

Fluorescence Measurement of Pyrene Wall Adsorption and Pyrene Association with Humic Acids

An Experiment for Physical Chemistry or Instrumental Methods

Edward C. Shane,*[†] Miranda Price-Everett, and Tonya Hanson

Chemistry Department, Morningside College, Sioux City, IA 51106

Rationale

There has been an interest in upgrading the undergraduate physical chemistry laboratory experience by incorporating contemporary experiments including fluorescence experiments (1). A number of articles in this *Journal* have covered the fundamentals of fluorescence techniques and applications of fluorescence techniques (2–7). In this experiment we explore the use of a fluorescence method to monitor the adsorption of the polycyclic aromatic hydrocarbon (PAH) pyrene to Pyrex, quartz, and low-density polyethylene (LDPE) surfaces and the use of a fluorescence quenching method to measure the association of pyrene with natural humic acids. The latter problem has important environmental consequences because PAHs are common organic contaminants.

This experiment has two parts. In part 1 the adsorption of pyrene on quartz is measured by following the decrease in pyrene fluorescence as it adsorbs onto the walls of a quartz fluorescence cell. In part 2 a fluorescence quenching method is used that follows the decrease in pyrene fluorescence when humic acid (HA) is added. A simple pyrene wall adsorption model is used to fit observed experimental adsorption data, and a simple association model is used to explain the observed pyrene fluorescence quenching by humic acid and to determine an association constant. The experiment is readily adapted to a project format.

The experiment demonstrates the significant effect that wall adsorption can have on the analysis of dilute solutions, the usefulness of nonlinear curve-fitting routines, and the importance of correcting for so-called “inner filter effects”.

Experimental Procedure

Chemicals

We used 99% pyrene, phenanthrene, and anthracene obtained from the Aldrich Chemical Company, without further purification. Humic acids were purchased from the International Humic Substances Society (<http://www.ihss.gatech.edu/>).

Pyrene Wall Adsorption

A 70-ppb pyrene solution is placed in a quartz fluorescence cell and fluorescence decline is monitored over a two-hour period, with readings taken every few minutes initially and every ten minutes thereafter. Several groups can share the fluorescence spectrophotometer, which we use in the fixed

wavelength mode to minimize the time to take a fluorescence reading; but each group will need its own fluorescence cell. We also adapt the basic method to measure pyrene adsorption on Pyrex and LDPE, so the experiment can be used as a project in which different groups measure pyrene wall adsorption on different surfaces.

Fluorescence Quenching Method

We use a method (8) that minimizes competition between pyrene adsorption on the walls of the quartz fluorescence cell and association with humic acids. In this method 1.5 mL of a humic acid solution and 1.5 mL of a 70-ppb pyrene solution are each transferred into the quartz fluorescence cell, the mixture is stirred for eight minutes, and a fluorescence reading is taken using an excitation wavelength of 325 nm and an emission wavelength of 391 nm. This is repeated with humic acid solutions of variable concentration. A background fluorescence measurement is taken with 1.5 mL of buffer and 1.5 mL of humic acid. The humic acids absorb at both the fluorescence excitation and emission wavelengths, so it is necessary to correct the observed fluorescence for the inner filter effect (2).

Hazards

Pyrene, phenanthrene, and anthracene are cited in MSDS literature as potentially harmful substances that are readily absorbed through the skin. Several other PAHs are characterized as carcinogens, so appropriate cautions should be taken. Gloves should be worn when handling PAHs and humic acids, and operations should be carried out in a fume hood.

Results

Surface Adsorption of PAH

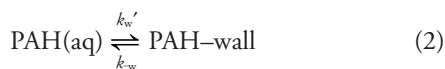
Backhus and Gschwend have described a kinetic wall-binding model in which the free PAH is fluorescent, but the wall-bound PAH does not fluoresce (9).



In this second-order reaction “wall” represents an available surface-adsorption site. If all the pyrene in 3.0 mL of a 70-ppb pyrene solution were adsorbed on the walls of the fluorescence cell, about 6×10^{14} pyrene molecules would occupy the surface sites. Assuming a surface area for pyrene of 20 \AA^2 , only about 10% of the exposed surface area would be occupied. In this limit of low surface coverage the term *wall* remains approximately constant; and the forward reaction in eq 1 may be

[†]Current address: School of Science and Mathematics, Office of the Dean, Millersville University, Millersville, PA 17551-0302; Edward.Shane@Millersville.edu.

represented as a pseudo-first-order reaction with $k_w' = k_w[\text{wall}]$.



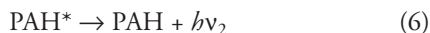
Solution of the general differential equation for the mechanism in eq 2 gives the following fluorescence relationship (10):

$$F = \frac{k_{-w}F_0}{k_w' + k_{-w}} + \frac{k_w'F_0}{k_w' + k_{-w}} e^{-(k_w' + k_{-w})t} \quad (3)$$

F_0 is the initial fluorescence, which is proportional to the initial [PAH]; and F is proportional to the [PAH] at any time, t . We use a nonlinear curve-fitting routine in Psi-Plot to analyze fluorescence-versus-time data and to determine k_w' and k_{-w} from eq 3. The equilibrium constant for the adsorption process in eq 1 can be expressed in terms of the forward and reverse rate constants, $K = k_w/k_{-w}$. Rewriting this in terms of the pseudo-first-order rate constant gives $K = k_w'/(k_{-w}[\text{wall}])$. If one defines [wall] with units of A_s/V , then a wall adsorption equilibrium constant, K , can be calculated using the measured surface area, A_s , and volume, V , of the cell and the relationship $K = \{k_w'/(k_{-w}(A_s/V))\}$ mL/cm² (9).

Association Constant

We use a static quenching model (11) in which the PAH and humic acid form a nonfluorescing complex, PAH-HA, and only the free PAH is available to fluoresce.



The first step represents the association between the HA and the free PAH. The second step represents excitation of the free PAH, and the third step represents the fluorescence of the free PAH. In this model the relationship between fluorescence, F , and humic acid concentration, [HA], is $F_0/F = 1 + K_a[\text{HA}]$ where the association constant, K_a , is

$$K_a = \frac{[\text{PAH-HA}]}{[\text{PAH}][\text{HA}]} \quad (7)$$

We use a linear regression fit of a graph of F_0/F versus [HA] to determine K_a from the slope of the line. It is possible to compare this static quenching model with a dynamic quenching model (11) in which humic acid is assumed to quench the excited PAH molecule: $\text{PAH}^* + \text{HA} \rightarrow \text{PAH}$. The dynamic quenching model is eliminated when it is found to require an impossibly high quenching constant for a diffusion-controlled reaction on the order of $10^{12} \text{ M}^{-1} \text{ s}^{-1}$.

To verify that the bound PAH-HA complex did not fluoresce, the quantum yield of the bound complex was measured using a method proposed by Backhus and Gschwend (9). A graph of F/F_0 versus [HA] follows the relationship

$$\frac{F}{F_0} = \frac{1 + \Phi K_a [\text{HA}]}{1 + K_a [\text{HA}]} \quad (8)$$

where Φ is the quantum yield of the bound PAH-HA complex. Fluorescence quenching data was fitted to eq 8 using a nonlinear curve fitting routine in Psi-Plot. The fitted value for Φ for a pyrene-Leonardite humic acid run at pH 5 was 0.029 ± 0.027 . For three merged pyrene-soil humic acid runs at pH 7 the value for Φ was -0.013 ± 0.001 . Thus Φ is essentially zero, as expected for a nonfluorescing PAH-HA complex.

The quenching experiments are readily adaptable to a project format in which different PAHs and humic acids are used. We found that Leonardite, peat, and soil humic acids have similar association constants when bound to pyrene; Suwannee River humic acid association with pyrene was too weak to detect. Phenanthrene and anthracene have measurable association constants. We found for experiments in which pH was varied from 5 to 10 that K_a increased as pH decreased. We checked for a salinity effect on K_a for [NaCl] up to 20 ppm but found none.

Acknowledgments

We thank the MidAmerican Energy Company for support of the summer research projects under which the PAH-HA experiments were initially researched, and high school students Brianna Macfarlane and Sara Johnson, who worked on those projects. The Hitachi fluorescence spectrophotometer was purchased with a NSF-ILI grant, USE-9152550. We also acknowledge helpful discussions with Larry D. Martin.

Supplemental Material

The student handout and notes for the instructor are available in this issue of *JCE Online*.

Literature Cited

- Schwenz, R. W.; Moore, R. J. *Physical Chemistry: Developing a Dynamic Curriculum*; American Chemical Society: Washington DC, 1993.
- Tucker, S. A.; Amszi, V. L.; Acree, W. E. *J. Chem. Educ.* **1992**, *69*, A8-A12.
- Tucker, S. A.; Acree, W. E. *J. Chem. Educ.* **1995**, *72*, A31-A33.
- Stothkamp, K. G.; Stothkamp, R. E. *J. Chem. Educ.* **1994**, *71*, 77-79.
- Ceballos, A.; Gonzalez-Valasco, F.; Inestal, J. S. *J. Chem. Educ.* **1994**, *71*, A297-A300.
- Gsponer, H.; Arguello, G. A.; Arguello, G. A. *J. Chem. Educ.* **1997**, *74*, 968-972.
- Buccigross, J. M.; Bedell, C. M.; Suding-Moster, H. L. *J. Chem. Educ.* **1996**, *73*, 275-278.
- Patterson, H. H.; MacDonald, B.; Fang, F.; Cronan, C. In *Humic and Fulvic Acids*; Gaffney, N. A.; Marley, N. A.; Clark, S. B., Eds.; American Chemical Society: Washington, DC, 1996; pp 288-298.
- Backhus, D. A.; Gschwend, P. M. *Environ. Sci. Technol.* **1990**, *24*, 1214-1223.
- Steinfeld, J. I.; Francisco, J. S.; Hase, W. L. *Chemical Kinetics and Dynamics*, 2nd ed.; Prentice Hall: Upper Saddle River, NJ, 1999; pp 22-24.
- Gauthier, T. D.; Shane, E. C.; Guerin, W. F.; Seitz, W. R.; Grant, C. L. *Environ. Sci. Technol.* **1986**, *20*, 1162-1166.