Parameters affecting Na-fluorescein (uranine) detection in mine water tracer tests

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Abstract Because of its conservative behaviour, low detection limits and low costs, Na-fluorescein (uranine, $C_{20}H_{10}Na_2O_5$) is a widely applied dye for tracer experiments. In this study, three factors potentially interfering with its fluorescence detection and thus limiting the applicability of Na-fluorescein at the standard excitation wavelength of 490 nm are examined (combined use of uranine and eosine ($C_{20}H_6O_5Br_4Na_2$); mineral precipitation due to buffering of acidic samples; dissolved contents of Fe and Mn ions). This study offers an approach to avoid these effects by making use of a second fluorescence maximum of uranine at pH 1.5 and an alternative excitation wavelength of 438 nm.

Key Words uranine, Na-fluorescein, interference, eosine, fluorescent dyes, dissolved Fe, acid mine water

Introduction

Since the initiation of the first quantitatively interpreted groundwater tracing tests at the end of the 19th century (Käss 2004), considerable improvements have been made in the technology of groundwater tracing. Historically, mostly salt tracers or particle tracers were applied while nowadays, there is a variety of different fluorescent dyes that can be detected even in low concentrations by irradiating the sample with light of a specific wavelength and measuring the intensity of the light emitted by the fluorescent components.

Owing to its numerous advantages in comparison to other dyes, Na-fluorescein ($C_{20}H_{10}Na_2O_5$) is one of the most commonly used fluorescent dyes. The detection of Na-fluorescein can be successful even in extremely low concentrations (detection limit in this study: 3 ng L^{-1} = 3 ppt; Naurath *et al.* 2011). Additionally, in most environments, Na-fluorescein is referred to as a nearly conservative tracer (Käss 1964, Käss 1967, Smart and Laidlaw 1977, Leibundgut 1981, Dervey 1985). Above all, the material costs are comparatively low due the multiple possible applications and thus the high demand of Na-fluorescein (Käss 2004, Smart and Laidlaw 1977).

However, there are interfering factors potentially limiting the detectability of the Na-fluorescein signal. In this study, three of these factors are examined. The first source of potential interference is the simultaneous use of eosine alongside Na-fluorescein in combined tracer tests (Käss 1982), whereas the second and third interfering factors are a low pH value and the content of dissolved Fe. Since the latter two factors often are related to acid mine waters, the use of Na-fluorescein in that kind of environment is usually considered problematic. Nevertheless, there are some cases where Na-fluorescein was used successfully even in mine water environments (Wolkersdorfer 2008). For example, tracer tests applying Na-fluorescein were recently conducted in a passive mine water treatment plant with maximum Fe concentrations of 15 mg L^{-1} and pH values between 6 and 7 as described by Wolkersdorfer (2011).

Another commonly used fluorescent dye is eosine ($C_{20}H_6O_5Br_4Na_2$). The molecular structure of eosine is nearly identical to that of Na-fluorescein, only with four hydrogen atoms being substituted by bromide (Käss 2004). Consequently, the characteristics of both substances are relatively similar. The intensity maximum of eosine fluorescence is observed at an excitation wavelength of 516 nm. However, a secondary maximum occurs at 480 nm which overlaps with the Na-fluorescein maximum excitation wavelength at 490 nm. The interference of the two dyes is also visible in the emission spectra which are added up in a way that leads to higher fluorescence intensities, resulting in an overestimation of Na-fluorescein concentrations if eosine is present and vice versa (Fig. 1, top).

Also the pH value has a major influence on the Na-fluorescein signal (Fig. 1, bottom). At the standard excitation wavelength (490 nm), the maximum intensity is achieved under slightly alkaline conditions (pH 9). Consequently, Na-fluorescein was presumed to be inapplicable in acidic environments for a long time. Buffering the samples to a suitable pH range as a possible solution for this problem leads to precipitation of dissolved metal ions and thus necessitates a time and cost intensive filtration of every sample to be analyzed.

It is, however, possible to quantify Na-fluorescein at a further excitation wavelength of 438 nm, where a second maximum of the fluorescence intensity emerges under acid conditions (pH 1.5; Behrens 1986; Fig. 1, bottom). With this approach, the problem of metal hydroxide precipitation occurring under alkaline conditions can be avoided. Furthermore, this also provides a solution to the problem of eosine influence on the Na-fluores-

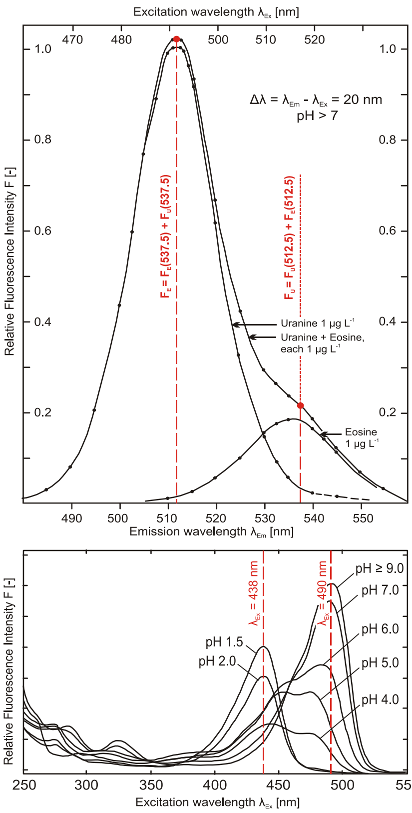

Figure 1 *Synchroscan spectra (constant interval between excitation and emission wavelength of $\Delta\lambda$ = 20 nm) of Na-fluorescein (uranine) and eosine (1 µg L^{-1} each) under standard alkaline conditions (top; after Käss 1982) and excitation spectrum of Na-fluorescein vs. pH (bottom; after Behrens 1986, cited in Käss 2004).*

cein signal because the fluorescence of eosine is suppressed in strongly acidic conditions. At pH 1.5, the eosine fluorescence intensity is reduced to less than 10 % of the maximum intensity (Käss 2004). To extend the applicability of Na-fluorescein as a tracer to acid mine water environments, recovery tests as well as interference tests concerning eosine were conducted at this shifted wavelength in water samples from several mining sites, buffered to pH 1.5.

During these tests it became apparent that the dissolved Fe content is a third limiting factor for Na-fluorescein detection with decreasing recovery rates in Fe-rich water samples which are significant based on a Mann-Kendall-Test (95 % level of significance). Therefore, further experiments with increasing Fe contents in the samples were designed to quantify this phenomenon.

Materials and Methods

Eight water samples were taken from five different coal or sulphidic ore mining locations in Germany. The characterization of the different matrices included on-site documentation of the physico-chemical and organoleptic parameters (electrical conductivity, dissolved oxygen, pH and Eh; colour, clouding and precipitates) as well as spectrophotometrical determinations of total Fe and Mn concentrations (Tab. 1). To prepare the samples for measurements at the two intensity-peaks of Na-fluorescein, two buffer solutions were prepared for pH 1.5 (KCl, HCl) and pH 9 (citric acid, HCl, NaOH). For measurements of eosine, a third buffer solution for pH 4.5, consisting of acetic acid (CH_3COOH) and Na acetate solution (CH_3COONa) was produced. All applied buffers were prepared after Küster and Thiel (2008). Buffering the Fe-rich samples 2 and 8 (cf. Tab. 1) to pH 9 always led to an orange-yellow precipitate, presumably Fe hydroxides, which had to be filtered after the buffer was added. The other combinations of samples and buffers showed no undesirable effects.

The fluorescence spectrometer Varian Cary Eclipse was calibrated in six linear calibration curves covering a span from 0.001 to 1,000 µg L^{-1} using a dilution series of Na-fluorescein (CI 45350, CAS 518—47—8, BASF) in deionized water with each standard buffered to pH 9. The same procedure was applied to calibrate the spectrometer for pH 1.5, and for eosine (CI 45380, BASF) at pH 4.5. However, due to the weaker fluorescences of eosine and of Na-fluorescein under acid conditions, here only five calibration ranges were defined reaching from 0.01 to 1,000 µg L^{-1}. The detection limits were calculated according to DIN 32645 of the German Institute for Standardization (2008) and yielded 0.003 µg L^{-1} for Na-fluorescein at pH 9, 0.015 µg L^{-1} for Na-fluorescein at pH 1.5 and 0.009 µg L^{-1} for eosine at pH 4.5.

Before conducting recovery and interference tests, background values of the samples for all three calibrations were measured. Subsequently, for the recovery tests, every sample was spiked with Na-fluorescein in a dilution series from 0.005 to 50 µg L^{-1}. Aliquots of the samples were buffered to pH 1.5 and to pH 9 and then measured using the corresponding calibration.

For the interference tests, every sample was spiked in the same way but with eosine instead. These samples were buffered to pH 1.5 and pH 9 as well, and the Na-fluorescein signal was measured. For validation of the eosine concentration, aliquots of the samples were additionally buffered to pH 4.5 and eosine was measured with the corresponding calibration.

Because a negative relation between Na-fluorescein recovery rate and dissolved Fe content emerged during the recovery tests, some addi-

Table 1 *List of samples with characterization of on-site matrix parameters (Eh [mV], eC: electrical conductivity [mS cm^{-1}], DO: dissolved oxygen [mg L^{-1}]), Fe and Mn concentrations [mg L^{-1}], n.a.: not analyzed, DL: detection limit (Fe) = 0.005 mg L^{-1}.*

No	pH	Eh	eC	DO	Fe	Mn	Colour	Clouding/Precipitate
1	5.9	410	1.36	7.57	n.a.	n.a.	pale yellow	red-brown precipitate
2	3.6	570	5.55	4.96	206	17.8	brown	brown clouding
3	4.8	432	3.29	5.65	16.4	9.54	brown	brown clouding
4	5.3	355	1.39	9.67	0.02	1.04	brown	brown clouding
5	3.6	616	4.22	10.1	9.69	201	pale red-brown	pale red-brown clouding
6	7.1	414	49.7	7.65	<DL	1.81	-	-
7	6.7	417	4.46	5.12	<DL	0.83	-	-
8	2.8	636	3.60	10.4	203	33.3	yellow	orange-brown precipitate

tional experiments were designed to confirm and further quantify this observation. Twenty solutions with different Fe contents between 0.0025 and 500 mg L^{-1} were prepared by dilution of a 1 g L^{-1} Fe standard solution. The solutions were spiked with three Na-fluorescein concentrations of 0.5, 5 and 50 µg L^{-1}, buffered to pH 1.5 and then the Na-fluorescein signal was measured. For comparison, ten of the 5 µg L^{-1} Na-fluorescein samples were additionally buffered to pH 9. Contrary to the natural samples, the buffering did not lead to a precipitation of Fe hydroxides and therefore it was not necessary to filter these artificial samples.

Results and Discussion

Even though all samples were collected from mining sites (samples 2 to 5 even from the same location but different sampling points), the matrices show very diverse characteristics. For instance, the photometric analysis revealed rather high Fe contents in samples 2 and 8 and a high Mn content in sample 5 whilst concentrations in the other samples were lower (Tab. 1). Sample 6, on the other hand, stands out because of its very high eC-value, presumably attributed to high contents of Na^+ and Cl^- from abundant saline waters in the sampling region (Grobe and Machel 2002), while the eC in the other samples is around 10 times lower. The pH values range from acidic to circumneutral.

Figures 2a and 2b show the results of the recovery tests. At pH 1.5 all samples exhibit a higher background value in comparison to the measurements at pH 9, causing the curves to bend upwards at lower input concentrations. The highest background value is measured for sample 4 in both pH ranges. At pH 9 rather good recovery rates were achieved for all samples, while at pH 1.5 the Fe-rich samples 2 and 8 showed far lower values compared to the input concentrations, although no visual effects like colouring or precipitation due to buffer addition could be observed. However, it is to be noted that the good recovery for these two samples at pH 9 was only possible through the filtration of every sample after the buffer was added (Fe hydroxide precipitation). Contrarily, sample 5 with a high Mn content showed none of the described problems. According to these results, the Fe content of the sample seems to restrict the detectability of Na-fluorescein at pH 1.5 but without visible effects.

In the interference tests, samples 2, 3 and 8 show only slightly higher background values at pH 1.5 than at pH 9 (Fig. 2c/d), whereas background values of the other samples are up to ten times higher which is potentially caused by other organic components in the sample (Smart and Laidlaw 1977). Nevertheless, if measured in acidic conditions, the Na-fluorescein signal apparently is independent of the eosine concentration in the sample, whereas at pH 9 the Na-fluorescein measured value in almost all samples obviously increases with higher eosine input concentrations. So the suppression of eosine at lower pH values seems to be successful while successive overestimation is obvious in the alkaline milieu. The validation of the eosine concentrations at pH 4.5 yielded average recovery rates of 94 %.

The Fe interference tests confirm a clear influence of the Fe concentration on the Na-fluorescein recovery rate (Fig. 3). At the upper two Na-fluorescein spikes at pH 1.5 (50 and 5 µg L^{-1}), the negative impact of Fe starts getting obvious even at Fe concentrations as low as 1 mg L^{-1}. Na-fluorescein recovery decreases only slightly with rising Fe content up to 50 mg L^{-1} Fe, where the recovery then exhibits a stronger decline. A rather rapid drop in the recovery rate starts at Fe concentrations of 200 mg L^{-1}. The curve for the lowest Na-fluorescein spike (0.5 µg L^{-1}) shows a more deviating progression reflecting the higher measurement error at these low concentrations.

In comparison, the recovery rate for the 5 µg L^{-1} Na-fluorescein spike at pH 9 already drastically decreases above an Fe concentration of 30 mg L^{-1}. At 100 mg L^{-1} Fe only 1 % of the Na-fluorescein input concentration was retrieved. Therefore, it is interpreted that at pH 9 interference of Fe with the Na-fluorescein measurement signal is already distinct at lower Fe concentrations com-

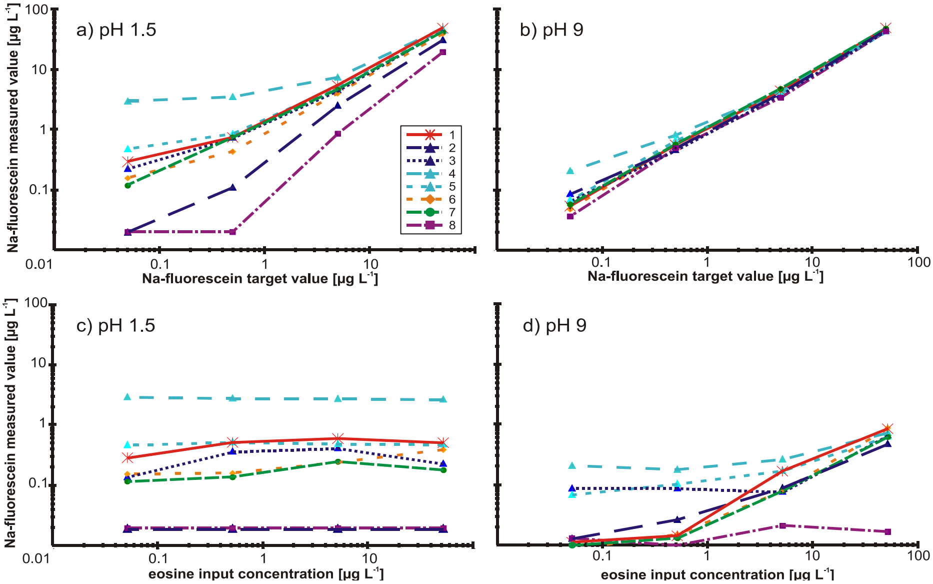

Figure 2 *Na-fluorescein recovery at pH 1.5 (a) and pH 9 (b) and Na-fluorescein signal as a function of the sample eosine concentration (interference tests) at pH 1.5 (c) and pH 9 (d).*

pared to the interference at pH 1.5. This, however, is contrary to the results of the recovery tests conducted in the natural mine water samples described above, as samples 2 and 8 had high Fe concentrations of about 200 mg L^{-1} and showed good recovery rates at pH 9. Obviously, the characteristics of natural mine waters were not successfully simulated with the rather simply constructed artificial samples.

Conclusions

The recovery tests for Na-fluorescein at two pH values (1.5 and 9) showed that it is generally possible to detect the dye even in different mine water environments. At moderate Fe contents below 20 mg L^{-1}, the Na-fluorescein concentration can be recovered to at least 80 % at both pH values. If samples are filtered after buffering, Na-fluorescein can be reliably quantified at pH 9. However, in the artificial samples the fluorescence signal already diminishes at Fe contents above 30 mg L^{-1}. At pH 1.5 the recovery rate remains on an acceptable level (≈80 %) with Fe contents of up to ≈100 mg L^{-1}, independent of the Na-fluorescein concentration. Above that level, the signal decreases rapidly even at pH 1.5, probably caused by generation of non-fluorescent Fe-fluorescein-complexes.

At pH 1.5 the detection limit of Na-fluorescein is five times higher than at pH 9 and most samples develop a higher background value, but nev-

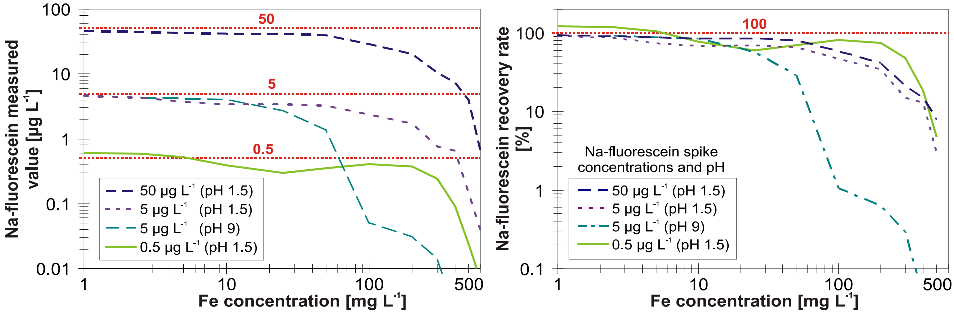

Figure 3 *Na-fluorescein signal of three input concentrations at different pH values in dependence of the sample Fe concentration as measured concentrations (left) and in terms of the recovery rate (right). Each curve represents a moving average (n = 3) of the measured datapoints.*

ertheless, the eosine fluorescence seems to be suppressed successfully at strongly acidic conditions and therefore can be examined separately.

It can be concluded that at Fe concentrations below 20 mg L^{-1} the Na-fluorescein concentration can be successfully recovered with both methods. When large sample numbers and sufficiently high dye concentrations (especially above the relatively high background value) are expected, the newly introduced method at pH 1.5 should be used to avoid the necessity to filter every sample after buffering which at large sample numbers is hard to manage and cost-intensive. Between 20 and 100 mg L^{-1} Fe, the new method yields better recovery rates, which for now seems to be restricted to artificial samples under laboratory conditions. Therefore, if Na-fluorescein is to be used in mine water environments, it is recommendable to test both methods in dilution series in the matrix to be examined in advance of the tracer test in order to make sure the method is applicable.

In consequence, above dissolved Fe concentrations of ≈100 mg L^{-1}, Na-fluorescein indeed proved not to be a good choice for tracer tests in mining waters whereby the presence of Fe, not pH, appears to be the limiting factor. Nevertheless, we showed here that in mine water environments containing low to moderate Fe concentrations, Na-fluorescein represents a feasible and cost-effective alternative which is also applicable in multitracer tests together with eosine.

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