A Computer Method for Separating Hard to Separate Dye Tracers

P.-A. Schnegg

Abstract Tracer tests are an irreplaceable tool for hydrogeologists. They are used to determine the paths of water flow between two spots in a catchment below the surface of the earth. Usually, hydrogeologists carry out tracer tests with only one tracer at a time, but sometimes two or more fluorescent substances are simultaneously injected into different spots and collected in a spring. Then, the resulting cocktail is analyzed by optical methods (fluorescence spectrometer) to separate the tracers and calculate their concentrations. Molecules with sufficiently different excitation spectra are easily separated. But two among the most frequently used tracers, uranine (Na fluoresceine) and eosine, are very close in this respect. Their separation is well-known to be difficult. Other examples are sodium naphthionate and amino G acid, two very useful tracers since they are colorless and therefore unnoticed in surface waters. The eluent of charcoal bags (fluocapteurs) is another example. Beside the released tracer, there is a very high fluorescence background of dissolved organic matter (DOM) from which it must be optically separated. The shape of the excitation spectrum of a fluorescent tracer can be approximated by a Gaussian curve. This curve is completely described by three parameters: peak wavelength, height, and width. The spectrum of a cocktail of two tracers is the sum of two such Gaussian curves. To separate these two curves, we use an algorithm based on the steepest descent in the parameter space to find the best set of $2 \times 3 = 6$ parameters of the model that best fits the measured curve. We achieve good separation even with a concentration ratio smaller than 1:10.

Keywords Separation · Fluorescence spectrometer · Tracer test

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1 Introduction

Groundwater tracing with dye tracers is of common usage in karst hydrology. This is a method of choice for identifying preferential flow paths in the underground. After the tracer solution has been injected into the ground, a fraction as small as one part per billion collected downstream is still detectable through its fluorescence. Sometimes in multitracer tests, two or three different dye tracers are simultaneously injected at neighboring sites. Often, collected water samples result in a mixture of these tracers. It is necessary to resolve overlapping peaks to establish the concentration of each tracer. This operation is carried out in the laboratory with the fluorescence spectrometer, or directly in the field with the portable filter fluorometer (Schnegg 2003). The success of this analysis depends on the choice of tracers. Their optical properties (wavelength of the excitation and emission bands) must be sufficiently different. Good examples are the following cocktails:

- Uranine—rhodamine (any rhodamine: sulfo B, amino G, WT). Spectral distance: 60–70 nm
- sodium naphthionate—uranine or rhodamine. Spectral distance: 120–180 nm

Unfortunately, a very good tracer such as eosine is not so easy to separate from other dye tracers because its spectrum displays intermediate wavelengths (spectral distance to uranine and rhodamine: 25–48 nm). Colorless tracers are also of great interest, but the spectral distance between two of them, sodium naphthionate and amino G acid (7-Amino-1,3-naphtalene disulfonic acid, monopotassium salt hydrate), is only 39 nm. For this reason, almost all tracer tests avoid these two last combinations.

Noting that the spectra (excitation, emission, or synchroscan spectra) can be represented by single Gaussian curves or the sum of two such curves, we developed a computing method for the separation of two tracers. A Gaussian curve is completely described by three parameters: its peak amplitude, peak position, and width. The spectrum of a mixture of two tracers is therefore the sum of two Gaussian curves (sometimes three if a tracer has two peaks). The separation problem is solved if the six parameters can be determined. As the involved mathematical relationships are highly nonlinear, an Algebraic solution is not available. We use the algorithm of steepest descent to extract the parameters. The program automatically varies the six parameters in turn and stops when the difference between the calculated and the measured response has reached the absolute minimum. Although the software of curve fitting for resolving overlapping peaks is commercially available (PeakFit, Eigenvector, etc.), there is no mention of such method in the area of tracer tests.

2 Minimization Routine MINDEF

The routine MINDEF was written at the Institute of Physics of the University of Neuchâtel (Beiner 1970) and used since then in various optimization problems. The following example (Rosenbrock's valley) illustrates this routine with only two parameters $x1$, $x2$ to have a good grasp of the principle. The function is

$$f(x_1, x_2) = 100\left(x_2 - x_1^2\right)^2 + (1 - x_1)^2 \tag{1}$$

The function has its minimum at point $[x_1 = 1; x_2 = 1]$ and its value is zero (Fig. 1).

Any pair of values can be assigned to $x1$, $x2$ as start point. Routine MINDEF varies the parameters and after some iteration finds its way to the lowest point, the minimum of the function. As an example, starting at point $[x_1 = -1.2; x_2 = 1]$ the minimization ends after 126 steps at point [1.015; 1.014] with a misfit of 2.96×10^{-2}. CPU time on a PC is a few seconds. Each parameter can be assigned a variation weight, so that if its weight is set to zero, the parameter is not allowed to vary. The minimization algorithm is extremely robust.

3 Application to the Separation of Two Tracers

In this article, the method was applied to the separation of two different tracer cocktails, each of them difficult to separate into individual tracer concentrations: (1) uranine with eosine and (2) sodium naphthionate with amino G acid (colorless

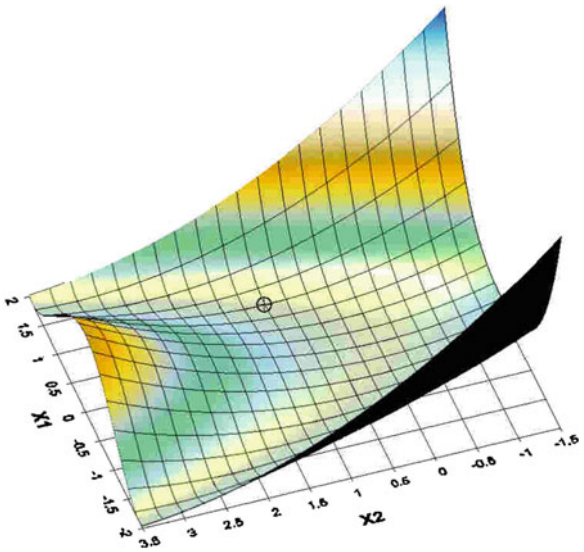

Fig. 1 Rosenbrock's valley (Eq. 1). The black circle is the minimum of the function

dyes). To check the efficiency of the method, we prepared known mixtures of the tracers. The starting solutions were prepared at a concentration of 10 µg/L (10 ppb).

The different cocktails were analyzed on a Perkin Elmer LS50B luminescence spectrometer, with excitation and emission slits of 10 nm and a scan speed of 500 nm/mn.

Figure 2 shows synchroscan spectra of uranine and eosine at 10 µg/L concentration along with their mixtures (from 9 µg/L uranine/1 µg/L eosine to 1 µg/L uranine/9 µg/L eosine). Figure 3 shows synchroscan spectra of sodium naphthionate and amino G acid at 10 µg/L concentration, as well as their mixtures (from 7.5 µg/L sodium naphthionate/2.5 µg/L amino G acid to 2.5 µg/L sodium naphthionate/7.5 µg/L amino G acid). The curves of single tracers show perfect Gaussian shapes (except for amino G acid whose curve is well approximated by the sum of two Gaussians). Therefore, the separation of uranine and eosine is a problem of finding $2 \times 3 = 6$ parameters. We could deduce that the number of parameters for the separation of sodium naphthionate and amino G acid would be higher (3×3). Fortunately, this is not the case since the secondary Gaussian curve of amino G acid is proportional to the main one. In addition, the total number of unknown parameters can be lowered since the center and width data can be measured with good precision by scanning the one-tracer solutions. Then, in both examples, the separation routine will handle only two parameters, the peak amplitudes.

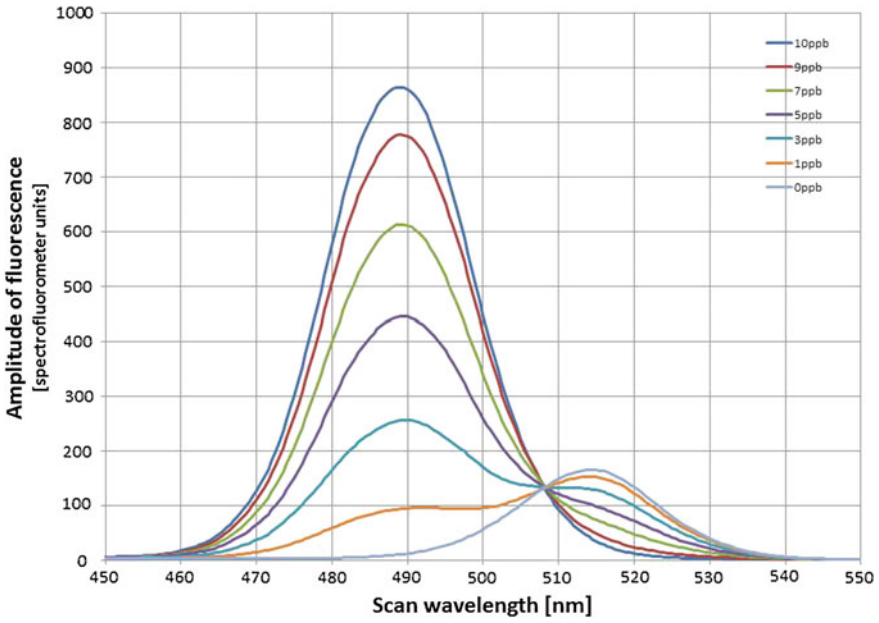

Fig. 2 Synchroscan spectra of mixtures of uranine and eosine 10 µg/L solutions. The concentration legend is for uranine. Corresponding eosine concentrations were 10 µg/L minus the concentration of uranine

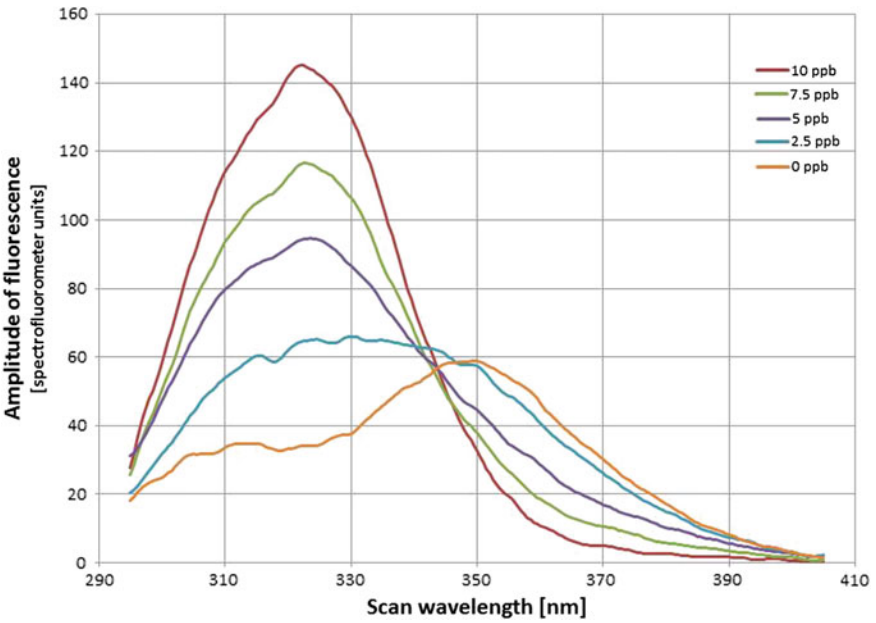

Fig. 3 Synchroscan spectra of mixtures of Na naphthionate and amino G acid 10 μg/L solutions. The concentrations legend is for naphthionate. Corresponding amino G acid concentrations were 10 μg/L minus the concentration of naphthionate

Table 1 Results of the separation with MINDEF of uranine and eosine from mixtures prepared with 10 μg/L solutions

% uranine (true)	% uranine (MINDEF)	% eosine (MINDEF)	% eosine (true)
0	0.69	100.00	100
10	10.45	89.63	90
30	29.05	72.56	70
50	49.02	53.66	50
70	70.49	34.76	30
90	89.55	17.07	10
100	100.00	7.93	0

Table 1 shows the results of the separation by MINDEF of uranine and eosine from mixtures prepared with 10 μg/L solutions. For uranine, the error is less than 1 % regardless of the concentration ratio, but for eosine it strongly depends on its concentration. The error is below 10 % for eosine concentrations in excess of 30 %, but it is close to 100 % when eosine concentration is lower than 10 %.

The case of the naphthionate-amino G acid cocktail is illustrated in Fig. 4. Suppose that all we know is that our water sample contains these two tracers, but at unknown ratio. We will run the program so that the response of our model best fits

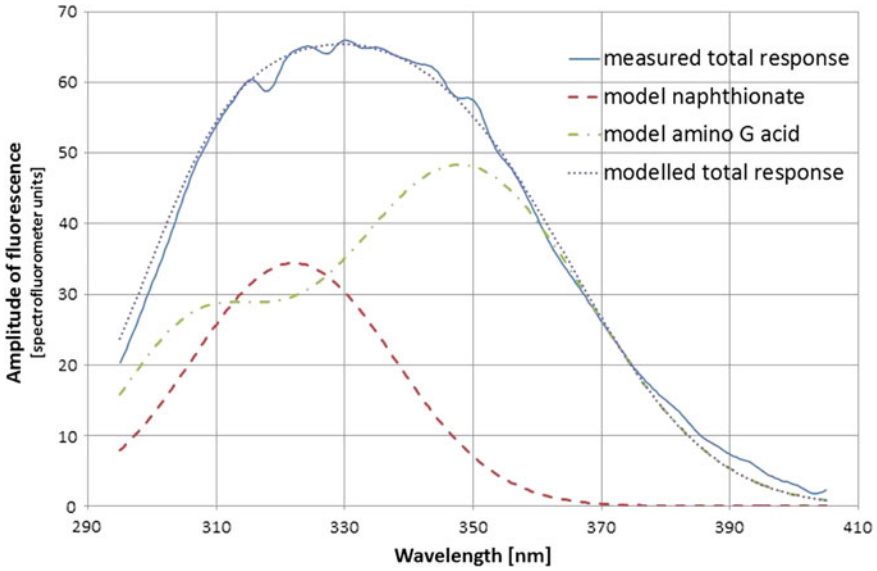

Fig. 4 Separation of a mixture of 2.5 µg/L naphthionate and 7.5 µg/L amino G acid

the curve measured with the spectrofluorometer. The total number of unknown parameters is six, since each Gaussian curve is fully determined by three parameters (the secondary peak of amino G acid is proportional to the main peak and therefore no additional parameter is required). But this number of parameters can be easily reduced to two: peak widths and wavelengths of both tracers can be determined once and for all by applying MINDEF on synchroscan spectra of one-tracer solutions. Then, these parameters are kept fixed in the final modeling phase of the tracer cocktail (variation weight set to zero) and only two unknown parameters, the peak amplitudes are varied and finally determined. Note that the common baseline was automatically subtracted by the luminescence spectrometer before applying MINDEF.

Table 2 shows the results of the separation by MINDEF of Na naphthionate and amino G acid from mixtures prepared with 10 µg/L solutions. From the values of the Tables 1 and 2, we see in both cases that the tracer with the highest quantum yield (peak amplitude) also displays the smallest error in the determination of its concentration.

The case of charcoal bags (fluocapteurs) is not illustrated in this work. However, we have also checked that the method is well suited for separating the tracer response from the background (luminescence of the dissolved organic matter (DOM)). In this problem, one Gaussian curve represents the response of the tracer and a second Gaussian curve the response of the DOM. Since DOM fluorescence appears in the UV to blue region of the spectrum, i.e., at wavelengths shorter than the tracer emission, only the long wavelength tail of the second curve (DOM) is normally represented in the spectrum of the eluent. However, to obtain a good fit

Table 2 Results of the separation by MINDEF of Na naphthionate and amino G acid from mixtures prepared with 10 µg/L solutions

% naphthionate (true)	% naphthionate (MINDEF)	% amino G acid (MINDEF)	% amino G acid (true)
100	100.00	7.02	0
75	75.52	28.07	25
50	54.55	50.88	50
25	23.78	84.21	75
0	0.00	100.00	100

of the measured curve, it is necessary to allow the variation of the three parameters associated with the DOM Gaussian curve, but at the end we are only interested in the amplitude of the tracer peak.

4 Discussion

We used synchroscan luminescence spectra of the tracers for the separation, but other spectra such as excitation or emission spectra can be used as well. However, the best result is always achieved when the peak separation is a maximum.

The assumption behind this separation method is the linearity of the synchroscan signals with regard to the concentration of the tracers. For excitation wavelengths above 500 nm, linearity is almost the rule. But for shorter wavelengths, a nonlinear term should be added. This is particularly true if secondary maxima located between 200 and 300 nm are used in the modeling instead of those of Fig. 4.

To improve the quality of the separation, some more parameters should be included in the MINDEF search, to account for the nonlinearity. The full polynomial dependency of the signal versus concentration could be measured and included in the optimization method.

The MINDEF routine is nice because it does not require the analytic expression of the derivative of the function to be known. But any other similar routine can be used for this work, such as MINUIT (James 2004), designed for particle physics in the 1970s by physicists of the CERN, and many others.

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