{(C_1q_1+C_2q_2+\dots+C_nq_n)}{c}$$

Note that these dilutions can be made in the field. Save a sample of each for later accurate dilution in the laboratory. The time and dye saved by such a method makes the extra measurement well worth the effort.

Note also that the dye to be injected must be completely mixed: a great deal of stirring is required for a 50-gallon container of dye. Therefore, if a choice exists between a high injection rate (low dye concentration) and a low injection rate (high dye concentration), the latter might be the best choice to insure adequate concentrations.

2. Slug Injection

The slug injection technique features a discrete amount of tracer poured or injected into the stream over a short time period. At the measurement site, the concentration is measured continuously from the first arrival of tracer until all has passed. The preferred method is by continuously pumping stream water through the fluorometer with a recorder attached (2, 4). Using this method:

$$(5) \quad Q = \frac{M}{\int_a^b c dt}$$

Where:

M is the total amount of dye added.

Q is the flow rate of the system and is assumed to be constant during the measurement.

t is time, usually in seconds.

c is the dye concentration passing the sampling location as a function of time.

d is the dilution factor used in preparing the standard.

This equation is not as formidable as it seems. The integral is the area of the recorder tracing of concentration versus time. This area may be measured in a variety of ways: using an integrating recorder, counting squares, using a compensating polar planimeter (fairly inexpensive and easy to use), or by cutting the curve out with scissors and comparing its weight (on an analytical balance) with that of a known area. The units of measurement may be anything, provided that c and M are constant: if c is in grams per liter, then M must be in grams. Or, if M is in pounds, c is in pounds per cubic foot, and t is in seconds, then the answer will be in cubic feet per second. Note that once again, the actual amount of the tracer M and the actual concentration c are not required. In practice, a known volume of an approximately known concentration of tracer will be added. Because the fluorometer will be calibrated with an accurately diluted sample of the concentrated solution, the dilution factor will be correct. Note also that the time required to inject the dye is unimportant, and is not part of the equation.

The slug method does have one major drawback: it is not as easy to use where dispersion is non-uniform. Concentration must be uniform throughout the cross section of the stream at the measurement site. In small turbulent streams, simply introducing the dye at one point is adequate. In wide sluggish streams, however, it helps to inject the dye from a boat angling downstream at a rate to match the current, as described later in time-of-travel studies.

Sampling with this method is done from one point. The best method is to continuously pump stream water through the fluorometer and automatically record the concentration. In large systems it is possible to take grab samples at timed intervals for individual measurement. Time between taking samples should be short enough to accurately define the curve. In either method, it is an absolute requirement that all the dye be accounted for. Sampling must begin prior to the arrival of the dye at the sampling point and continue until all traces of dye have disappeared.

The slug method has recently lost favor in natural stream measurement due to the availability of simple, portable, constant-rate injectors. In very large systems, however, the slug technique might be chosen because of its significantly smaller dye requirements. In addition, slug injection is used in time-of-travel studies, and will, as previously mentioned, give the flow rate with some additional calculation.

3. Velocity Method

In systems where the cross section is constant (and measurable) and the velocity is constant across the profile (except for negligible surface effects), the rate of flow may be accurately determined from the velocity. The velocity may be determined easily and accurately by calculating transit time between two points.

The use of various chemical tracers for this purpose has been studied in open canals (5, pg. 164) and in closed pipes (5, pg. 192). Salt (detection with conductivity), colored dyes, and fluorescent dyes were used. Unless the points of injection and detection are quite far apart, accuracy requires that the injection of the tracer be extremely rapid in order to yield a sharp profile, and that the response time of the detection equipment be negligible.

Fluorescent dyes can be used in small quantities, a great advantage, making rapid injection easy. For all but extremely short transit times, the rapid response of the Turner Designs Model 10 Series Fluorometer (one second to 63%) will be more than adequate. A simple modification (which requires only 20 minutes) will shorten the response time to 0.1 second.

The standard one-second response time is regulated by internal damping and is required only when the highest sensitivity settings are used. Reduced damping would effectively lower the ultimate sensitivity (by increasing readout noise) by a factor of about three, leaving the instrument with at least 30-100 times the sensitivity required for such studies.

The time required to pump the sample to the instrument is another factor to be considered in velocity studies. Although this time will be fairly constant, determinable and deductible from the observed time, it is better to have it short and best to have it negligible. The optical flow cell in the fluorometer is designed to accept large hoses and very high flow rates. Because the fluorometer is portable and may be operated very close to the channel, it is relatively easy to keep the time lag below one second.

4. Multi-channel Situations

Up to this point in the discussion the assumption has been that no water leaves or enters the stream or system between the injection point and the sampling point. If there is a multi-channel situation, you need to determine the point in the system where the measured flow rate exists.

The simplest example is one where the stream diverges just after the complete mixing of the tracer, but before the sampling location. The flow measured is prior to the diversion. Because the concentration is not altered by the diversion, there is no effect on the measurement. Thus, the measurement may be made at whichever stream is more convenient. Be sure, however, that you don't use this method if the diversion occurs prior to complete mixing.

The most common multi-channel occurrence is the convergent system, i.e. a river and its tributaries, sewers flowing into mains, or infusion (leakage of groundwater into the system). The flow measured is the sum of the flows - that flow existing at the point of sampling. In other words, you need to measure the flow past the last point of convergence. The location of sampling should be sufficiently downstream to permit complete mixing of the new water, and is easily checked when constant-rate injection is used.

Note that the introduction point of the dye is unimportant; it may be a convenient canal or pipe entering the main stream.

5. Infusion and Leakage Studies

Frequently, you will want to detect unseen convergence or divergence. Such changes in the flow rate may be discrete (leaks in sewers letting water either in or out), or continuous (porous sand in a natural system). In either case the procedure is the same: multiple overlapping reaches are individually measured. With a discrete leak, a discontinuity appears in the flow between two studies. The cause may be localized by further studies in the reach that contains the discontinuity. In continuous gains or losses, the flow rate steadily increases or decreases in successive downstream reaches.

Leakage measurements are simple to calculate, and very valuable in sewer systems. For example, a one-gallon-per-minute infiltration leak in a sanitary sewer costs about \$150 to \$600 in capital investment to process (6). Careful preliminary studies showed that sediment in sewage had no effect on measurement of the fluorescent dye, that background readings were low, and that the constant-rate injection method compared favorably with measurements by parshall flumes.

Smith and Kepple also measured sewage flows in Anderson, California, a city with a population of about 6,000 people that experienced substantial groundwater infiltration during the winter months (6). Measurements were made between 3:00 and 5:00 a.m., at a time when most of the flow came from infiltration and identifiable continuous users. A baseline study was conducted when the water table was below the sewer invert elevations, when the flows would be primarily from continuous users. A second study was conducted in January when the water table was high, and infiltration contributed significantly to the flow. Sampling at 35 locations required about two hours, and the researchers localized the areas of infiltration and estimated the magnitude of each. Their method of plotting and handling the data is excellent, and their study is recommended reading (6).

The popularity of dye-dilution methods for measuring sanitary-sewer flow has grown rapidly. It has many advantages: speed, accuracy, one-man operation (no-need to crawl a manhole), and applicability to flows varying from a trickle to a full pipe, and even surcharged manholes. Turner Designs offers a manual designed to teach correct performance of all phases of operation to a field crew (33).

6. Ground Water

Flow rate, time-of-travel, and water budget studies in simple Karst systems have had excellent results. When rhodamine B, rhodamine WT, sodium chloride, potassium chloride, and tritium were compared as tracers in studies of groundwater flow of treated sewage in coarse sand, rhodamine WT was found to be the most effective (24, 25). The researchers were able to follow rhodamine WT further than tritium although it was somewhat attenuated (presumably by absorption). In addition, in two studies rhodamine WT was found to be an effective tracer to prove that leachate from a highway deicing salt storage area reached adjacent wells.

Despite these studies, the use of fluorescent dye tracers in typical groundwater systems has not been thoroughly investigated. No data is available on the extent to which the various dyes will be absorbed on soil or subsurface strata. Only a few quantitative studies of sorption have been published (2, 4, 7). In all the studies, the sorption was studied by allowing a solution of the dye to stand in the presence of the substrate. Percolation through the substrate would be more appropriate to groundwater studies. The substrates studied were varied sediments, organic material, and sand. In the sand study, only rhodamine B was tried and was partially adsorbed (4). Subsequent washing recovered most of the adsorbed dye. Rhodamine B is useless for groundwater studies because of absorption. But pontacyl brilliant-pink B and rhodamine WT are vastly superior for these studies, showing little sorption.

Fluorescein has been used for years to study short groundwater reaches from outhouses to wells. Although it is almost never used in surface systems because of its photosensitivity and relatively high background, it shows no sorption in limited tests (7). To provide good detectability, fluorescein can be considered for use in certain groundwater studies

where background fluorescence is determined to be sufficiently low. There is no question, however, that rhodamine WT is the dye of choice for further study.

While the success of rhodamine WT in groundwater studies cannot be predicted, its sensitivity and low cost makes a trial worth the effort and positive results are mostly self-checking. If the dye is injected at a constant rate and there is little or no sorption, the concentration profile at the sampling point will resemble that obtained in studies in streams; i.e. relatively rapid rise in concentration, then a plateau, and lastly, after injection has stopped, a relatively rapid drop in concentration. If sorption is high, then there will be a slow rise in concentration as sorption sites are filled, followed by considerable tailing. If the underground channel is constricted, a mass balance could be obtained by comparing the flow rate from the plateau value with integration of the area under the concentration curve (which should also yield the total amount of dye added).

Brown and Ford review the available literature on groundwater studies and discusses Karst studies conducted (23). Fluorescent dyes have been only partially successful in this area. In some cases negative results or partial recoveries can be attributed to sorption; and in others, the failure to recognize the size of the system, and to using insufficient tracer. In addition, Karst systems can be very complex, with many branches. On occasion, large amounts of tritium (a conservative tracer) were not totally accounted for and in some cases, were lost entirely in such systems.

D. FLOW-RATE PRACTICE

1. Injection Rate

This section describes the simple calculations required to determine a suitable dye-injection rate for the constant-rate method. The requirements for slug injection are discussed in the section on time-of-travel.

Because the fluorometer is capable of accurate measurement over a wide range of concentration, there is no optimum injection rate - only a widely separated minimum and maximum requirement. The following factors must be considered:

- 1) Accuracy required in the determination of the flow rate.
- 2) Background (the natural reading of the water before the dye is added).
- 3) Estimated maximum and minimum flow rates.
- 4) If applicable, the maximum allowable concentration flowing past an intake to a potable water system.
- 5) Ease of measurability. (Below 100 parts per billion, concentration is linear with instrument reading. Above this number, concentrations are read from a calibration curve.)

The significance of these factors is best described through example: you backpack into the woods to measure the discharge of a number of small streams, which have some sediment and organic matter due to runoff, but are relatively clear. An accuracy of $\pm 5\%$ is considered to be satisfactory. You are limited to the equipment you can carry. Dye cost will be negligible regardless of the amount you use, but you wish to use the smallest possible amount to make handling easier and to conserve a limited supply.

At the first stream, you proceed as follows:

- 1) You estimate that the flow rate is between 50 and 500 cubic feet per second.
- 2) You take several water samples over a period of about 15 minutes.
- 3) You read the fluorescence of these samples with your pre-calibrated fluorometer, and determine that the fluorescent "blank" before dye addition is equivalent to $0.15 \pm .05$ ppb of the dye. (This is unusually high for purposes of illustration.)

The minimum required dye concentration will be set by the experimentally determined variability in the "blank". Because the required accuracy of measurement is $\pm 5\%$ (or one part in 20), the dye

concentration must be at least 20 times the "blank" variability (or one ppb).

To give an adequate safety margin, a minimum concentration of two ppb is chosen. Now, return to the basic equation already illustrated in Flow Rate Theory:

$$(1) \quad Q = q \frac{C}{c}$$

Where:

Q is the stream flow rate.

q is the injection rate

C is the concentration of the injected dye, (here assumed unity, because your fluorometer was originally calibrated on a dilution of this dye.)

c is the dye concentration in the stream.

Rearrangement of the equation yields:

$$q = Q \frac{c}{C}$$

Because the largest Q will yield the lowest c, the top estimated flow rate of 500 cubic feet per second (cfs) is used and q must be:

$$500 \times \frac{2 \times 10^{-9}}{1}$$

or 10^{-6} cfs. In more easily visualized terms, this is about 1.7 ml/minute.

Now let's examine the situation. First, there is no dye problem, as a liter of dye will last for nearly 10 hours and you will sample only until a definite plateau is established. This period will only be a few minutes for fast, smooth channels at 100 cfs, and perhaps 15 minutes for a cobble stream. Second, if the flow rate is only 50 cfs, the dye concentration in the stream will be 20 ppb. This is well within the linear range of the fluorometer.

One more adjustment is necessary. As you are limited in portable equipment, you chose an injector

with the relatively high injection rate of 50 ml/minute. You need to inject 1.7 ml/minute of the dye. You can now dilute by 50/1.7, or approximately 30-fold (with stream water). The dilution need not be accurately made, but the dye must be thoroughly mixed. Save a small sample of the mixed dye so that you can calibrate your fluorometer with a precise dilution of the dye actually injected.

Next, consider the calibration of a sewage plant flow meter at 5, 10, and 20 million gallons per day. (It is presumed that an accurately calibrated variable speed injector is available).

- 1) You check the background and find it is unusually high -- 0.8-1.2 ppb.
- 2) You decide to reduce this source of error to 0.2% or less.

These conditions set the desired dye concentrations for measurement at a minimum of 100 ppb, and an injection rate of about 5.5 ml/minute, with the 20 million gallon-per-day rate. If this same injection rate were used at the lower flow rates, the dye concentration would be 200 ppb at 10 million gallons per day, and 400 ppb at 5 million gallons per day. (These latter concentrations are above the linear range.) Three approaches are possible:

- 1) Prepare a multi-point calibration curve for the fluorometer. This requires multiple precise dilutions, and at the higher concentrations, accuracy suffers slightly because equal increments in concentrations yield progressively smaller increments of meter readings.
- 2) Precisely dilute the dye to be injected. For the five-million-gallon situation, perform a five-fold dilution. Otherwise, dilute approximately and recalibrate the fluorometer.
- 3) Reduce the rate of injection (the simplest approach).

2. Site Location

In constant-rate injection studies, it is necessary to have the concentration at the measurement point reach a plateau. The plateau must continue long enough to verify its existence. For a fast-flowing canal (which takes only a few minutes from first appearance of dye to the plateau), only a few additional minutes of measurement are necessary. With long reaches or in slow-moving streams, longitudinal dispersion causes the rise of dye concentration to take longer, and definite proof of the plateau takes longer.

In natural streams, rise time is also affected by storage areas. These storage areas slowly take dye from the main stream. Until the entire system is in equilibrium, the dye concentration downstream will be lower than it should be and the calculated discharge higher. Whenever possible, site selection should avoid obvious storage areas between the injection and the sampling points. Many storage areas are not obvious, and in practice a true plateau usually requires much longer injection in natural streams. As an aside, storage is a probable reason for the poorer results with the slug method in natural systems.

It is axiomatic that the injection should continue long enough to produce the desired plateau. In small systems, it is practical to continue the injection until the measurement is complete. In large systems, such a practice could needlessly expend a large amount of dye because distance between the sampling point and the injection point has a definite effect on the rate of the rise to the plateau. Increasing distance slows the rise. In other words, if dye is injected for a fixed period of time, the further downstream it is measured, the shorter will be the plateau, with a corresponding increase in rise and tailing time. The optimum point of measurement is no further downstream than safely below the region of complete lateral mixing.

Optimum sampling position is not as critical as it sounds, although rules of thumb and calculations will be discussed. In small fast-moving streams, one can afford to be conservative and sample further downstream than the estimated minimum distance. The additional dye and time required are trivial. In large slow-moving streams, there is plenty of time to test for complete mixing and move further downstream if complete mixing has not been achieved. With the portable Model 10 Series

Fluorometer, you can take readings from a rowboat as easily as you can in the laboratory. You aren't doing dilutions or calculations at this point, but simply checking that readings are constant across a profile. Start at the calculated spot or even closer, wait for the plateau, and test for uniformity. If things aren't right, head the boat downstream and try again.

Kilpatrick, et al. gives an equation for calculation of the distance required for complete mixing (1). Rules-of-thumb are found in a number of articles (1, 2, 8). The most common rule is to sample 100 stream widths below the injection point. Some typical observations and factors to be considered are:

- 1) "Typically, on small cobble-strewn streams, using a single-point (dye-injection) source, a 30-minute injection will yield a 15-minute plateau (of dye concentration) at the required mixing distance. Greater distance will produce a shorter plateau, but improve mixing. On larger, more sluggish streams, several hours of injection may be required and the method becomes impractical unless improved techniques (also discussed) are used. In a swift-flowing, concrete-lined canal, a 30-minute injection has been found to yield a 28-minute plateau, at a distance of 250 channel widths. Thus, canal flows are well suited to any of the dye dilution measuring techniques." (1, parenthetical notes added).
- 2) Study the stream carefully before starting. Note the location of tributaries and their possible effect on complete mixing of the tracer at the measurement site. Take best advantage of the terrain, with easy access for injection and measurement. Take note of turbulent sections with the idea of injecting above them.
- 3) Where the system is completely or partially filled by a pump, inject the dye upstream of the pump. This greatly accelerates mixing (2, 3). Injection in riffles or turbulence is helpful.

- 4) With single-point injection, the length required for mixing increases roughly as the square of the stream width for smoothly flowing streams (1). The rule of 100 stream widths is probably more than enough for small turbulent streams, but may be too short for large sluggish streams.
- 5) Holly presents an experimental, theoretical, discussion of mixing tracer that is added at steady state to a full smooth pipe (35). For a single-injection port at the wall and a single-measurement port at the pipe wall, any measured concentration at a distance greater than 220 pipe diameters is within 0.5% of the average. For 1%, the distance is about 175 pipe diameters. Multiple and jet-injection effects are also discussed.

3. Dye Loss

The characteristics of current fluorescent dye tracers, particularly rhodamine WT, are such that one rarely encounters significant loss of dye during a measurement. The possibility does exist in certain situations, however, and one should know how to check, recognize, and correct for dye loss. There are three general mechanisms to be considered: photodecomposition (the destruction of dye by sunlight), chemical degradation, and loss by sorption on sediment or stream bed.

With the exception of fluorescein, photodecomposition will be negligible unless the study proceeds for several days. Such studies will probably be time-of-travel determinations (discussed later) where loss of dye does not affect the answer as long as enough tracer remains to identify its passage. Should it be necessary to estimate loss, a relatively large tank with no top, such as a fish tank (preferably all glass) may be filled with a solution of dye made up in distilled water at about 100 ppb. The level should be marked so that evaporative loss can be replaced. This is measured, left in the open for the duration of the study, and measured again. This yields the maximum possible loss. Stream depth and turbidity will reduce loss.

Chemical degradation is rare. Strong oxidizing agents could destroy the dyes, but because their discharge is discouraged, high concentrations are unlikely. Low concentrations of oxidizing agents would probably be consumed by other organic matter present, as the dyes are relatively unreactive. An extreme variation in pH could cause what would appear to be reversible loss of dye. But because the fluorescence of all dyes except fluorescein is stable over a pH range of at least 4-10.5 (8), the loss is unlikely. All aquatic life would be dead before the measurements were affected.

The only likely candidate for chemical destruction of dye is chlorine. Many researchers report that chlorine destroys both rhodamine B and rhodamine WT dye. In all likelihood, these tests were done with elemental chlorine and not "residual" chlorine. High concentrations of residual chlorine do degrade the dyes, but Deaner demonstrates that the loss is quite slow at normally encountered chlorine levels (9). For example, a chlorine residual of 2.3 mg/l (2.3 parts per million), caused the loss of only 3% of a ten parts per billion concentration of rhodamine WT over a 20-hour period. This study was very carefully done, and chlorine residuals were measured both at the beginning and end of each experiment. However, the studies were done only in water of high organic content (sewage), which might have had a protective effect (although it is unlikely, as significant chlorine residuals always remained).

Data indicates that free chlorine may cause problems when bromine salts are present, as they are in sea water. Free chlorine reacts with the bromine salts, which in turn react with the rhodamine dyes (40).

Sorption has been studied and discussed extensively in the reference literature, probably because the first truly practical tracer, rhodamine B, shows significant sorption in certain situations. However, sorption is not considered a significant problem with rhodamine WT, although it will be discussed later in some detail.

Loss of dye through sorption is usually reversible. For example, the substrate moves with the water (and dye), as suspended sediment. Sorption will affect readings, but is correctable or, the substrate does not move with the water, or is associated with the stream bed. This situation affects the rise time and tailing,

but probably won't cause an error in the determination.

Loss of dye by sorption on suspended sediment is easily checked. Correction for dye loss can be made with the constant-rate injection method. To do so, make up the same (but not necessarily exact) dilution of dye with both stream water and clear water with the same salinity as the stream water (7). The dye concentration should be close to that in the study, as any loss will be concentration dependent. Allow the stream sample to stand for the length of time required for the study, stirring occasionally if necessary, to keep the sediment suspended. Measure both samples. If a small difference is discovered, correct the stream data by multiplying the fluorometer reading by the ratio of the readings of the clear sample to that of the stream sample. In the unlikely event that a large difference is discovered, additional experiments will be necessary to empirically discover a concentration that ultimately will yield a reading close to that of the main study. Obviously, the same may be accomplished by calibrating the fluorometer with a dye dilution made with stream water. The dye dilution used for calibration should yield about the same reading as that found in the stream.

Sorption is not an instantaneous process. In a study of fine sediment, a one-hour period was found to be sufficient to allow free concentration to stabilize (7). In the stream itself, if the time lapse is short between injection and measurement, a second measurement downstream will show a lower reading if sorption is occurring. This could, however, be due to sorption on the stream bed. In a long reach, sorption on suspended sediment will likely be stabilized prior to measurement.

Significant sorption on the stream bed is likely in shallow streams with fine sediment on the bottom. Cobbled streams and similar systems seldom present a problem. In severe cases of sorption on the stream bed, the concentration will never stabilize, as no plateau will be found. More commonly, the sorption (if it occurs at all) will probably be slight and reversible, and you will observe a slower than normal rise to the plateau as the sorption sites are saturated, followed by extended tailing after the plateau passes. The plateau concentration may be slightly low due to secondary sorption sites, but if a true plateau is

identified, any error is probably negligible. It may be useful to obtain a mass balance as discussed in groundwater studies.

In the slug-injection method, the treatment of sorption is somewhat more complex. Sorption by the stream bed can be detected by measurement at several downstream points, yielding decreasing curve areas. If sorption is not severe, a correction can be made simply by extrapolation. Sorption on suspended sediment may be handled with the steady-state method, but will require multiple sediment studies because of the concentration dependence.

E. TIME-OF-TRAVEL PRACTICE

1. Introduction

Time-of-travel measurements are needed to provide a better understanding of how a stream copes with injected waste, in order to aid Civil Defense planning in the case of a sudden introduction of a harmful contaminant into a stream (8), and to determine what action should be taken in case of release of radioactive materials from a power plant (10).

The U.S. Geological Survey ran time-of-travel studies on nearly 100 streams in 30 states (11). The method they used may be implemented rapidly in an emergency, such as the Mississippi River at flood stage.

Detailed descriptions of specific experiments are given for work on the Wind/Bighorn River in Wyoming (12), the Missouri River (13), the Umpqua River in Oregon (8), and the Ottawa River in Canada (10).

An excellent handbook, with chapters covering dyes, monographs to determine amounts of dye to inject, selection of sites, and sampling techniques is also available (14). Kilpatrick gives more complete information on amounts of dye required (15).

In many cases, the information desired is not only time-of-travel of the stream or system as a whole, but also the rate of dispersion and mixing. How long, for instance, will an accidental spill take to disperse into a harmless concentration? Will industrial waste, discharged continuously at one point, disperse rapidly, or will it remain a concentrated plume for

many miles? Do contaminants discharged into an estuary flush steadily to the sea or circle the estuary? Fluorescent dyes have been used extensively in such studies. In fact, much of the early use of these tracers was to define problems of this type. Although this topic will not be discussed further in this monograph, it is the subject of another monograph entitled "Circulation, Dispersion and Plume Studies," which is available upon request from Turner Designs (29).

Time-of-travel studies tend to be of most interest in large systems. Fluorescent dyes are unquestionably the best tracers to use because of the low cost and quantities required, ease of measurement, time, equipment, and manpower. Nonetheless, a large study requires careful planning to ensure success. Not only should the public be informed (through the press) of the purpose of the study, but all applicable agencies should be advised of all operational aspects. In the early stages before dispersion, dye is so visible that it needs to be explained. Justifying a test to the public is often a great way to test whether the study you are doing is necessary or not.

2. Dye Requirements

Kilpatrick summarizes the results of 400 studies using rhodamine B and BA dyes, and 85 studies using rhodamine WT (15). Curves that allow easy selection of the proper amount of tracer as a function of discharge, length of reach, and mean velocity are shown. Because of its low sorption loss, rhodamine WT requires only a single curve. As an alternative method to the curve, an empirical formula is presented for calculation of the amount of dye needed.

The Kilpatrick reference should be used by anyone who does time-of-travel measurements. We will not, therefore, duplicate its curves. We also recommend a chapter by Collings in a booklet published by the U.S. Geological Survey (14).

3. Dye Injection

In small streams the dye is usually simply poured into the main current of the stream. In large and sluggish streams where lateral mixing is quite slow, the dye is usually given horizontal distribution by the researcher. If a bridge is available, simultaneous pouring or

injection from several points is used. Commonly the dye is continuously injected from a boat traversing the stream at an angle to compensate for the current (8). Injection just upstream of riffles will speed lateral and vertical distribution. When concentrated dye is added to the stream, its specific gravity should be adjusted to near that of the stream by dilution or other techniques (8). See section J on DYES for further details.

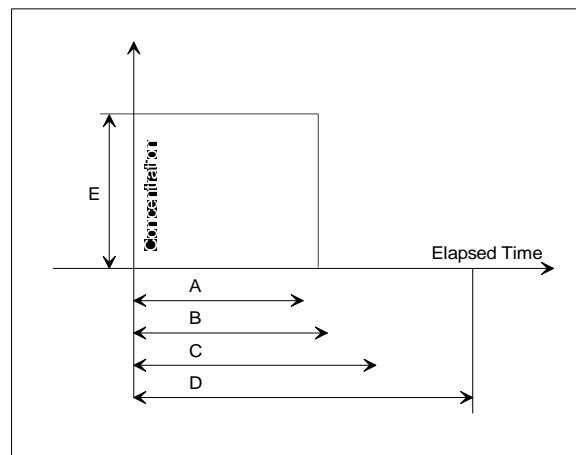
4. Site Location

The chapter by Collings in the USGS booklet discusses the factors involved in selecting the best sites for injection and measurement (14). Collings emphasizes that every reach is unique in some way, and the importance of careful examination prior to inauguration of a time-of-travel study. Since this chapter is recommended reading, we won't try to summarize it.

In order to decrease the concentration of tracer passing a given point, long reaches are frequently divided into sub-reaches with separate, but possibly simultaneous, injections of dye. Other benefits are discussed. Secondly, this monograph and other papers go into some detail on recognition of dye loss due to sorption (15). With rhodamine WT, this is sharply reduced.

5. Interpretation of Data

As the dye slug passes a downstream point, a typical plot of concentration versus time will have the following form (11, 14):



A, B, C and D are travel times respectively of the leading edge, peak concentration, centroid (mean travel time), and complete passage.

The shape of the curve is affected by the type of stream. Canals or rivers with fast, smooth flows yield almost symmetrical curves with only slight tailing. In other words, B and C coincide and A and D are nearly the same distance from B. Slow-moving streams or passage through vegetation or swampy areas yield long tails. Reservoirs, lakes or ponds in the reach can lead to very broad curves with irregularities and even multiple peaks (14).

The shape of the curve may be used to calculate the longitudinal dispersion coefficient of the stream, frequently an item of interest (13). Distortion of the curve by sorption effects (occasionally observed with rhodamine B, but seldom with rhodamine WT) would cause error.

F. RESIDENCE TIME

In the ideal chlorine contact chamber, chlorine would be mixed with sewage and the sewage would traverse the chamber at a uniform rate, without dispersion, so that each drop of water is treated for the same length of time before the chlorine is diluted by discharge. In practice, of course, some water exits in a shorter or longer time than the majority. What is surprising, however, is that studies in California showed that even the mean residence times are lower than expected, varying from 30-80 percent of the theoretical times (16).

Deaner develops a simple fluorescent-dye procedure for determining the distribution of residence times. The procedure is described in detail and the measurements are interpreted (16).

G. MEASUREMENT TECHNIQUES

1. Sample Characteristics

Certain factors may affect the measurement of the sample. The readout of the fluorometer is proportional to a linear concentration from the smallest detectable concentration to about 0.1 parts per million. (See section J on Dyes for further details.) As the concentration is further increased, the readout

rises at a decreasing rate, and eventually reverses at a very high concentration. There is no possibility of confusion. At the point where readings become non-linear, the sample will have a faint but definite pink color when viewed through a one-inch test tube. If you can't see the pink, it is definitely linear.

At concentrations below 0.1 parts per million, a single-point calibration (or any known lower concentration) may be used to calibrate the instrument because if one sensitivity range is calibrated, all are calibrated. For concentrations between 0.1-0.5 parts per million, a multi-point calibration curve is used or the sample is diluted. Above 0.3 parts per million, dilution will be more accurate. Above 0.5 parts per million, dilute the sample before calibration.

Any other material in the sample that absorbs light will give a reduced reading: dissolved colored material or suspended solids, for example. However, suspended solids that are light in color reflect rather than absorb the green light used for excitation, and may not affect readings at all, even at quite high levels of turbidity. If an effect is suspected, it is easily checked and a correction applied (see section G3 on Turbidity).

The one slight drawback to the fluorometry method is the problem of temperature. All the dyes are inversely affected by temperature: increasing the sample temperature reduces fluorescence, and decreasing temperature increases fluorescence. One can either control the sample temperature (which is easy in a laboratory) or one can correct for it. Because the correction is independent of concentration, it isn't difficult. Even controlling the temperature in the field is feasible. Simply hang the standard (carefully sealed) over the side of the boat in the water until sample and standard are the same temperature.

Temperature effects on dyes are already widely recognized. Field measurement of discharge rate, as routinely practiced, can also be affected by temperature, and accuracy can be improved substantially. Some of the variations assumed to be due to uncontrollable or unknown effects are due to temperature. The optical filters used in all filter fluorometers show an inverse change in characteristics as a function of temperature. Pre-

calibration is strictly valid only if the ambient temperature is the same and the instrument has been allowed to come to full operating temperature. Depending on the type of photomultiplier tube and the choice of optical filters, the observed change is -0.15% to -0.33 % in °F. What is important is filter temperature rather than ambient temperature. The Turner Designs Model 10 Series Fluorometer has a uniquely low filter temperature rise (above ambient), but no definite figure can be given because factors such as wind, flowing sample, and direct sunlight will affect this rise. Conveniently, however, the front panel temperature is nearly the same as the filter temperature. This refinement may also explain the perplexing, occasionally reported recoveries of dye in excess of 100%.

2. Calibration

Operation, calibration and the taking of measurements are fully described in the operating manual accompanying the Turner Designs Model 10 Series Fluorometer. Only a few general points will be made here.

Although reading the operating manual will probably save you time and frustration, you will not harm the instrument by touching it first.

Calibration consists of simply inserting a known concentration or known dilution of the dye into the instrument and taking a reading. You may accept the reading you get and calculate all others by ratio. If, for instance, a one ppb solution reads 4.5, a two ppb solution reads 9.0. You may also adjust the instrument to read the concentration of the standard numerically. The reading of a sample (with due regard to decimal point) is the concentration of the sample.

Finally, if you know the method, dilutions are simple to make accurate to better than 0.2%. You can be sloppy and do better than 1%, but it is best to do the dilutions properly, as a chemist would. You should also have the proper volumetric glassware to do the dilutions.

3. Turbidity

If you are in the field and suspect you may have to make a correction for turbidity, it is easy to check on-the-spot without volumetric dilution equipment.

- 1) Collect two samples of stream water. Add a drop of dye to one and mix. If it is more than a faint pink, pour some out and add more stream water.
- 2) Blank the fluorometer on the sample without dye, and read the sample with dye.
- 3) Allow both samples to settle.
- 4) Repeat step 2 on the clear portions. You probably won't have to adjust the blank, but it is good practice to check. Confirm that the temperature hasn't changed.
- 5) If the clear sample reads higher than the turbid one, use the ratio of the readings (clear divided by turbid) to correct all the readings from the stream.
This correction is valid for all dye concentrations with the assumption that the turbidity is constant.

If the sample won't settle, go ahead and make your study, but take a stream sample back to the laboratory where you can filter, centrifuge, or alternatively calibrate by an accurate dilution with the stream water. This latter course would be used in the rare case of a dissolved colored substance.

Turbidity rarely resists such corrections. Once, however, it occurred in a sewage treatment plant during calibration of magnetic flow meters by the constant-rate dye dilution technique. In this case, it was necessary to filter the samples prior to measurement.

An alternate solution, if the dye concentration is high enough to permit it, is to dilute the standard and samples with clear water. If, for example, the light loss due to turbidity or color in the original water were 30%, an unrecognized 10% change in turbidity or color would yield a 3% error in the answer. A 10-fold dilution will reduce the effect to 3%, reducing the

potential error to a negligible 0.3%. Remember that blanks should also be diluted 10-fold.

Turbidity causes a second effect which is generally unrecognized and is a potential error, although only in rare situations. All glass and gelatin optical filters are somewhat fluorescent. Scattered excitation light strikes the emission filter and causes it to fluoresce. If the blank and standard are prepared from the sample water, this additional fluorescence simply yields a higher blank than the same water without turbidity and there is no error. If the dye concentration is low enough so that the added contribution is significant, then a change in turbidity will cause an error in the opposite direction from that previously discussed. The effect is usually small and seldom a problem with dye concentrations above one ppb (actual ingredient). It should be borne in mind, however, that some of the worst scattering can occur in the absence of visible particles.

In the volumetric flume study (32), the injection rate was set to yield a rhodamine B concentration of one ppb at the highest flow (about 200 cfs). There was violent turbulence and many visible air bubbles in the early sections of the flume. Samples and blanks were collected far enough down the flume that the visible bubbles were absent. For convenience, however, water for the standards was collected from the river just prior to entry. At 200 cfs, the dye technique consistently calculated a flow 8% lower than the volumetric determination. The error reduced approximately linearly with reduction in flow. The problem was massive levels of micro air bubbles that were causing a blank increase of 0.08 ppb. In other words, the apparent dye concentration was 0.08 ppb higher than the true dye concentration.

Upon discovery, there was sufficient time to invent a practical means of removing the bubbles. Thus, water taken at the sample point was used to prepare standards and all standards, samples and blanks were measured 30 minutes after the water collection. This ploy was probably not totally successful due to the different treatment given to the standards (pipetting, mixing, etc.). (The researchers didn't think to chill the samples, an ultrasonic bath was not available, and the director of the hydraulic laboratory indicated that it would take from 24-48 hours for the bubbles to disappear). Note that if a dye

concentration of 10 ppb had been used in the beginning, the bias at 200 cfs would have been 0.8%, about 0.4% at 100 cfs, and would have probably been ignored. Unfortunately, sufficient dye was not available to make this increase after the problem was diagnosed.

4. Sample Temperature

The final problem is sample temperature. It is simplest to have your calibration standard and your samples at the same temperature. Float your carefully sealed standard in the stream. In the laboratory, place all the samples together, away from any source of heat. For precise studies, it is best to incubate both standard and samples in a water bath. The sample compartment of the fluorometer will be a few degrees above ambient temperature, but there will be no effect on the sample in the few seconds required for measurement. However, don't attempt to remeasure any sample until it has re-equilibrated, because the cuvette will have begun to warm up. If you wish to periodically recheck samples, or for some reason wish to have them in the fluorometer for an extended period, use the 10-030 Sample Holder, which is temperature-regulated.

If you need to correct various samples to a standard temperature, Wilson contains a simple graph from which an accurate correction is easily obtained (17). On the other hand, if you have a pocket calculator handy, the equation is:

$$F_r = F_s e^{[n(T_s - T_r)]}$$

Where:

F_r is the calculated fluorescent reading at the reference temperature, T_r .

F_s is the observed fluorescence reading of the sample at the time of reading the sample temperature, T_s .

e is the base of natural log.

n is a temperature coefficient for whatever dye is used. For Rhodamine WT, rhodamine B, pontacyl brilliant pink B, and fluorescein, the values have been

determined to be 0.026, 0.027, 0.029, and 0.0036 respectively (2,7).

T_s is the sample temperature at time of reading F_s .

T_r is the reference temperature.

Note that usually when presented, n has a negative value, but here the equation was rearranged in simpler form, with n being positive. All temperatures are in degrees centigrade. For small temperature differences, the values may be used directly. For a two-degree rise in temperature for rhodamine WT, the reading will drop 5.2% (2×0.026 , expressed as a percentage).

H. SAMPLING SYSTEMS

1. Grab, Manual

Hand-taken samples are satisfactory in all types of studies, even those requiring the profile of concentration versus time. The frequency of sampling will depend on the expected rate of rise and fall of the concentration. In the slug-injection technique, hand sampling has the disadvantage of requiring considerable clerical work, always with the possibility of human error. A running graph is valuable for catching inconsistencies, anticipating the need for more frequent sampling, or, conversely, showing that frequent sampling is not necessary.

Hand samples may be taken by simply dipping the clean storage container or cuvette below the surface, or by use of the many samplers available for sampling at depth.

Samples may be stored in polyethylene, polypropylene or glass. Prolonged contact with metals should be avoided. Copper, brass and iron have been shown to degrade some of the dyes, particularly when the water has a high salt content. But degradation is a slow process, so brief contact during collection is permissible.

Hand sampling may be the only practical method when one is monitoring simultaneously at several locations, and the budget doesn't warrant more than one fluorometer.

In unusual circumstances, some sample processing may be desirable. Although equipment is available that will perform almost any type of processing on a continuous basis, it is not generally applicable to the field. Conditions such as extremely high turbidity requiring filtration, concentration in the non-linear range requiring dilution, and extremely high acid or alkaline water requiring pH adjustment, need hand sampling.

The normally used 25 x 100 mm cuvettes require a minimum volume of 25 ml and will hold a maximum of about 50 ml. Accordingly, a sample volume of at least 100 ml (about 4 oz.) is recommended to allow for rinsing the cuvette.

If you are measuring the individual samples in the continuous flow cuvette, the volume required will depend on your arrangement for manual introduction of the sample (see Model 10 User's Manual). In most cases, a pint will be more than adequate.

2. Grab, Automatic

Automatic samplers for sewage and pollution studies are well developed and readily available. Such devices could easily supply the equivalent of grab samples (unattended and over a long period of time). We have noted advertisements for such devices from the following firms: Sigmamotor, Inc., 3 No. Main St., Middleport, NY, 14105, (716) 735-3115; Brailsford Co., Inc. 670 Milton Road, Rye, NY, 10580, (914) 967-1820; ISCO, Inc., P.O. Box 5347, Lincoln, NE, 68505, (402) 464-0231; Manning Corp., 2555 N. Interstate 35, Round Rock, TX 78664, (512) 388-9100.

3. Continuous

Where it is possible to do so, continuous pumping of sample through the fluorometer's flow cell (preferably with an attached strip-chart recorder) is recommended. Continuous measurement saves most time and dye, and also provides a graphic picture, with an illustration of the entire concentration as it passes the sampling point. In constant-rate injection, the plateau is clearly shown. In slug injections, the curve needed to interpret time-of-travel or to calculate rate of flow is automatically provided.

In time-of-travel studies, it is necessary to know the time lag between the sample intake and the instrument. This is easily determined in a number of ways. Perhaps the simplest and most accurate is to inject a small amount of dye directly into the intake and note the time lapse to instrument response. If there is a single operator, time the filling of a five-gallon bucket, and, from the inside diameter and length of the sampling hose, calculate the time lapse. Because the flow cell of the Turner Designs Model 10 Series Fluorometer will accept extremely high throughput, the time lag will be so low as to be negligible in most studies.

The rate of sampling (the velocity of the sample through the instrument) has absolutely no effect on the reading recorded by the instrument.

If sufficient head can be provided, siphoning can be used. Sampling, however, is normally done with a pump. Centrifugal pumps are the least expensive and best suited to the purpose. In any sampling system, one of the few things that will always affect measurement is the presence of air bubbles. An occasional bubble is not a problem, but a continuous, massive infusion of air bubbles will completely invalidate the measurement.

Submersible pumps are often used and are very satisfactory (2, 10). A commonly-used pump for shallow sampling is a battery-operated bilge pump, which you can buy from a local boat store. A typical and adequate capacity is 400 gallons per hour. Capacity is unimportant as long as the pump will operate against the head. Above-water pumps, if properly used, are also satisfactory. Such pumps frequently introduce bubbles by air leakage and by cavitation. Therefore, mounting the pump on the discharge side of the fluorometer is recommended (17). You should remember, however, that in this case the sample is under suction and there is some danger of bubble formation if the sample is saturated with air. Accordingly, the rate of sampling should be kept relatively low and the operating head should be kept as low as possible. Centrifugal pumps are not damaged by restricting the flow, so an oversize pump can be regulated by a valve on the outlet, pinching the hose, etc. Occasional opening of the restriction is desirable to prevent plugging by debris. If bubbles are

a problem, a bubble trap aided by an ultrasonic bath is fairly effective.

The intake is generally in the main part of the stream and should be well clear of the bottom. Removal of large pieces of debris (which may lodge in elbows and constrictions) is generally accomplished by intake filtration. A simple and effective intake system, consisting of a pipe perforated with many holes and wrapped with plastic screen, has been reported (2).

The type of intake hose generally used is polyethylene or plastic garden hose. Although to our knowledge, no thorough study of materials has been made, it is known that the highly-plasticized soft vinyl tubing, frequently used in the laboratory, will absorb some of the dyes, and later, as concentration falls, release it. The use of rubber hose is not recommended (17). If the hose is not completely opaque, the portion attached to the inlet and outlet of the fluorometer must be wrapped carefully with black tape. A distance of three or four feet is generally satisfactory, depending on the hose diameter. The object is to prevent outside light from reaching the photomultiplier tube. This is easily checked by shading the hose with the instrument set on a sensitive range. Direct sunlight and shade should give the same reading.

I. EQUIPMENT

1. Fluorometers

The Model 10 Series Fluorometers, the Model 10 Analog and its successor the Model 10-AU Digital, manufactured by Turner Designs, are designed with the requirements of field use in mind. These instruments include numerous convenient features recommended by people experienced in the use of fluorescent-dye tracers. Complete details will be found in the descriptive brochure (18), which outlines the unique features of these instruments.

LOW POWER NEEDS. A Model 10 Series Fluorometer may be operated interchangeably on 115 volt AC, 230 volt AC, or 12 volt DC power, without an inverter. When operating from 12 volts DC, the current demand is only 3 amperes (about the same as the back-up lights on a car).

DESIGNED FOR FIELD USE. The Model 10 Series Fluorometer is available in a rack-mount version for cabin boat, van, or remote field station installation. For really rugged service on land or small boats, a water-resistant instrument is available. For the laboratory, the rack-mount version is also available mounted in a laboratory case.

AUTOMATED LAMP START. Lamp start is automatic. In case of power failure, data is lost only while the power is off.

AUTOMATED RANGE SELECTION. The Model 10 Series Fluorometer automatically selects the appropriate sensitivity range. The instrument has a very wide dynamic range, which allows both low and high concentrations to be read accurately.

For convenience, the instrument can also be operated in the manual mode.

AUTOMATED BLANK SUBTRACTION. When the blank is suppressed on one range, it is suppressed on all ranges, without readjustment.

NO CUVETTE FOGGING. When using the continuous-flow cuvette, the area outside the cuvette is sealed, and desiccant is supplied. Even on a hot, muggy day, with a cold sample, no condensation can form.

STABILITY. The three-period optical design automatically compensates for dark current, variation in light source intensity and shift in photomultiplier gain. When properly calibrated, readings will remain stable for long periods of time, drifting less than 1% (0.5% for the Model 10-AU) in a month.

RAPID AND REPEATABLE RESPONSE TIME. Response time is 1 +0.2 seconds to 63% response, 4 +1.0 seconds to 98% response. Faster response, with minor loss of sensitivity, is available on special order.

DIRECT CALIBRATION. The range multipliers are extremely accurate -- a calibration of one range is a calibration of all ranges.

A new one-piece injector-style flow cell is available for the Model 10-AU, which greatly simplifies calibration.

Ask Turner Designs for more information about P/N 10-AU-020.

RAPID WARM-UP. The lamp "cold-spot" temperature is controlled by a thermostated heater to ensure starting and arc position stability even at low temperatures. Low total power dissipation (only 24 watts on 12 volts DC) results in low sample compartment temperature rise.

SPECIAL FEATURES ON THE MODEL 10-AU. The Model 10-AU Digital Fluorometer, successor to the Model 10, has several convenient features:

a. Direct Concentration Readout. After calibration, the instrument will perform all calculations and display the actual concentration of the sample.

b. Temperature Compensation (option). The instrument can be set to correct the fluorescent output for changes in sample temperature, eliminating a potential source of error.

c. Internal Data Logging. The instrument will log data directly without the need for another data collection device. The data can be downloaded easily into a computer in the field or back in the lab. A program is provided to download and convert data to ASCII format for use with most spreadsheet programs.

d. Self-Diagnostics. Internal instrument functions such as lamp operation and internal temperature are displayed on the Model 10-AU's diagnostic screens. This simplifies troubleshooting in the field.

DATA COLLECTION. The Model 10 Fluorometer has a "telemetry output", which allows data to be collected with most analog data loggers or chart recorders.

The Model 10-AU Field Fluorometer has three methods for data collection: 1) The analog voltage output can be used with a logger or chart recorder; 2) The RS-232 serial data output can be used with a computer or other serial device; or 3) The optional Internal Data Logger (10-AU-450), where the Model 10-AU will log data directly into the instrument for later downloading and analysis (converted to ASCII format). This feature is particularly useful for studies

where additional data collection equipment is unavailable and when many data points are to be recorded.

2. Power Sources

Only power sources for the Turner Designs Model 10 Series Fluorometers will be discussed. Injectors, pumps, recorders, and other accessories should be chosen with consideration of the type of power available and the manufacturer's recommendations.

The Model 10 will operate on either AC or DC current. For AC operation, any source that will provide 50-400 Hz and 105-130 volts at 0.3 amperes is satisfactory. Conversion to 210-260 volts, at 0.25 amperes requires only a simple power cord change. The instrument is completely internally protected against voltage surges. No additional precautions need be taken, although prolonged operation above 130 volts (or 250 volts) is not advisable.

For DC operation, any portable generator or battery that will provide 11-16 volts at 3 amperes may be used. The negative lead should be grounded.

We assume that batteries are more commonly used for portable application. The prime requirement of the battery is that it must deliver 3 amperes for the period of expected operation without the voltage dropping below 11. Most batteries are capable of this.

Any battery will produce fewer total ampere hours at high current drain than at low drain. The loss is dramatic if the drain exceeds the purpose for which the battery was designed. For example, size "D" alkaline flashlight batteries produce 1.5 volts and some have a capacity of ten ampere hours. A bank of eight would produce 12 volts, and might be assumed to yield two amperes for five hours. In fact, a two-ampere drain would cause the voltage to drop below 11 in only a few minutes. However, in a pinch, paralleling four banks of eight, or even better, nine, should provide many hours of operation.

A 12-volt lead-acid battery is probably the best choice for most applications. One example is a battery designed for snowmobiles, with special caps to prevent any battery acid loss. The Gould SN-9L is rated at 32 amperes, weighs 21 pounds, and

measures 7-3/4" x 5-1/4" x 7-1/4". It has a life of about 10 hours.

One disadvantage of this battery and all automotive-type batteries is that they are not designed for complete discharge without damage. Several dozen complete charge-discharge cycles is all that can be expected, unless they are recharged immediately after each discharge.

A battery designed especially for field work, permitting complete discharge without immediate recharge is the Globe GC-1220B 20-ampere hour battery. The electrolyte is gelled to prevent spilling. It weighs 16 pounds, and measures 7" x 6-1/2" x 5". It has a life of about 6 hours.

Lead-acid batteries of under 20-ampere hour capacity should not be used, because they will not supply a steady 3 ampere drain. Nickel-cadmium batteries have the same limitations.

3. Dye Injectors

There are three basic types of constant-rate injectors: constant displacement pumps, constant-head (gravity-feed) devices, and regulated pressure systems.

Constant displacement pumps are frequently used. One series of commercially available pumps which appear to be nearly ideal for field work is the RP-BG series manufactured by Fluid Metering, Inc., and available through Turner Designs. All units feature continuously variable flow rate -- from full forward to full reverse. Models with maximum flow rates of 6.7, 16, 20.2 and 48 ml/minute against back pressures up to 75, 30, 15 and 5 PSIG respectively are available. Current drain is between 0.06 and 0.1 amperes from a 12-volt battery. They are self-priming, and warranted to deliver constant flow to 1% from full flow down to 10% of full flow.

The pump that we recommend as the hardest is the Model RP-BG 75-2CSY (Turner Designs catalog #10-008). A laboratory test yielded constant flow to better than 1% over a battery voltage range of 10-15 volts, against a zero pressure head. One of our sales engineers has carried one of these pumps for five years. He keeps it at the same adjustment (full flow).

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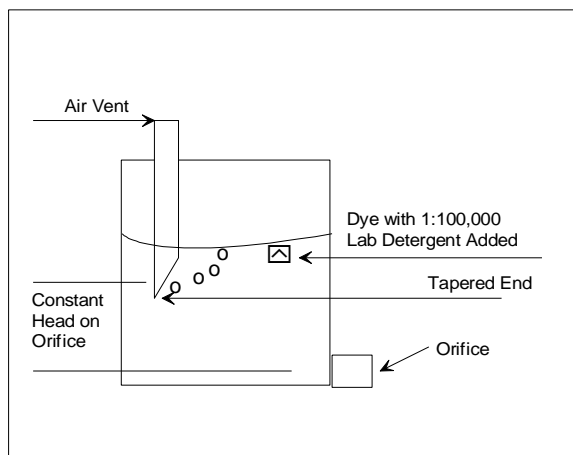
It has been used about 100 times, has a total of 200-300 hours on it, and has run dry for 30-60 minute periods several times. The injection rate has varied less than 0.5%. One of these pumps makes a very tidy field package, weighing about six pounds and measuring 9-3/4" x 5-1/8" x 3-1/8". It should run for about 100 hours on eight size-D alkaline flashlight batteries (ten-ampere hours at 1.5 volts per cell). Eight of these batteries weigh 2.6 pounds.

Until you do a little calculation, the maximum injection rate of 40 ml/minute may strike you as too low for practical use. Using undiluted rhodamine WT, however, will yield a concentration of five ppb (one ppb active ingredient) in a discharge of nearly 6,000 cfs. In terms of sewer studies, this rate yields 100 ppb in a 200 mpg flow. One minor drawback is that the flow is pulsating, 25 strokes per minute for the low capacity pump, and 75 strokes for the high capacity. This is smoothed out and is not a disadvantage in the flow-rate test itself, but calibration of the pump requires longer than if it were continuous. The usual calibration procedure is to time the filling of a volumetric flask. With a pulsating flow, the volume collected must include, for example, at least 100 pulses, for 1% accuracy.

Fluid Metering also manufactures pumps driven by synchronous motors. If AC power is commercially available, these pumps should be considered, as they are supposedly accurate to a small fraction of a percent against a constant head.

We do not mean to imply, by the aforementioned recommendations, that these are the only suitable injection pumps. Of the pumps we have used up to this point, however, these models seem to be the most practical for field use.

Another common approach to injecting is the constant-head device. The simplest constant-head injector is the Mariotte Vessel, sketched below.



In use, air enters the air vent and bubbles through the solution to the air chamber above. Within a few minutes of turn-on, the pressure at the bottom of the air vent stabilizes at atmospheric pressure. Because the exit of the orifice is also at atmospheric pressure, the liquid head is constant as shown, and independent of liquid level.

The major virtues of the Mariotte Vessel are that it requires no power, and combines reservoir and regulator as a compact unit. The major drawback is that because flow is a function of temperature (viscosity of water varies by 2.5%), measurements are not always completely accurate. In addition, there is no way to refill it without interrupting injection.

In high flows, you don't need much sophistication. Construction details are given by Chase, et al., (14, pg. 16). Considerable research has been devoted to developing units for low flows. A unit has been described which operates satisfactorily at two ml/minute (19, 20, 21). Very careful attention was given to orifice shape, filtering, elimination of bubbles from dissolved gasses, etc.

Chase, et al., (14, pg. 21) describes a constant-head (floating) siphon. The advantage of this device is that it may be filled without disturbing the experiment. Unfortunately, to the best of our knowledge, these devices are not commercially available.

Kilpatrick used a pressure-actuated injector for his experiments (1). This is an adaptation of a chlorine feeder, which is available from Aerofeed Inc., P.O. Box 303, Chalfont, PA 18914. You can pressurize it in

the field with a foot or tire pump. The flow rate is adjustable from 60 to 250 ml/minute. Its useful volume is nine liters and it weighs only nine pounds when empty. The flow is controlled by a special pressure regulator, which maintains a constant pressure difference of five pounds per square inch across a metering valve. Probably here, too, flow rate is temperature sensitive due to viscosity variations discussed above in connection with the Mariotte Vessel.

4. Sample Pumps

For continuous sampling, some sort of pump will be used (see section H3 on Sampling Systems, Continuous). Although we hesitate to make specific recommendations, we have noted that pumps manufactured by Gelber Pumps, 3721 West Morse, Lincolnwood, IL 60645; and Cole-Parmer, 7425 North Oak Park Avenue, Chicago, IL 60648, seem to be in relatively common use for such studies. Both companies have extensive catalogs.

Turner Designs offers a reliable sample pump, which has been found effective (P/N 10-590).

Because there are 1/2" IPS female threads on both intake and exhaust fittings, connection to the flow cell is simple. For laboratory studies, where smaller inlet tubing might be desired, Turner Designs has an adaptor that will accept 3/16" to 1/4" (ID) plastic tubing.

Turner Designs also offers various other cuvette sizes for continuous flow measurements. Contact Turner Designs.

Special situations may call for the miscellaneous sampling devices already mentioned (see section H on Sampling Systems).

J. DYES

1. Introduction

In the following section, we will consider the properties of the different dyes available for research. The properties of rhodamine WT will be emphasized, as it has been shown to be the best tracer for most applications. However, we will also consider

rhodamine B, pontacyl brilliant pink (sulfo-rhodamine B), and fluorescein.

2 Toxicity and Approval

Rhodamine WT is related to rhodamine B, a tracer in common use in the sixties. It was developed to overcome a disadvantage of rhodamine B: absorption on suspended sediment. The same modification was expected to reduce toxicity, and limited testing has confirmed this.

The eggs and larvae of the Pacific oyster were exposed to concentrations of one and ten parts per million of rhodamine WT for 4-8 hours. They developed normally (22). Trout and salmon held for 17.5 hours at 10 parts per million, and an additional 3 hours at 375 parts per million showed no distress, and remained healthy in dye-free water when checked a month later. Wilson mentions some unpublished oral injection studies by the U.S. Geological Survey (17).

Rhodamine WT was an immediate success as a tracer in marine systems and in wastewater. While it was also used in potable water, such use was occasionally forbidden on the grounds that it did not have formal federal approval. Rhodamine WT is currently approved for such use.

While the EPA has sole responsibility for identifying those substances that may be used as tracers (36), the Food and Drug Administration (FDA) does issue policy statements. The FDA issued such a policy statement on April 22, 1966 concerning rhodamine B (37). A temporary tolerance limit for ingestion of rhodamine B was set at 0.75 mg per day. Based on normally expected water consumption, the tolerance would not be exceeded unless the concentration approaches 370 parts per billion. Noting that 30 ppb may be detected visually in a glass of water, and 10 ppb is visible in a larger volume, such as a clear reservoir, the FDA pointed out that if the dye is not visible, the tolerance would not be exceeded. The USGS, a large user of fluorescent-dye tracers, directed that the concentration should not exceed 10 ppb at the intake of a water supply (15). The visual and instrumental detectability of rhodamine WT, based on active ingredient, is about the same as

rhodamine B (rhodamine WT is supplied as a 20% aqueous solution).

Ten parts per billion may not sound like much to the uninitiated, but it is a thousand times the limit of detectability guaranteed by Turner Designs on its Model 10 Series Fluorometers (39). Background fluorescence caused by fluorescent materials in the water being studied usually limits detectability. But even so, measurements can be made to 0.1 parts per billion of rhodamine WT (active ingredient) in raw sewage!

On April 10, 1980, Dr. Joseph A. Cotruvo of the EPA issued a memo stating that the EPA considers rhodamine WT to be equivalent to rhodamine B (38). More recently, the following policy statement was sent to Crompton and Knowles, a dye manufacture (41).

The Criteria and Standards Division (Office of Drinking Water) has reviewed the available data on chemistry and toxicity of Rhodamine dyes. We would not anticipate any adverse health effects resulting from the use of Rhodamine WT as a fluorescent tracer in water flow studies when used with the following guidelines.

- *A maximum concentration of 100 micrograms/liter Rhodamine WT is recommended for addition to raw water in hydrological studies involving surface and ground waters.*
- *Dye concentration should be limited to 10 micrograms/liter in raw water when used as a tracer in or around drinking water intakes.*
- *Concentration in drinking water should not exceed 0.1 micrograms/liter. Studies which result in actual human exposure to the dye via drinking water must be brief and infrequent. This level is not acceptable for chronic human exposure.*
- *In all of the above cases, the actual concentration used should not exceed the amount required for reasonably certain detection of the dye as required to accomplish the intended purpose of the study.*

The Criteria and Standards Division recommends that Rhodamine B not be used as a tracer dye in water flow studies.

This advisory supersedes all earlier advisories issued by EPA on the use of fluorescent dyes as tracers in water flow studies. This advisory is granted on a temporary basis only.

EPA is terminating its voluntary additives advisory program as announced in the Federal Register (53 FR, 25586, July 7, 1988). A copy of the Federal Register Notice is enclosed for your convenience. All EPA advisory opinions issued within the framework of the additives program will expire on April 7, 1990.

Our opinion concerning the safety of this tracer dye does not constitute an endorsement, nor does it relate to its effectiveness for the intended use. If this letter is to be used in any way, we require it to be quoted in its entirety.

Rhodamine B, which until recently was used mostly as a tracer, has some undesirable biological effects at high concentration. At the low concentrations of tracer use, it is probably harmless. The effects on oyster eggs and larvae, and on fish have been studied (22), and other biological effects and precautions are summarized by Wilson (17).

Little information is available for pontacyl brilliant pink B, and because of the superiority of rhodamine WT, certification is not being actively sought. Fluorescein was, at one time, certified for unrestricted drug and cosmetic usage, but is not listed in the Federal Register under the same limitations as rhodamine B. Presumably, it is also tolerated at low levels.

Finally, it should be mentioned that although all four dyes are chemically related, each is a distinct chemical entity. Rhodamine WT is a different chemical substance than rhodamine B. By analogy with other chemicals, the structural change made to produce rhodamine WT would be expected to reduce biological activity.

3. Stability

There is surprisingly little quantitative data on the vulnerability of the various dyes to destruction by light, other chemicals, oxidation, bacterial action, etc. All appear to be reasonably stable to chemical attack, oxidation, and bacterial action. Chlorine is the chemical most likely to be encountered. Undocumented reports indicate that chlorine, in its elemental form, rapidly destroys the fluorescence of rhodamine WT (and probably rhodamine B). This would be expected from the chemical structures of WT and B. However, elemental chlorine exists only transiently in solution. It rapidly dismutates, and the dismutated form is commonly called "residual" chlorine. Deaner shows that levels of residual chlorine considerably in excess of those normally found in potable water or treated sewage have no effect on the fluorescence of either rhodamine B or rhodamine WT (9). Preliminary data indicates that free chlorine may cause problems when bromine salts are present, as they are in sea water. Free chlorine reacts with the bromine salt, which in turn reacts with the rhodamine dyes.

Although several authors cite quantitative studies of the effect of light, the studies are contradictory. Some of the studies (including both high and low losses) were obviously not representative of actual field conditions. It is certain that sunlight causes an irreversible loss of dye, but the rate is uncertain. There is no question that rhodamine WT, rhodamine B, and pontacyl brilliant pink possess adequate stability for quantitative field measurement. In a week-long time-of-travel study, there would undoubtedly be substantial loss from photo-decomposition, but dye loss does not affect such measurements. Rate-of-flow measurements seldom take more than a few hours. High concentrations of dye (0.2%) appear to be indefinitely stable under laboratory lighting, probably because of a self-shielding effect. Lower concentrations may diminish significantly (perhaps as much as 20%) in a year. Stored in the dark, all samples are stable. Buchanan notes that field samples stored in the dark for six months showed no change (8).

In any study of photo-decomposition, it should be remembered that glass will shield the sample from ultraviolet light to a degree dependent on the type of

glass. As mentioned previously, fluorescein decomposes rapidly in light.

4. Solubility

Solubility is important to ease of handling. Concentrated solutions are easier to transport. Rhodamine WT has the best water solubility of all the dyes and is supplied only as a 20% aqueous solution of specific gravity 1.19.

Rhodamine B and pontacyl brilliant pink are powders, soluble in water to about 2% (7). Rhodamine B is also available as a 42% solution in acetic acid, with a specific gravity of 1.2. Preparation of the stock solution from the powder can be a messy operation and should be done in a windless area that can be hosed down. Field preparation is inadvisable, as windblown particles seem inevitably to reappear to invalidate the study. Insoluble particles have been reported to clog injectors (19).

Rhodamine WT or the concentrated rhodamine B solution are recommended as dilutions are easy to make without mess, even in the field. The high specific gravities of these solutions cause layering unless they are injected into turbulence (such as near a ship's propeller). In small studies, they are usually diluted prior to injection, and the diluted sample is used as the standard for calibration. In large studies, a preliminary dilution may be accomplished by injecting the dye into water being pumped from, and returned to the stream. The pumping rate of the water need not be known, only the rate of injection of concentrated dye.

The specific gravity of the rhodamine B solution may be adjusted with methanol, and various pre-adjusted solutions have been available in the past, but may be discontinued. The use of methanol with rhodamine WT has not been reported in literature, but is widely used.

5. Water Conditions

The pH of the water has little effect on the fluorescence of rhodamine WT, rhodamine B and pontacyl brilliant pink B in the range of pH 4-10.4 (8), or perhaps more conservatively pH 5-10 (2, 7). Fluorescein has no definite plateau of fluorescence

versus pH (7). Standards should be made with stream water, or, if the pH is not constant, all samples should be buffered.

Ionic strength affects the fluorescence of rhodamine WT slightly. A salinity of 35 parts per thousand (sea water) decreases the fluorescence by about 5%.

6. Detectability and Background

Turner Designs guarantees that with the Model 10 Series Fluorometers, the ultimate detectability of rhodamine B dissolved in pure water will be at least as good as 10 parts per trillion. Production tests actually indicate this guarantee to be conservative by a factor of five. The Models 10-000R and 10-005R (different photomultiplier tube for chlorophyll determinations) may be slightly less sensitive for rhodamine B, but are more than adequate in sensitivity for such use.

The ultimate detectability of rhodamine WT (based on active ingredient), rhodamine B, and fluorescein, dissolved in pure water, are all about the same. The detectability of pontacyl brilliant pink is less sensitive by a factor of three.

Any discussion of the detectability of fluorescent dyes with a properly designed fluorometer is inseparable from a discussion of background or blank. The practical limit of sensitivity is set by variation of background. Background consists largely of two things: the fluorescence of extraneous materials in the water, and a small but inescapable emission from water itself (called Raman shift). The great value of rhodamine WT, rhodamine B and pontacyl brilliant pink is that the fluorescence of these dyes falls in an unusual region of the spectrum. The fluorescence of fluorescein falls in a more common region of the spectrum, and its practical application is limited to use in relatively pure water.

Background found in natural systems, even relatively polluted systems, is remarkably low. The background in pure water (about 20 parts per trillion) may rise to only 100-150 parts per trillion in relatively polluted water such as San Francisco Bay. Even in raw sewage, a background of only one part per billion was encountered.

Of naturally occurring materials, only the pigment present in blue-green algae yields substantial background. Large concentrations of these algae will significantly affect the detectability of the tracer, unless its presence is recognized, and the proper optical filters used. (See Filters and Light Sources below).

Common industrial chemicals will seldom contribute to excessive blank. For example, the fluorometer will be almost totally blind to high concentrations of brighteners used in detergents. The fluorescence characteristics of these compounds are far removed from those of the red dyes.

Although background, and its variability, will generally be low, it cannot be predicted with accuracy. This is why one of the steps in any study is to measure the background (blank) of the water prior to injection of dye.

7. Filters and Light Sources

Filters and light sources for the rhodamine dyes and pontacyl brilliant pink are described in the Filter Selection Guide (27). Filters and light sources for fluorescein are in a monograph entitled "Fluorescein" (28).

8. Aesthetics

At the point of the initial dye release, the red color of the rhodamine dyes is very obvious, and may be considered objectionable by the public. This may be overcome by "masking" the red color with the brilliant green fluorescence of fluorescein, which seems more acceptable (28).

9. Sources of Dyes

Rhodamine WT and Rhodamine B -- Industrial grade
of D & C Red #19

Crompton & Knowles Corporation
P.O. Box 33157
Charlotte, NC 28233-3157
1 (800) 432-6188

FORMULABS
1710 Commerce Drive
Piqua, OH 45356
(513) 773-0600

Fluorescein (also referred to as Uranine)

Pylam Products Company, Inc.
100 Stewart Ave.
Garden City, NY 11530
(516) 222-1750
(800) 645-6096

Sulpho Rhodamine B Extra

Pylam Products Company, Inc.
100 Stewart Ave.
Garden City, NY 11530
(516) 222-1750
(800) 645-6096

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