Application of a multi-LED field fluorometer for simultaneous detection of hard to separate dye tracers and fluocapteurs

P.A. Schnegg
Albillia Co, Neuchatel, Switzerland

L. Thueler
University of Neuchatel, Switzerland

ABSTRACT: Tracer tests are an irreplaceable tool of hydrogeologists. They are used to determine the paths of water flow between two spots in a catchment through or under the surface of the earth. Often hydrogeologists carry out tracer tests with only one tracer at a time, but frequently two or more fluorescent substances are simultaneously injected into different spots (sink holes, wells, boreholes, fissures) and collected in a spring. Then, the resulting cocktail is optically analyzed to separate the tracers and calculate the concentrations. Molecules with sufficiently different characteristics (excitation and emission spectra) are easily separated. But two among the most frequently used tracers, uranine and eosine, are very close in this respect. Their separation is well-known for being hard. Other examples of hard to separate tracer pairs are naphtionate and amino-G acid, two very useful tracers since they are colorless and therefore pass unnoticed in surface waters. The eluent of charcoal bags (fluocapteurs) is another example. The released tracer shows a very high fluorescence background of dissolved organic matter (DOM) from which it must be optically separated.

In this work, the separation power of two optical methods is compared: 1) The lab spectrofluorometer and 2) The flow-through field fluorometer. It is shown that this second, inexpensive technique compares very favorably with the first one. In the case of tracer pairs, this method allows perfect separation, whereas the spectrofluorometer requires additional data handling. Our design of the field fluorometer displays 3 LEDs with increasing wavelength (a dark-red forth LED is used for the turbidity measurement). The tracer separation is achieved in real-time by solving a set of linear equations of the unknown concentrations. Today, the field fluorometer can do the job that was previously entrusted at higher cost to the laboratory.

KEYWORDS: fluorometer, tracer test, tracer separation, uranine, eosine, fluocapteur, charcoal bag

1 INTRODUCTION

1.1 *Purpose of tracer tests in hydrogeology*

Tracer tests provide a simple mean for tracking water flow in the underground. A tracer is a substance that can be injected into the ground and then, detected in extremely small quantities in other sites, thus revealing subterranean path linking the injection to the detection area.

1.2 *The method: Fluorescence measurement*

There are tracers of various classes: Natural tracers such as dissolved organic matter (humic acids) or inorganic (ions), turbidity, electrical conductivity and temperature of water, stable isotopes. Biological tracers: Bacteriophages, spores. But perhaps the best known and simplest to use are the fluorescent dye tracers. They are extremely sensitive since their detection level is of a few parts per trillion (PPT) for some of them (uranine). What makes them so sensitive is the measurement method. The emitted fluorescence is measured at 90 degrees of the excitation beam. At smaller concentrations the fluorescence signal is proportional to the dye concentration. The detection is carried out on samples collected in the water over the time (hours or days) and analyzed in the laboratory with a spectrofluorometer. A more modern alternative is the direct detection in the field with a flow-through portable fluorometer.

1.3 *The multi-tracer experiment*

Since the measuring instruments (spectrofluorometer or flow-through field fluorometer) have spectral capabilities, more than one tracer can be used at a

time. In case of mixing of the waters before the tracers reach the detector, optical separation can be achieved. The more different are the tracers, the best is the separation. Optimal tracer pairs are uranine / rhodamine or naphtionate / uranine, with fluorescence maxima distant by 70 nm and 92 nm. Uranine, also called sodium fluoresceine, is certainly the best dye tracer in terms of sensitivity and sorption properties. Therefore most tracer tests make use of this substance. Eosine is also an excellent dye tracer, with better sorption properties than rhodamine. Unfortunately, its optical properties are very close to those of uranine (distance to uranine: 26 nm), thus difficult to separate from it. Figure 1 shows the excitation spectra (synchroscan) of a cocktail of uranine and eosine with increasing eosine and decreasing uranine concentration. Obviously values below 10% make separation difficult.

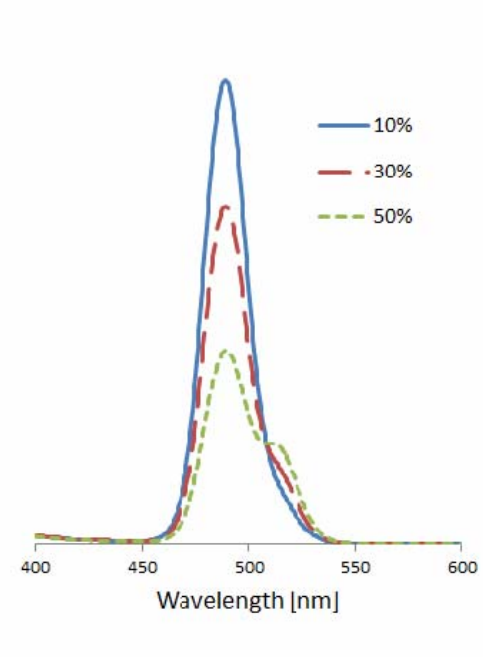

Figure 1. Fluorescence of a uranine-eosine cocktail with increasing eosine concentration.

In a similar way, it would be nice to use simultaneously amino-G acid (AGA) and sodium naphtionate, since both substances give discreet colorless solutions. But their spectral separation is even smaller (9 nm, however their excitation is better separated: 39 nm). Some authors (Jozja et al. 2011) claim that such combinations of tracers cannot be used, because the concentration of one of the tracers will be poorly estimated. In the following sections we show that this is not true.

2 THE SEPARATION METHOD

2.1 *Principle of the flow-through field fluorometer*

An optical cell (Pyrex or quartz glass tube) placed along the axis of a stainless steel cylindrical waterproof casing (flow-through field fluorometer) measures the tracer concentration in the water flowing through the tube.
The components used for the measurement of dye concentration are installed along the orthogonal axes of two square crosses in two parallel planes (Figure 2).

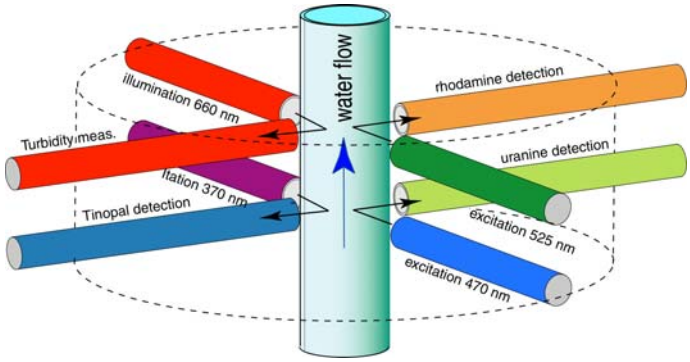

Figure 2. Setup of the flow-through field fluorometer.

The measurement system consists of a four-fold excitation section, with a quasi-monochromatic light source, a filter and a condenser lens and a detection section, orientated 90° to the excitation beam, with a lens, a filter and a photo-detector. The light sources and the filters are selected according to the absorption-emission spectra of the dyes. Such geometry allows for installation of up to four measuring systems on two levels. One of the sets is dedicated to the measurement of the water turbidity while the three others are used for measuring the dye concentrations.

The separation of two to three tracers is achieved by solving a set of 2 or 3 linear equations. Each equation gives the amount of fluorescence signal V_i on photodiode P_i produced by each tracer under excitation by lamp $L_{i,}$ with *i=1,2 or i=1,2,3*. For small tracer concentrations such as found in many hydrogeological tests (< 1 ppm), tracer signals are additive. As an example we take the hypothetical case of a water containing three different tracers with concentrations α, β and γ. Previous calibration of the fluorometer yields the fixed coefficients C_j^i of three different sets *i* of lamps, filters and photodiodes for a fixed concentration (100 ppb) of each tracer *j*. The set of equations

$$C_1^i\alpha + C_2^i\beta + C_3^i\gamma = V_i \quad , i=1,2,3 \tag{1}$$

has the following solution:

$$\alpha = \frac{\begin{vmatrix} V_1 & C_2^1 & C_3^1 \\ V_2 & C_2^2 & C_3^2 \\ V_3 & C_2^3 & C_3^3 \end{vmatrix}}{\begin{vmatrix} C_1^1 & C_2^1 & C_3^1 \\ C_1^2 & C_2^2 & C_3^2 \\ C_1^3 & C_2^3 & C_3^3 \end{vmatrix}} \quad \beta = \frac{\begin{vmatrix} C_1^1 & V_1 & C_3^1 \\ C_1^2 & V_2 & C_3^2 \\ C_1^3 & V_3 & C_3^3 \end{vmatrix}}{\begin{vmatrix} C_1^1 & C_2^1 & C_3^1 \\ C_1^2 & C_2^2 & C_3^2 \\ C_1^3 & C_2^3 & C_3^3 \end{vmatrix}} \quad \gamma = \frac{\begin{vmatrix} C_1^1 & C_2^1 & V_1 \\ C_1^2 & C_2^2 & V_2 \\ C_1^3 & C_2^3 & V_3 \end{vmatrix}}{\begin{vmatrix} C_1^1 & C_2^1 & C_3^1 \\ C_1^2 & C_2^2 & C_3^2 \\ C_1^3 & C_2^3 & C_3^3 \end{vmatrix}} \qquad (2)$$

Stability of this solution depends on the choice of cut-off wavelengths for the various filters, on the central wavelength of the light sources and also, on the choice of tracers in the cocktail. Tracer cocktails that result in a small determinant (denominator in Equation 2) are more difficult to separate than those with larger determinant.

2.2 *Results of the tracer separation with the flow-through field fluorometer*

The separation with the flow-through field fluorometer was tested in the laboratory by mixing uranine at a concentration of 10 ppb with eosine at the same concentration. Seven mixtures ranging from 0 to 100% uranine were measured. The result is shown on Figure 3.

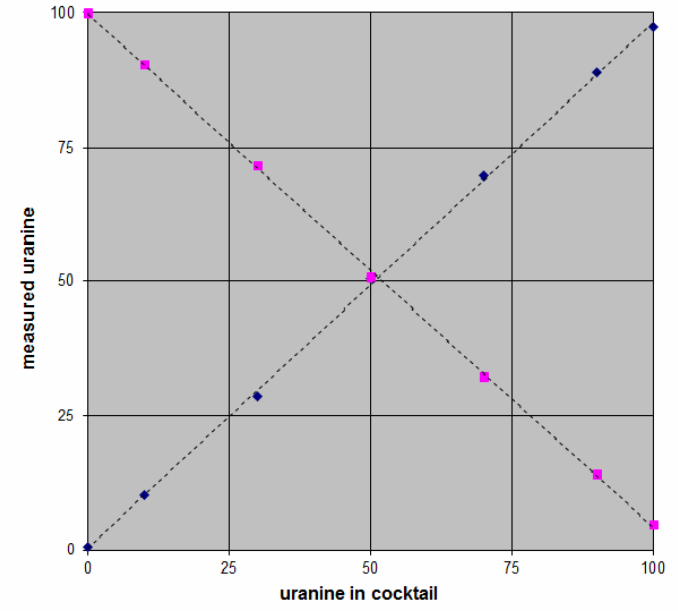

Figure 3. Measured vs. actual uranine and eosine concentrations in a cocktail. Results from the flow-through field fluorometer as obtained by solving Equation 1.

2.3 *Results of the tracer separation with a spectrofluorometer from the laboratory*

The same samples have been measured by a Perkin-Elmer lab spectrofluorometer. For the separation of the two dye components, we assume a Gaussian shape of each synchroscan spectrum. Such curve is described by three parameters: peak wavelength, height and width. We use a steepest descent algorithm to extract the six parameters of the two curves. This algorithm varies each parameter in turn, until the computed curve matches the measured one in the R.M.S. sense. Figure 4 shows the result of the optimization after 100, 200, 400 and 600 iterations.

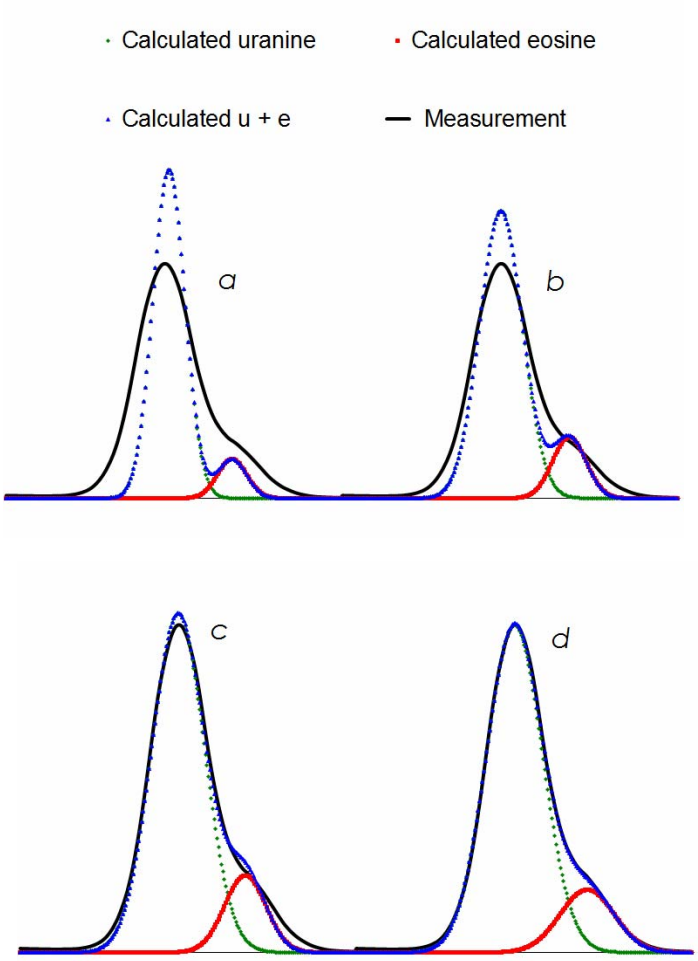

Figure 4. Separation of uranine and eosine from a 50-50% cocktail with the help of an optimization program based on the steepest descent in the space of the parameters. After 600 iterations, the calculated curve matches the measured one. a to d: 100, 200, 400 and 600 iterations. Horizontal axis: wavelength.

Figure 5 shows the result of the separation of the seven uranine-eosine mixtures. Obviously the determination of the eosine concentration is less good than in Figure 3. It is overestimated at low eosine concentration. We explain this less good result by the actual shape of the spectroscan curves. They may slightly differ from a Gaussian. The effect is amplified at low eosine concentration.

Wernli (2005) proposes a method for separating tracers with overlapping spectra. The method is based on the measurement of the spectrum at specific wavelengths. For comparison, the results of the method are shown on Figure 5. The quantification of uranine is good, but again, eosine is overestimated at lower concentrations.

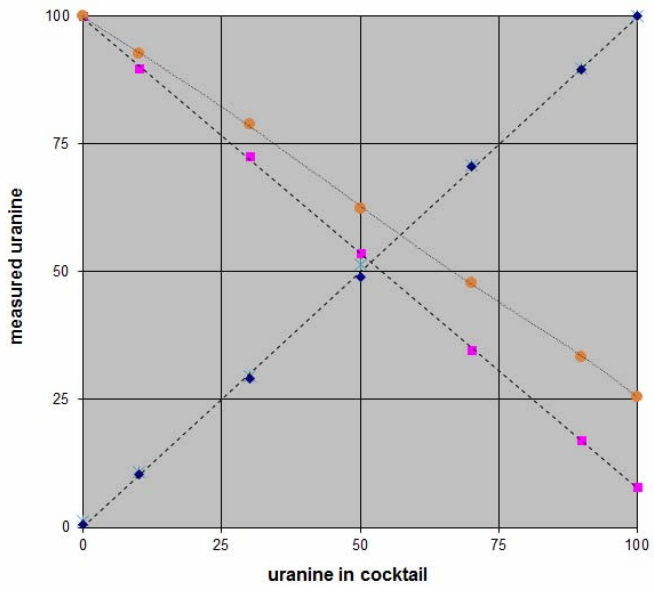

Figure 5. Measured vs. actual uranine and eosine concentrations in a cocktail. Results from the Perkin-Elmer spectrofluorometer and separation by fitting of two Gaussian curves. For comparison, the circles represent the result of the separation by the method of Wernli (2005).

2.4 *Another useful case: The naphtionate – AGA mixture*

Amino-G acid (AGA) is chemically close to sodium naphtionate. Both substances are used as colorless tracers, but rarely together because they are hard to separate. Figure 6 shows that in spite of overlapping spectra these tracers can be accurately separated from each other with the flow-through field fluorometer.

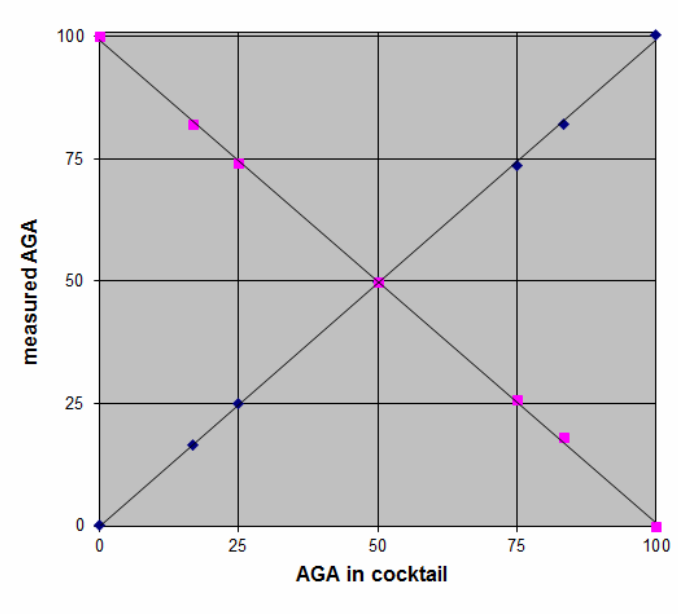

Figure 6. Measured vs. actual AGA and naptionate concentrations in a cocktail. Results from the flow-through field fluorometer as obtained by solving Equation 1.

2.5 *Analyzing fluocapteurs with the flow-through field fluorometer*

Fluocapteurs (charcoal bags) are a handy and economical tool for water tracing. Although their information is mostly qualitative (tracer – no tracer), they are of interest because a large target can be surveyed at a time by multiplying the sampling sites at low cost. The flow-through field fluorometer can be used in the laboratory for analyzing the eluent of the fluocapteurs. In fact this eluent not only contains the absorbed tracer, but also a large quantity of dissolved organic matter (DOM). If the tracer is uranine, then we are faced with a separation problem. There is some overlap between the spectra of uranine and DOM. We apply Equations 1 and 2 to the eluent measured in the flow-through field fluorometer. The full measurement requires a calibration of the fluorometer. Three calibrating solutions are prepared and measured: 1) The eluent of a new fluocapteur that has never been used. This is our blank sample. 2) A 100 ppb uranine solution prepared with that eluent. This is the standard solution for calibrating the uranine. 3) The eluent of a fluocapteur left during the same period upstream of the tracer injection. This is the standard solution for calibrating the DOM. Table 1 shows the results of the method. Eluants 4 and 5 are from fluocapteurs that have been in contact during 6 hours with stream water containing 10 and 100 ppb of uranine.

Table 1. Analysis of fluocapteurs with the flow-through field fluorometerr.

Sample	Uranine	DOM
	ppb	relative
Eluent 1	0.00	0.00
Eluent 2	100.00	0.00
Eluent 3	0.00	100.00
Eluent 4	0.88	98.64
Eluent 5	6.23	33.47

The uranine concentration found in eluents 4 and 5 are ten times smaller than measured in the stream water. This is not surprising, since we have seen that the extraction method (mixture of ethanol and ammonia) was able to release only 10% of the tracer.

3 CONCLUSIONS

It was unexpected for us that a portable, inexpensive sensor could compete and even compare favorably with dedicated, high precision laboratory instruments such as the spectrofluorometers. Obviously the present tests have been done in excellent conditions of turbidity. However, above 10 NTU this parameter tends to disturb the separation of two or three tracers. In the lab, it is always possible to filter the samples before measuring them with the spectro-

fluorometer. This is not feasible for a flow-through field fluorometer, unless there is a local power supply for a pump. But the main advantage of the field instrument is the immediate availability of the data (flash memory and/or GPRS transmission) and the total absence of contamination.

4 REFERENCES

Wernli, H.R. 2005. Einführung in die Wasserbeschaffenheit: Hydrologisches Praktikum. Geographisches Institut, (eds).

Jozja, N., Mondain, P.H. & Muet, P. 2011. Réflexions sur la fiabilité des traçages au regard des difficulties analytiques. In Catherine Bertrand, Nicolas Carry, Jacques Mudry, Michiel Pronk & François Zwahlen (ed.), *9th conference on limestone hydrogeology*; *Proc. H2Karst., Besançon, France, 1-4 sep. 2011, p. 249-252.*

Resumen en español

Los ensayos de trazadores son herramientas irremplazables del hidrogeólogo. Ellos se utilizan para determinar los caminos del agua de superficie y subterránea entre dos puntos. El hidrogeólogo lleva a cabo el ensayo con un solo trazador, pero con frecuencia dos o más sustancias fluorescentes se inyectan simultáneamente dentro de varios puntos (agujeros, pozos, fisuras) y se recogen en manantiales. Después de eso, el cóctel resultando se analiza para separar los trazadores y calcular las concentraciones. Las moléculas con características ópticas suficientemente diferentes (espectros de excitación y detección) se separan fácilmente. Pero dos entre los trazadores más utilizados, uranina y eosina, son muy vecinos. La separación esta reconocida por ser delicada. Otros ejemplos de pares de trazadores que también son delicados: Naftionato y acido-amino-G, dos trazadores muy útiles porque sin color, así que pasan inadvertidos en las aguas. Otro ejemplo: El líquido de elución de bolsas de carbón activado (fluocaptores). El colorante extraído muestra un nivel muy alto de fluorescencia de fondo producido por la materia orgánica disuelta de la cual debe ser separada.

En éste trabajo, comparamos el poder de separación de dos métodos ópticos: 1) El espectro-fluorimetro de laboratorio 2) el fluorimetro de campo. Se puede constatar que la segunda técnica, muy barata, se compara ventajosamente con la primera. En el caso de pares de trazadores, el método permite una separación perfecta, mientras que el espectro-fluorimetro necesita más manipulaciones de datos. Nuestro diseño del fluorimetro comprende 3 LED de longitud de onda creciente y una cuarta roja para medir la turbidez. La separación se obtiene en tiempo real resolviendo un conjunto de dos ecuaciones de dos (concentraciones) incógnitas. Hoy, el fluorimetro de campo puede hacer el trabajo que antes se confiaba, por un coste más alto, al laboratorio.

Palabras clave: fluorimetro, ensayo de trazadores, fluocaptor, bolsa de carbón activado.

Presented at:

XI Congreso Latinoamericano de Hidrogeología, Cartagena de Indias, Colombia, 20-24 August 2012