ABSTRACT
Fluorescein (Acid Yellow 73) dye-dilutions ranging from $10^{-7}$M to $10^{-11}$ M concentrations were analyzed in a solution of gasoline-saturated distilled water. Spectrofluorophotometric analyses confirmed that fluorescein was readily detectable at the $10^{-11}$ M concentration. Sorption of fluorescein to activated carbon also produced sufficient fluorescence intensity at the $10^{-8}$ M concentration for easy analytical detection. This study shows that gasoline-contaminated water does not substantially reduce the fluorescence capabilities of fluorescein. Fluorescein should still be considered a useful dye for tracing ground-water flow even in the presence of gasoline contamination.

INTRODUCTION
A recent paper examined fluorescein (Acid Yellow 73; CI 45350) and Rhodamine WT (Acid Red 388) for reduced fluorescence in the presence of gasoline-contaminated water [1]. Fluorescein and Rhodamine WT readily dissolve in water, migrate at the prevailing ground-water flow rate, and are easily detectable at very low concentrations by spectrofluorometric methods making them highly useful for tracing ground-water flow.
desirable ground-water tracers. The study was significant for the field of contaminant hydrogeology because both of these fluorescent dyes are commonly used for tracing ground-water flow and possible contaminant migration. The use of fluorescent dyes to trace ground-water flow is appropriate because the dyes may be considered as surrogate pollutants [2-5]. If these two fluorescent dyes are adversely affected by hydrocarbon-contaminated ground water, then their utility as tracing agents is greatly reduced.

According to [1], Rhodamine WT is not substantially affected by the presence of gasoline-contaminated water, but fluorescein is severely affected; it was recommended that fluorescein not be used in tracing studies where petroleum hydrocarbons are present. However, some important factors were not considered in [1]. The most important factor is the lack of uniform behavior associated with activated carbon. Activated carbon does not behave uniformly from sample to sample; adequate control in experiments is problematic. Additionally, not having conducted a standard serial dilution for either of the two dyes also led to analytical complications in [1].

The seriousness of the potential impact of hydrocarbon-contaminated ground water on fluorescein required replication of the study. We examined the effect of gasoline-contaminated water on the fluorescence of fluorescein and the effect on the sorption of fluorescein onto activated carbon. Given the nonuniformity of sorption by activated carbon, it was deemed necessary first to establish whether its fluorescence signature would be reduced in water, and then to examine sorption onto activated carbon.

**CHARACTERISTICS OF FLUORESCIN**

Fluorescein is an ionic salt that readily dissolves in water. It has been well reviewed in the literature [6-9]. Each of these papers describes the behavior of fluorescein and other dyes in various environmental conditions and under various analytical instruments.

Substantial detail on fluorescein in environmental tracing and its detection by fluorometers is available, see Figure 1 [6]. A pH < 5.0 significantly reduces fluorescein fluorescence, because fluorescein has a quinoid structure under alkaline conditions; but, under acid conditions, it changes to a leucocompound. The quinone ring in the quinoid structure in fluorescein is fluorescent, the lactone ring of the leucocompound is not fluorescent [6]. Recent experiments have shown that fluorescein exhibited no reduced fluorescence with increasing saline conditions, although it had previously been suggested that fluorescein fluorescence will be significantly decreased as salinity increases [8, pp. 111-112; 10]. Various background problems, such as suspended sediments, natural fluorescence from algae (e.g., *Chlorella* sp.) and other natural plant pigments, and dissolved and colloidal organic matter also adversely affect fluorescein fluorescence or its detection. Increasing ground-water temperatures may also adversely affect
fluorescein fluorescence, but experiments have reported no significant effect [9, p. 112, 11].

Fluorescein naturally photodecays, which may be regarded as a desirable property when tracing karst ground waters. Upon discharge at a spring, fluorescein will eventually decay to nonconsequential (i.e., nondetectable) levels. Chemical degradation, biodegradation, and sorption processes will also affect fluorescein negatively. However, fluorescein may be less likely to be affected than Rhodamine WT and other fluorescent dyes by these factors [6].

Fluorescein can be detected at concentrations as low as 0.29 μg L⁻¹ using a typical filter fluorometer [6]. However, more modern instruments allow reliable detection of fluorescein at concentrations down to 10 ng L⁻¹ from field tests [12]. Others have determined still lower detection limits of fluorescein with newer equipment, down into the pg L⁻¹ range [T. Aley, personal communication].

Limited investigations of fluorescein toxicity have been conducted. In-depth toxicity reviews for the more commonly used fluorescent dyes for tracing studies have been performed [13, 14]. Neither study has found any significant evidence of toxicity for fluorescein or any of the other dyes evaluated.

**MATERIALS AND METHODS**

Distilled water was saturated with an unleaded eighty-nine octane gasoline in the laboratory. Saturation was accomplished by adding a quantity of gasoline to the distilled water in a closed flask and allowing the solution to mix by use of a stirring bar for several hours. By saturating the water with gasoline, it was possible to conclude that the worst possible contamination problem had been achieved, assuming no light floating phase.

Next, a serial fluorescein-dye dilution was conducted using the distilled water-gasoline solution. These dye dilutions ranged from 10⁻⁷ M to 10⁻¹¹ M concentrations. Fluorescein reportedly exhibited reduced fluorescence below 1000 μg L⁻¹.
when analyzed on a Schimadzu scanning spectrofluorophotometer (model number not reported) [1]. Attempts to measure fluorescein fluorescence at 50,000 μg L⁻¹ were made, but this concentration resulted in a fluorescence intensity greater than the capability of the instrument. The serial dye dilutions developed for this investigation resulted in fluorescein-gasoline-water samples at substantially lower concentrations. These lower concentrations should not have yielded any detectable fluorescence if the previous study was correct.

All five of the dye dilutions were analyzed on a Perkin-Elmer Model LS-5B scanning spectrofluorophotometer for fluorescence intensity. Twenty samples of the gasoline-water solution blanks also were analyzed for fluorescence intensity to identify any possible fluorescence effects from the gasoline. Finally, a packet of activated carbon (6-14 mesh) was placed in the gasoline-water solution with a dye-dilution concentration equal to 10⁻⁸ M. This carbon packet was allowed to sit in the solution (closed container) in total darkness with constant stirring for one week. After one week, the packet was removed and eluted with 70 percent two-propanol containing 6 grams of potassium hydroxide. The resulting elutant was then analyzed spectrofluorometrically.

RESULTS AND DISCUSSION

The results of the fluorescein analyses are listed in Table 1. Table 1 and Figure 2 demonstrate that fluorescein was readily detectable by its fluorescence at all the dilutions. Fluorescein was also detected in the elutant from the activated carbon. Twenty gasoline saturated water samples were also examined with no quantifiable fluorescence detected in any of the gasoline-water solution samples tested.

Examination of Figure 2 shows that the data do not form a straight line. The 10⁻⁷ M fluorescein dye concentration in the gasoline-water solution appears to deviate significantly from the other data points. The lack of linearity is most likely a result of the 10⁻⁷ M concentration exceeding the limit of Beer’s law. Figure 3 is a plot of

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Fluorescein Concentration in Distilled Water Saturated with Gasoline and Measured Fluorescence Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Molar)</td>
<td>(ng L⁻¹)</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>3.76 x 10²</td>
</tr>
<tr>
<td>10⁻⁸</td>
<td>3.76 x 10¹</td>
</tr>
<tr>
<td>10⁻⁹</td>
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<td>10⁻¹⁰</td>
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<tr>
<td>10⁻¹¹</td>
<td>3.76 x 10⁻²</td>
</tr>
<tr>
<td>Intensity in Water</td>
<td>728.00</td>
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<tr>
<td>Intensity in Elutant</td>
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the $10^{-8}$ M to $10^{-11}$ M concentrations. Although the data do not form a perfect straight line, they are sufficiently close as to be within expected error. A plot of just the $10^{-8}$ M to $10^{-10}$ M concentrations would yield a straight line.

Sorption of fluorescein onto activated carbon and subsequent eluting of the carbon also showed that gasoline-contaminated water does not substantially affect the sorption of fluorescein. The greater intensity of fluorescein in elutant (Table 1) was expected because the sorption capacity of activated carbon concentrates fluorescein.

The Ozark Underground Laboratory (OUL) has reportedly used fluorescein numerous times to trace ground-water flow in instances where gasoline was present in the ground water; many of these have been associated with fuel leaks and spills. The fluorescein performed well under field conditions. Many of the traces conducted by the OUL were successful, even when the monitored well had a layer of free product floating on top of the gasoline contaminated ground water (sampling was never conducted within the free product zone). Successful fluorescein dye traces involved dye recovery both in grab samples of water and in carbon samplers. OUL also found fluorescein to be a useful tracer when used in aquifers contaminated by diesel fuel [T. Aley, personal communication].
Figure 3. Straight line fit through a plot of fluorescein concentration (ng L\(^{-1}\)) versus intensity for the four lowest dye dilutions analyzed in this study.

The concentrations employed in this study are much lower than those that are typically used in tracing studies. Fluorescein should still be regarded as a useful tracing agent in ground-water tracing studies provided that several environmental factors are considered prior to initiating a ground-water tracing study. These environmental factors include:

1. Analyzing background fluorescence for a suitably long period in advance of dye injection;
2. Ensuring a neutral or higher \(\rho H\) (not generally an issue in carbonate aquifers);
3. Accounting for salinity and temperature effects;
4. Estimating dye losses through sorption, dilution, and photodecay; and
5. Conducting a standard serial dilution of the chosen dye.

These five items are not exhaustive but are the minimum that should be considered. Proper consideration will guide the selection of an appropriate dye for a ground-water tracing study and the appropriate injection quantity necessary to ensure positive recovery of the dye at downgradient discharge sampling stations [12]. A procedure for preparing a serial dilution of fluorescent dyes to be used in water tracing has been described [15].
CONCLUSIONS

Fluorescein dye dilutions in the presence of gasoline-contaminated distilled water were shown to be readily detectable down to the $10^{-11}$ M concentration by scanning spectrofluorophotometric methods. Sorption of fluorescein onto activated carbon and subsequent elution and analysis also showed that fluorescein was readily detectable when using activated carbon as a detector. Recommendations that fluorescein dye not be used in the presence of petroleum hydrocarbons in water are not warranted. It would be more appropriate to follow recommendations which include the collection of site waters for dye-dilutions and analysis, and to carefully evaluate field conditions prior to initiating any dye-tracing study. Following a standard methodology for making serial dye dilutions and evaluating field conditions will yield more valid results and allow for more confidence in the applicability or lack thereof of the dye(s) in question.

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REFERENCES


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