NOTE

Octanol-water partition coefficients (K_{ow}) vs. pH for fluorescent dye tracers (fluorescein, eosin Y), and implications for hydrologic tracer tests

YASUHIRO OBA* and SIMON R. POULSON

Department of Geological Sciences & Engineering MS-172, University of Nevada-Reno, Reno, NV 89557, U.S.A.

Values of the octanol-water partition coefficient (K_{ow}) have been measured vs. pH for two fluorescent dyes (fluorescein, eosin Y) commonly used in hydrologic tracer tests. Values for both fluorescent dyes are similar, with slightly larger values for fluorescein vs. eosin Y at any given pH. Measured values of log K_{ow} show a negative correlation vs. pH, and range from 2.78 for fluorescein (pH 4.29) to −1.22 for eosin Y (pH 7.63). Values of log K_{ow} have been used to calculate example values of the organic matter-water partition coefficient (K_{om}) and the retardation coefficient (R), and suggest that fluorescein and eosin Y act as excellent hydrologic tracers at pH > 7, but become increasingly non-conservative at pH < 7. The results illustrate the importance of pH upon fluorescent dye speciation, and upon fluorescent dye adsorption behavior. Hence, measurement of pK_a and the variation of log K_{ow} vs. pH for other fluorescent dye tracers is recommended.

INTRODUCTION

Artificial tracer compounds are commonly employed to investigate water flow in a variety of hydrologic environments, where a tracer is introduced into the flow system, and can then subsequently be recognized, identified, and possibly quantified at a different location. As pointed out by Käss (1998) and Flury and Wai (2003), an ideal artificial tracer will have the following characteristics: will be absent in natural waters (or present in very low concentrations); will be toxicologically and ecotoxicologically safe; will have a high solubility in water; will be resistant to chemical, physical, and biological degradation; will be cost-effective in their use; will have a low detection limit during analysis; and will have little or no interaction with the flow medium (i.e., adsorption, ion exchange). In large part, fluorescent dye tracers satisfy these characteristics, and consequently, fluorescent dye tracers have been extensively used in hydrologic tracer experiments.

Adsorption is a key process in determining how effectively (or not) a dye tracer behaves in the same man-

ner as ground water. Ideally, a dye tracer will exhibit zero adsorption onto aquifer substrate material, and hence would be expected to show similar behavior (e.g., flow velocity) to ground water. Adsorption onto aquifer material will effectively retard a dye tracer, making it less desirable as a ground water tracer. In the case of organic compounds such as fluorescent dye tracers, organic matter in the aquifer is the most important substrate for adsorption (Schwarzenbach *et al.*, 1993). As fluorescent dye tracers have one or more functional groups present that may protonate/deprotonate (e.g., carboxylic acid, sulfonic acid, hydroxyl, amine groups), dye tracers may be positively charged, negatively charged or neutral, depending upon the pH and the values of the acid dissociation constants (pK_a) for each functional group. Hence, adsorption behavior and values of the organic matter-water partition coefficient (K_{om}) may also change vs. pH. A number of studies have measured values of K_{om} during the adsorption of fluorescent dye tracers onto various substrates (e.g., Smart and Laidlaw, 1977; Kasnavia *et al.*, 1999; see also studies compiled in Käss, 1998; Flury and Wai, 2003), but these studies do not always measure the pH of the sorption experiments, or measure the variation of K_{om} vs. pH. In some instances, studies did not directly measure the value of K_{om}, so it may be difficult to apply the results determined for the specific substrates used in these studies to other substrate material. An alternative approach (e.g., Kasnavia *et al.*, 1999) is to measure the octanol-water partition coefficient (K_{ow}) for the dye tracer over a

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*Corresponding author (e-mail: oba@lowtem.hokudai.ac.jp)
*Present address: Institute of Low Temperature Science, Hokkaido University, N19W8, Kita-ku, Sapporo, Hokkaido 060-0819, Japan.
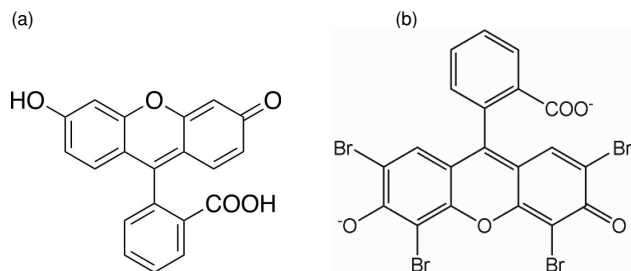
Fig. 1. Chemical structure of: (a) fluorescein, neutral form ($H_2Fl°$); (b) eosin Y, deprotonated form.

Fig. 2. Values of log K_{ow} vs. pH for fluorescein (this study; Kasnavia et al., 1999) and eosin Y (this study). Data points represent average of 3 to 5 experiments, and error bars represent 1 standard deviation. Some error bars are smaller than the data symbol.

range of pH values, which can then be correlated with the value of K_{om}, and subsequently to calculate a retardation coefficient (R), the ratio of the velocity of the ground water to the velocity of the dye tracer (e.g., Poulson et al., 1997).

This study has measured the values of log K_{ow} vs. pH for fluorescein and eosin Y (Fig. 1), two of the most commonly used fluorescent dye tracers, both of which are considered to be toxicologically and ecotoxicologically safe (Behrens et al., 2001).

METHODS

Fluorescein (sodium salt, CAS #51847-8) and eosin Y (CAS #15086-94-9) were obtained from Sigma-Aldrich and Riedel-De Haën, respectively. The value of the octanol-water partition coefficient (K_{ow}) was determined by the shake-flask method (e.g., Leo et al., 1971). Experiments were performed in 50 ml glass centrifuge tubes with polypropylene-lined caps, and used ~10 ml of deionized water, ~2 ml of 1-octanol (99+% ACS spectrophotometric grade, Sigma Aldrich), with concentrations of 0.05–0.10 mmol for eosin Y or 0.16–0.56 mmol for fluorescein. The pH was adjusted with HCl or NaOH. Three to five experiments were performed at each pH. Tubes were shaken for 10 minutes using a Fisher Scientific Vortex Mixer, and allowed to equilibrate. After equilibration, the tubes were centrifuged to separate the 1-octanol and the water. Dye concentrations in water were measured using a UV-visible spectrophotometer (Cary 50 Bio, Varian, CA) at visible wavelengths of 514.9–517.0 nm for eosin Y and 465.0–480.1 nm for fluorescein. Standards of known dye concentrations were prepared at each pH, and used to perform calibrations of absorbance vs. concentration. Dye concentrations in 1-octanol were calculated by mass balance. After the dye concentrations were measured, 1-octanol was removed using a plastic pipette, and the final pH of the aqueous solution was measured.
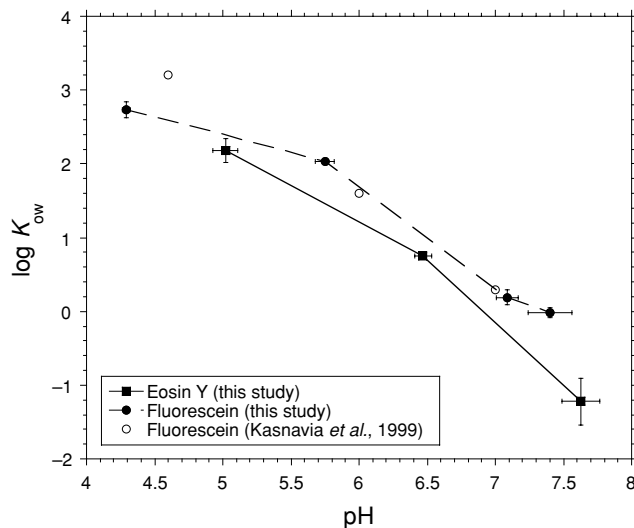
RESULTS AND DISCUSSION

Values of log K_{ow} vs. pH are plotted in Fig. 2. Data for fluorescein from this study show good agreement with the data from Kasnavia et al. (1999). There are no previous measurements of log K_{ow} vs. pH for eosin Y available in the literature. Values of log K_{ow} show a negative correlation vs. pH, with values of log K_{ow} for fluorescein being slightly larger than log K_{ow} for eosin Y (at a given pH) over the range of pH values considered in this study. The similarity of the values of log K_{ow} for fluorescein and eosin Y is consistent with these compounds having very similar chemical structures and functional groups, with the exception of the presence of four bromine atoms in eosin Y that substitute for aromatic hydrogen atoms (Fig. 1).

Least-squares best fit lines between log K_{ow} and pH show good correlation coefficients for both fluorescein and eosin Y ($r^2 = 0.95$ and 0.98, respectively: best fit lines not plotted). However, values of log K_{ow} probably do not vary linearly vs. pH over this entire pH range, due to the change of fluorescein speciation vs. pH. Fluorescein speciation (H_3Fl^+ vs. $H_2Fl°$ vs. HFl^- vs. Fl^{2-}) vs. pH has been calculated using values of acid dissociation constants (pK_a) taken from Diehl and Horchak-Morris (1987), and is plotted in Fig. 3. Over the range of pH 4 to 8, Fig. 3 illustrates that the Fl^{2-} species dominates at pH > 6.36, that the HFl^- species dominates pH < 6.36, that the $H_2Fl°$ species becomes significant (i.e., mole fraction > 0.1) at pH < 5.6, and that the H_3Fl^+ species is insignificant (very

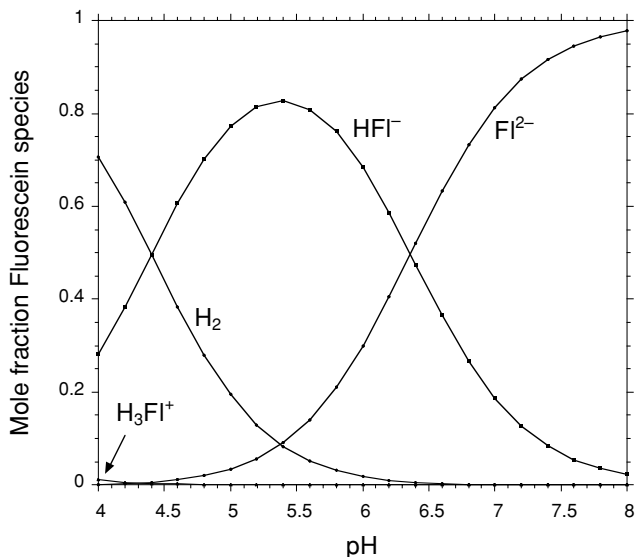*Fig. 3. Speciation of fluorescein vs. pH, using values of pK_a taken from Diehl and Horchak-Morris (1987).*

pH	Interpolated log K_{ow}	Calculated log K_{om}	Calculated R
Fluorescein			
5.5	2.16	1.46	4.04
6.0	1.70	1.00	2.04
6.5	1.00	0.29	1.20
7.0	0.34	−0.38	1.04
7.5	−0.08	−0.80	1.02
Eosin Y			
5.5	1.68	0.98	2.00
6.0	1.20	0.49	1.33
6.5	0.66	−0.05	1.09
7.0	−0.18	−0.90	1.01
7.5	−1.00	−1.73	1.00

small mole fractions at pH < 4.2). The observed changes of log K_{ow} vs. pH are consistent with the changes of fluorescein speciation vs. pH, and because the different fluorescein species are likely to have different values of log K_{ow}: the doubly-charged Fl^{2-} species is expected to be the most hydrophilic species; the neutral $H_2Fl°$ species is expected to be the most hydrophobic species; and the singly-charged HFl^- species is expected to have an intermediate hydrophilic/hydrophobic character. Values of pK_a for eosin Y are not available in the literature, although the similarity of chemical structures and many functional groups might suggest that pK_a values for eosin Y may be similar to pK_a values for fluorescein, in which case speciation of eosin Y would also change over the range of pH values used in this study.

Values of the sorption partition coefficient between water and organic matter (K_{om}) can be calculated using the strong linear correlation between log K_{om} and log K_{ow}. For aromatic hydrocarbons, this relationship is given by (Schwarzenbach *et al.*, 1993):

$$\log K_{om} = (1.01 \times \log K_{ow}) - 0.72. \tag{1}$$

For dye tracers, one of the most important properties is the value of the retardation coefficient (R), which is the ratio of the velocity of the ground water to the velocity of the dye tracer. A value of $R = 1$ indicates that a dye tracer is acting as a perfect tracer (i.e., the dye tracer has the same velocity as the ground water), whereas a value of $R > 1$ indicates that the dye tracer has a slower velocity compared to the ground water. Values of R during ground

water flow can be calculated using:

$$R = 1 + (K_d \times [\rho_{bulk}/\Phi]) \tag{2}$$

where ρ_{bulk} is the bulk density, Φ is the porosity, and K_d is the distribution coefficient. For the case of organic compounds, K_d is typically given by

$$K_d = f_{om} \times K_{om} \tag{3}$$

where f_{om} is the weight fraction of organic matter in the aquifer sample. Using example values of aquifer parameters (e.g., $\rho_{bulk} = 2.1$, $\Phi = 0.2$, and $f_{om} = 0.01$), and considering a pH range of 5.5 to 7.5 (common pH range of natural waters = 5.5 to 8.5: Drever, 1997) calculated values of retardation coefficients are presented in Table 1. Calculations illustrate that values of R for fluorescein are greater than the value of R for eosin Y at any given pH, indicating that eosin Y behaves somewhat more conservatively than fluorescein at any given pH.

At pH ≥ 7.0, example calculated values of $R ≤ 1.04$ for both compounds, indicating that both fluorescein and eosin Y exhibit excellent tracer behavior, with less than a 4% deviation of transport velocity for the tracers as compared to ground water. However, values of R increase significantly as pH decreases to values less than 7, with maximum calculated values at pH 5.5 of $R = 4.04$ for fluorescein, and $R = 2.00$ for eosin Y, indicating that both fluorescein and eosin Y become increasingly non-conservative in their behavior as pH decreases below 7.

Since all fluorescent dye tracers have one or more functional groups that may protonate/deprotonate (e.g.,

Käss, 1998), the adsorption behaviors of other dye tracers are also expected to depend upon the relevant values of pK_a for each dye tracer, as these are the critical parameters in determining the hydrophobic vs. hydrophilic nature of each dye tracer vs. pH. In particular, dye tracers with values of pK_a that lie in the range of 5.5 to 8.5 (as is the case for fluorescein) are particularly affected, as these dye tracers will have different speciation (and hence different hydrophobic/hydrophilic characteristics) over the pH range of 5.5 to 8.5 that is common for natural waters (Drever, 1997). Unfortunately, pK_a data for dye tracers other than fluorescein are unavailable, so determination of these values would be desirable. Measurements of the variation of log K_{ow} vs. pH are available for some additional dye tracers (rhodamine WT and rhodamine B) in Kasnavia *et al*. (1999) over a range of circum-neutral pH, but these data are unavailable for many other fluorescent dye tracers, and determination of these values would also be desirable.

SUMMARY

Most fluorescent dyes used as hydrologic tracers have one or more acid/base functional groups present in their chemical structure. Hence, the speciation of these fluorescent dyes depends upon values of pK_a and upon pH, which in turn can affect the values of properties such as the octanol-water partition coefficient (K_{ow}) and the organic matter-water partition coefficient (K_{om}), which in turn have an effect upon the retardation coefficient (R) for the tracer during ground water transport.

Values of log K_{ow} have been measured vs. pH for two commonly used fluorescent tracer dyes (fluorescein, eosin Y), and demonstrate a negative correlation of log K_{ow} vs. pH, with slightly larger values of log K_{ow} for eosin Y vs. fluorescein at any given pH. Over the range of pH values used in this study, the correlation of log K_{ow} vs. pH is not quite linear, due to the change of fluorescent dye speciation vs. pH. Using example values of aquifer properties, values of log K_{ow} can be used to calculate example values of K_{om} and R. These calculations suggest that both fluorescein and eosin Y serve as excellent dye tracers at pH > 7, with values of $R < 1.04$. However, values of R

increase as pH decreases pH < 7, indicating that both fluorescein and eosin Y become increasingly non-conservative at lower values of pH, and that these dyes may not be good choices for hydrologic tracer experiments under acidic conditions. Future research to quantify values of pK_a and the variation of log K_{ow} vs. pH for other fluorescent dye tracers is recommended.

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