Comparison of Spectrofluorimetry and Capillary Electrophoresis/Laser-Induced Fluorescence for Detection of Fluorescent Dyes in Ground Water

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1. Abstract

Fluorescent dyes injected into wells on a RCRA site were subsequently adsorbed by activated charcoal contained in packets suspended in monitoring wells on an adjacent Superfund site. Both spectrofluorimetry and capillary electrophoresis with laser-induced fluorescence detection (CE/LIF) revealed Fluorescein and Tinopal at low parts-per-trillion (ppt) levels in extracts of the charcoal. While a high fluorescent background obscured the analyte signals using synchronous scanning spectrofluorimetry, CE/LIF isolated the analyte signals, provided migration times unique to each dye, and permitted use of internal standards to improve data quality. Fluorescein was detected using a Beckman P/ACE 5000 equipped with an Ar ion laser and Tinopal was observed with a HeCd laser and an optical bench similar to that of Nie *et al* (1). The CE/LIF data provided more compelling evidence for migration of contaminants between the sites.

2. Map of Adjacent Contaminated Sites

Multiple sources of groundwater contamination are common in industrialized areas. To assess risk to humans and the ecosystem, the EPA must correlate the contamination observed with each source. Sources posing serious risk are cleaned up and liability is determined. In judicial proceedings, a preponderance of evidence establishes liability. Hence, confirmation of results by multiple analytical techniques provides important additional evidence. Figure 1 is a map of an industrial region of Chattanooga, TN. The EPA and parties responsible for clean-up sought to determine if pollutants from one contaminated area could migrate underground to a second contaminated site. A dye tracer study was performed by injecting one of three dyes or a fluorescent brightening agent into four injection wells on the first site. Dye (10-30 lbs in 2000 L of water) was injected and flushed into the surrounding area with another 8000 L of water. Tinopal CBS-X and Eosine Y were injected into the wells farthest from the monitoring wells. Two weeks later, Rhodamine WT and Fluorescein were injected into the other two wells. The monitoring wells were sampled for about 2 months. The minimum distance between monitoring well A and the closest injection well was greater than 100 meters. Thus, any dyes reaching the monitoring wells would be diluted by many orders of magnitude. Fluorescence detection provided high sensitivity to detect traces of the dyes. An environmental consulting firm injected the dyes, collected samples, and analyzed charcoal extracts and water samples for the dyes by spectrofluorimetry. Portions of the samples were sent to the EPA's Environmental Sciences Division in Las Vegas for confirmation.

Fig. 1

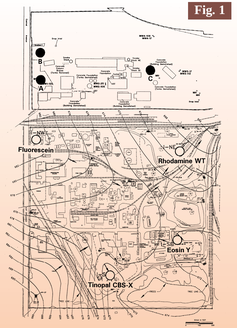


Figure 1. Map of RCRA site (bottom) and adjacent Superfund site (top). Injection wells are labeled with the names of the dyes. Monitoring wells are labeled A-C.

3. Sample Preparation

To detect the dyes, activated coconut charcoal was sewn into fiberglass-mesh packets and suspended close to the bottom of the collection wells. The packets were changed periodically, and a water sample was collected each time a packet was replaced. If present, mud was washed off the charcoal packets with a stream of deionized water. Activated charcoal (1 g) was added to 10 mL of a 5:3:2 solution of 1-propanol:water:ammonium hydroxide, stirred once, and decanted after 1 hour to fill a 3 mL cuvette. Blanks were prepared by soaking clean sand in the same solution. For CE analyses, 1 mL each of 50 mM $Na_2B_4O_7$ and an internal standard solution were added to 3-mL of extract. Dye standards containing 10, 100, and 1000 ppt of each dye were used to quantify the dyes in the extracts. The amount of water from which dye was collected by the charcoal packets was unknown and variable, depending on the time immersed, the amount of precipitation during a sampling period, and the size and number of underground channels that intercepted each bore hole. Quantitation of the dye extracted from the charcoal indicated only that the dye plume was detected as it passed through a well.

4. Spectrofluorimetric Analysis

We first analyzed the samples using a Jobin-Yvon Spex Fluorolog II spectrofluorimeter. Synchronous scanning of the two double grating double monochrometers isolated narrow bandwidths of excitation radiation provided by a 450 Watt Xenon lamp and of the light fluoresced from a 3-mL cuvette filled with a blank, standard, or sample. The excitation radiation was varied from 350 to 600 nm as the fluoresced light was monitored by a water-cooled photomultiplier tube from 365 to 615 nm to observe a constant Stokes shift of 15 nm. The largest slits, 5-mm wide, provided maximum sensitivity and a bandpass of 8.5 nm.

5. Fluorescein and Tinopal Standards

In Figure 2 are displayed synchronous scan data for a deionized water blank and standard solutions containing 10 ppt each of Tinopal, Fluorescein, and Rhodamine WT and 10 ppt each of Tinopal, Eosin Y, and Rhodamine WT. The water blank scan was subtracted from raw data to provide the other two scans. Broad peaks were observed for each dye.

If both Fluorescein and Eosin Y were present, their signals would interfere. Applying the concept of peak capacity to this data, no more than 8 such peaks could be observed across this wavelength domain without substantial peak overlaps. The detection limits for these dyes in deionized water were estimated to be 3 to 20 ppt (about 10^{-11} M) based on their peak heights and a S/N ratio of 3.

Figure 2. Synchronous scans of (a) water blank; (b) 10 ppt each of Tinopal CBS-X, Fluorescein, and Rhodamine WT; and (c) 10 ppt each of Tinopal CBS-X, Eosin Y, and Rhodamine WT.

Fig. 2

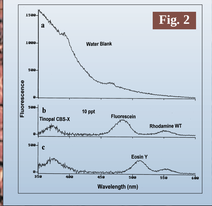


Fig. 3

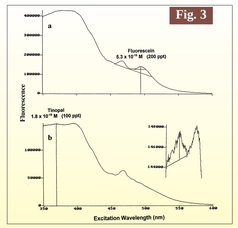


Figure 3. Synchronous scans of charcoal extracts containing the largest amounts of (a) Fluorescein and (b) Tinopal observed.

6. Detection of Fluorescein and Tinopal by Spectrofluorimetry

In Figure 3 are shown the synchronous scans for the highest concentrations of Fluorescein and Tinopal observed in the charcoal extracts. A scan from a clean sand blank was subtracted from the raw data to provide these scans. Most of the fluorescence was due to interferences, and the Fluorescein and Tinopal signals were superimposed on a major background signal. In fact, the Tinopal signal was barely discernable without magnifying the y-axis. For Fluorescein, the broad spectral features made choosing a baseline difficult. Attorneys could choose either one, depending on whom they currently represented. The two drawn in the Figures would provide concentrations different by more than a factor of two. Fluorescein was detected in 6 charcoal extracts from 2 monitoring wells. Tinopal was found in 3 charcoal extracts from 1 monitoring well. The other two dyes were not detected, and none of the 4 dyes was observed in the water samples collected when the packets were replaced.

7. CE - a Simple Confirmatory Analysis

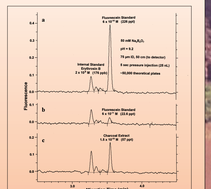
The spectrofluorimetric data provided evidence that two of the dyes migrated from the first contaminated site to the second. But how well would it hold up in court? We felt confirmation by another analytical technique was desirable to provide additional evidence, preferably one that could discriminate against the interferences observed. Others have used HPLC/LIF to detect fluorescent dyes in water. For example, van Soest *et al.* (2) found Rhodamine B in North Sea water sampled near the Netherlands. We decided to apply CE, since it is a simpler technique for this application. No ion pairing agent is required to encourage retention (by the reversed phase) and no organic waste is generated by the analysis. The different mobilities of the dye anions in aqueous buffers provides the separation mechanism. To the best of our knowledge, this work is the first application of CE/LIF to groundwater migration studies of fluorescent dyes. Confirmation of the fluorescein results was obtained using a Beckman P/ACE 5000 CE instrument with an Ar laser that provided excitation radiation at 488 nm and a bandpass filter centered at 520 nm in front of the fluorescence detector, which was a photomultiplier. The 75- m i.d., fused-silica capillary was 57-cm long with the detector 50 cm from the inlet vial. The running buffer was 50 mM sodium tetraborate, pH 9.2. A 5-sec pressure injection of 25 nL yielded an electropherogram that provided about 50,000 theoretical plates, which was sufficient for our application.

8. Fluorescein Results

Shown in Figure 4 are electropherograms for standard solutions containing $6x10^{-10}$ and $6x10^{-11}$ M of Fluorescein and $2x10^{-6}$ M Erythrosin B as an internal standard. The third trace was obtained for a charcoal extract. The two extra peaks were due to impurities in the Erythrosin B. The absence of other peaks indicated that other compounds extracted from the charcoal were not anions or did not absorb and fluoresce under these conditions. In the electropherograms, prominent peaks associated with the internal standard and the Fluorescein were observed above a level baseline for both the standards in deionized water and the charcoal extracts.

Fig. 4

Figure 4. Electropherograms: (a) $6x10^{-10}$ M Fluorescein standard, (b) $6x10^{-11}$ M Fluorescein standard, and (c) a charcoal extract. Erythrosin B ($2x10^{-6}$ M) was the internal standard.

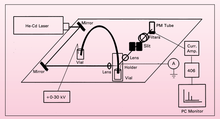


9. Optical Bench

The presence of Tinopal was confirmed using an optical bench similar in design to that of Nie *et al* (1). As illustrated in Figure 5, a He-Cd laser provided excitation radiation at 354 nm, which was focused by a quartz lens into the channel of the 75- m i.d., fused-silica capillary. The cylindrically symmetrical capillary holder remained in place to preserve the alignment when a capillary was replaced. The emitted light was focused by a second lens through a vertical slit, passed through two bandpass filters centered at 450 nm, and was detected by a photomultiplier tube. The current from the PM tube was amplified and converted to a voltage by a Keithley current amplifier, digitized by a Beckman 406 interface module, and displayed as an electropherogram by a personal computer using Beckman Gold software. The optics, PM tube, CE vials, and capillary were in a light-tight aluminim box, painted flat black inside. The open design of the optical bench permits use of different lasers (we now have 6) and could be used for multiple column experiments in the future.

Fig. 5

Figure 5. Diagram of the optical bench.



10. Tinopal Results

In Figure 6 are shown the electropherograms for $1x10^{-9}$ M Tinopal in deionized water and for a charcoal extract. The running buffer was 40 mM sodium tetraborate, pH 9.2. To inject 130 nL by gravity, the inlet end of the capillary was inserted into the sample vial and raised 30 cm for 30 sec. The internal standard was $2x10^{-8}$ M 7-hydroxycoumarin-4-acetic acid, which saturated the detector. Also observed were two peaks due to impurities in the internal standard. The larger one was used to quantify the Tinopal. A magnified portion of the lower trace shows a Tinopal peak with a S/N ratio of 2.5:1 due to 88 ppt of Tinopal. About 30,000 theoretical plates were observed for the Tinopal standard, which provided separation of the peaks.

Fig. 6

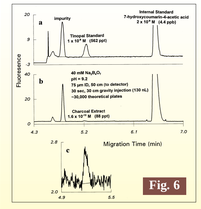


Figure 6. Electropherograms: (a) 10^{-9} M Tinopal standard, (b) a charcoal extract, and (c) a magnified portion of (b). The int. std. was $2x10^{-8}$ M 7-hydroxycoumarin-4-acetic acid.

11. Estimated Concentrations of Fluorescein and Tinopal

The spectrofluorimetric method provided rapid screening for the dyes. CE isolated analyte responses and provided confirmation for the dyes based on migration times. Table 1 illustrates that similar amounts of Fluorescein and Tinopal were found by both methods in the charcoal extracts.

Table 1

Table 1. Estimated Concentration (ppt) of Fluorescein and Tinopal in Charcoal Extracts

Sample	Dye Found	Spectrofluorimetry	Capillary Electrophoresis
15BR	Fluorescein	90	95
16BR	Fluorescein	200	153
17BR	Fluorescein	90	180
181BR	Fluorescein	100	62
182BR	Fluorescein	200	147
181CR	Fluorescein	30	ND
15CR	Tinopal	70	196
16CR	Tinopal	70	
17CR	Tinopal	100	171
181CR	Tinopal	ND	88
182CR	Tinopal	ND	112
22CR	Tinopal	ND	99

12. Advantages of Spectrofluorimetry

Spectrofluorimetric analyses were faster. Each blank, standard, or sample was poured into a disposable cuvette, which was inserted into the instrument, and a scan was made in 6 min. In contrast, capillary rinse cycles required 5 min on the automated CE instrument, and each data acquisition required another 5 or 6 min depending on the analyte s migration time. Borate buffers and rinse solutions also had to be prepared. However, the automation provided by the Beckman P/ACE 5000 permitted unattended operation, which allowed other tasks to be done during column rinses and data collection. The optical bench system was labor intensive, requiring manual rinses and injections.

The spectrofluorimeter was rugged and simple to use. No mechanical problems occurred during these analyses. Dust, air bubbles, and buffer deterioration can cause problems in CE. Although most such problems may be due to operator errors, the spectrofluorimeter was easily operated by someone with no prior experience.

For spectrofluorimetry, the excitation wavelength was variable over a wide range, and several compounds were targeted for a given analysis. With CE/LIF, multiple lasers were required to optimally observe each analyte in turn. Because the peak capacity is so low for the spectrofluorimetric method, however, only a limited number of analytes can be quantified at a time.

13. Advantages of CE/LIF

CE/LIF provided greater specificity. Component separation with even a modest 30,000 theoretical plates provided peaks much narrower than those observed in synchronous scans. The much greater peak capacity ensured separation of the analyte signal from fluorescing interferences.

The spectrofluorimetric detection limit for Fluorescein was 3 ppt in deionized water free of interferences and 60 ppt using the Beckman P/ACE 5000 with a 5 sec injection. Solid phase extraction of large water samples could reveal much lower concentrations of dyes. Evaporation of the extract from a solid phase extraction disk to a few tenths of a mL or less before injection on a CE column (rather than to 3 mL, the volume of a cuvette) would provide at least a 10-fold greater sensitivity increase for CE relative to spectrofluorimetry. Field amplification could provide another 100-fold improvement in sensitivity for CE and yield a much lower detection limit than that observed using spectrofluorimetry.

The high peak capacity of CE permitted use of an internal standard that did not interfere with analyte signals. Referencing of analyte signals to an internal standard signal provided better quality data. Baseline resolution greatly reduced the uncertainty in assigning a baseline for quantification. The CE data with well defined peaks atop a level baseline provided more compelling evidence for the presence of the dyes than the small broad peaks superimposed on a very large and variable baseline caused by fluorescing interferences in the spectrofluorimetric data.

14. Conclusion and Future Work

Both spectrofluorimetry and CE provided evidence that groundwater migration occurred between two contaminated sites. Two compounds that fluoresced with the correct wavelength maxima in synchronous scans also displayed the same migration times as standards in electropherograms. Using the optical bench and a new ArKr mixed-gas laser, which provides excitation lines at 514 nm and 531 nm, we will determine if the other two dyes can be detected in charcoal extracts. We will also determine if Fluorescein can be detected in the water samples after concentration by 2 or 3 orders of magnitude by SPE. If these dyes are detected, the preponderance of evidence for migration between contaminated sites will be increased.

References

1. Nie, S.; Dadoo, R.; Zare, R.N. *Anal. Chem.* 1993, 65, 3571.
2. van Soest, R.E.J.; Chervet, J.P.; Ursem, M.; Suijlen, J.M. *LC GC International* Sept., 1996, 586.