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Interactions of dissolved humic substances with oppositely charged fluorescent dyes for tracer techniques

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ABSTRACT

To investigate interactions between oppositely charged fluorescent dyes and dissolved humic substances, fluorescence quenching of fluorescein and rhodamine 6G with dissolved humic substances was performed. Binding coefficients were obtained by the Stern–Volmer equation. The fluorescence of rhodamine 6G was largely quenched by the addition of humic acid and a non-linear Stern–Volmer plot was obtained. This strong quenching may be caused by the electrostatic interaction between cationic rhodamine 6G and humic acid and strengthened by the hydrophobic repulsion. In contrast, the quenching and interactive effects of dissolved humic substances for fluorescein were relatively weak.

1. Introduction

Fluorescent dyes have been widely employed in various technologies such as dye tracing, bio-imaging, chemical sensing, and dye lasers (Yuan et al., 2013; Basabe-Desmonts et al., 2007; Li and Psaltis, 2008; Harden et al., 2008). Dye tracer techniques are powerful tools that enable better understanding of solute transport in the liquid phase under a variety of hydrological conditions by tracking natural tracers and/or spiked artificial tracers. In the field of water and environmental engineering, tracers have been applied to investigate water flow in surface and ground water systems, as well as in laboratory experiments (Goppert and Goldscheider, 2008; Jones and Smith, 2005; Sabaliunas et al., 2003). To be effective, tracers must be absent from (or present in very low concentrations) natural aquatic environments, have high water solubility and low sorptivity to soil and sediment, be safe, highly detectable, and chemically, physically, and biologically stable. Among a variety of tracers, fluorescent dyes composed of synthetic organic compounds possess most of these characteristics, are cost effective and easy to use, and can be easily identified on-site using a portable fluorometer. Fluorescein is a popular fluorescent dye tracer commonly used in aquatic systems (Sabaliunas et al., 2003; Harden et al., 2008). Rhodamine 6G is also sometimes used as a fluorescent dye tracer (Kwak et al., 2013); however, this compound has been shown to have toxic effects and is therefore generally limited to use in laboratory experiments (Alford et al., 2009). The difference of two dyes is that fluorescein is anionic and rhodamine 6G is cationic.

Smart and Laidlaw (1977) investigated the sensitivity, detectability, effect of water chemistry, decay rates, adsorption resistance, toxicity, and cost of eight fluorescent dye tracers, including fluorescein. Since then, ecotoxicological assessments (Behrens et al., 2001), photobleaching (Larsen and Crimaldi, 2006), and effects of solvents and pH (Saini et al., 2009; Oba and Poulson, 2012) of fluorescent dye tracers have been investigated. However, no studies have focused on the effects of dissolved humic substances (DHS) on fluorescent dyes. Although fluorescent dyes can be used for qualitative analysis, the interactions might inhibit the optical detection of fluorescent dyes due to the potential quenching caused by the presence of DHS. DHS is known to interact with organic compounds such as herbicides, pesticides, and polycyclic aromatic

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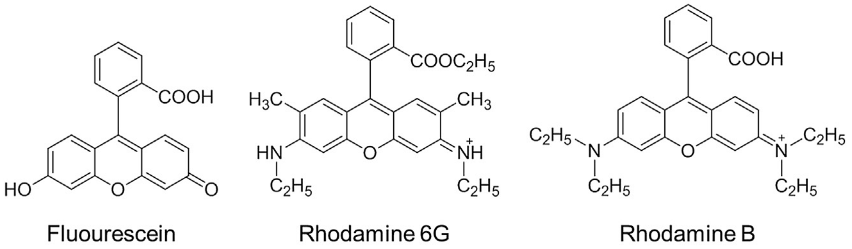

Fig. 1. Chemical structures of fluorescein, rhodamine 6G, and rhodamine B.

hydrocarbons (PAHs) in surface water (Ravacha and Rebhun, 1992; Schlautman and Morgan, 1993; Hesketh et al., 1996; Spark and Swift, 2002), and to affect their fate and transport. Various methods such as simple adsorption experiments, dialysis, microcalorimetry, reverse phase separation, solubility enhancement, and fluorescence quenching have been used to investigate the interactions between DHS and these organic compounds (Hesketh et al., 1996; Spark and Swift, 2002; Raber et al., 1998; Yamamoto et al., 2003; Schlautman and Morgan, 1993). Fluorescence quenching is a simple and useful method commonly employed to investigate interactions of DHS with PAHs because some PAHs show fluorescence and DHS acts as a quencher. The Stern–Volmer equation (also known as the fluorescence static quenching equation) can be plotted by conducting fluorescence quenching experiments (Gauthier et al., 1986). The binding coefficient can be obtained easily by determining the slope of the Stern–Volmer plot. In this study, the fluorescence quenching method was applied to analyze interactions of oppositely charged fluorescent dyes, fluorescein and rhodamine 6G, with DHS to investigate its effects on the dyes. This paper would contribute to the practice of selecting suitable fluorescent dye tracers in aquatic and hydrology studies.

2. Materials and methods

2.1. Fluorescent dyes

The chemical structures of fluorescein, rhodamine 6G, and rhodamine B are shown in Fig. 1. All fluorescent dyes are xanthene derivatives with many valuable characteristics, including sharp and intense absorption and fluorescence bands, high fluorescence quantum yields, high molar absorption coefficients, good photochemical stability, relatively long emission wavelength, and good water solubility (Chen et al., 2012). Fluorescein sodium salt (MW: 376.27), rhodamine 6G (MW: 479.01), and rhodamine B (MW: 479.01) were purchased from Sigma–Aldrich (St. Louis, United States) and used without further purification.

2.2. Sample preparation and fluorescence quenching experiments

Fluorescence quenching experiments were conducted in batch

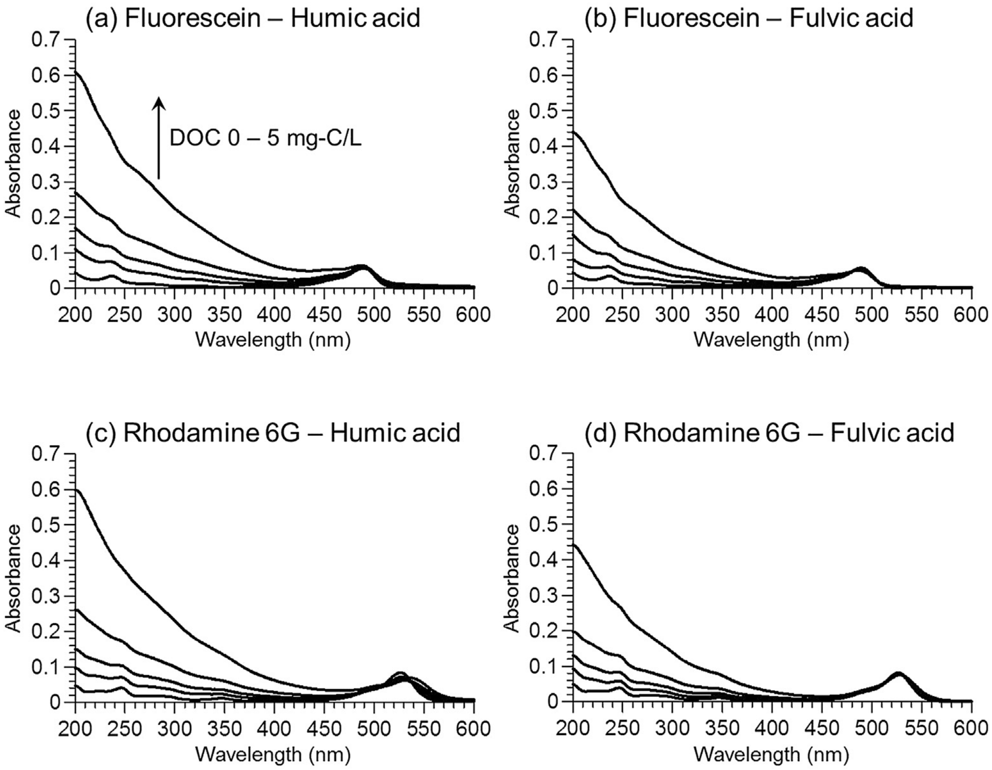

Fig. 2. Absorption spectra of fluorescein (a, b) and rhodamine 6G (c, d) with increasing DOC concentrations of humic acid or fulvic acid. The spectra were taken at DOC concentrations of 0, 0.5, 1, 2, and 5 mg-C/L. The concentration of the fluorescent dye was 1.0 μM (0.38 mg/L for fluorescein and 0.48 mg/L for rhodamine 6G).