Supporting Information

Experimental evidence of non-complete fluorescence quenching of pyrene bound to humic substances: implications for K_{oc} measurements

E.A. Shirshin,^a* G.S. Budylin^a, N.Yu. Grechischeva^b, V.V. Fadeev^a, and I.V. Perminova^c

^{a.} Department of Physics, Lomonosov Moscow State University, Leninskie Gory 1-2, 119991 Moscow, Russia Phone/Fax: +7(495)9391653; E-mail: <u>shirshin@lid.phys.msu.ru</u>

^{b.} Russian State University for Oil and Gas named after I.M. Gubkin, Leninskiy Prosp. 65-1, 119991 Moscow, Russia

^{c.} Department of Chemistry, Lomonosov Moscow State University, Leninskie gory 1-3, 119991 Moscow, Russia

Protocol of fluorescence measurements

Fluorescence emission spectra were recorded with a FluoroMax-4 (Horiba-JobinYvon) spectrofluorometer. For $S_0 \rightarrow S_1$ pyrene excitation the excitation wavelength was 360 nm, and the excitation and emission slit widths were 5 and 1 nm, respectively. Fluorescence emission was collected in the range from 368 to 600 nm.

For $S_0 \rightarrow S_2$ pyrene excitation, the excitation wavelength was 334 nm and the excitation and emission slit widths were 1 and 1 nm, respectively. Fluorescence emission was collected in the range from 350 to 600 nm.

It was estimated that pyrene sorption onto the walls of quartz cuvette and Teflon magnetic stirrer accounted for almost 30% fluorescence intensity decrease during the time course of our experiments (Fig. S1). As a result, all samples were equilibrated for 60 minutes in the quartz cell under continued stirring prior to spectral measurements. There was no further loss in the fluorescence observed after 60 minutes exposure. All experiments were conducted under continued stirring of the sample.



Fig. S1. The changes of pyrene fluorescence spectra due to sorption on the cuvette walls during 500 s.

Determination of equilibrium time in the system Py-HS

In our experiments equilibrium time for pyrene-HS system after the addition of HS aliquot was found to be about 1 minute. At the same time, intermolecular interactions with participation of HS (e.g., conformational changes) can take as long as 1 hour.¹ Sierra *et al.*² discuss the possibility of pyrene-assisted rearrangement of HS molecules into micelles. Moreover, some authors perform fluorescence measurements in pyrene-HS system, e.g., I_1/I_3 estimation³, 24 hours after the sample preparation. This motivated us to examine thoroughly changes in the fluorescence spectra of pyrene taking place in the presence of 20 mg L⁻¹ of HS within the time course from 1 minute to 48 hours. The corresponding experiment showed the invariability of pyrene and HS fluorescence spectra shapes on time (Fig. S2). As a result, all further fluorescence measurements were conducted 1 minute after addition of HS aliquot.



Fig. S2. Fluorescence spectra of pyrene in the presence of 20 mg L^{-1} of HS, normalized to the intensity of the fluorescence peak at 373 nm. The black line corresponds to the initial fluorescence spectrum, and the red line corresponds to fluorescence spectrum obtained in 48 hours.

Determination of optimum acquisition times

We observed the formation of photodegradation byproducts with fluorescence band centered at ca 400 nm when pyrene was irradiated at 334 nm during several hundred seconds. This effect could not be neglected when processing fluorescence spectra obtained upon excitation into the forbidden $S_0 \rightarrow S_1$ transition because emission of this byproduct overlaps with the weak pyrene fluorescence and deforms the band shape. In case of 334 nm excitation into the allowed $S_0 \rightarrow S_2$ transition, no deformation of pyrene spectra was observed. This fact can be explained by the almost 100 times higher signal-to-noise ratio in case of excitation into allowed $S_0 \rightarrow S_2$ transition compared to the excitation of pyrene into the forbidden $S_0 \rightarrow S_1$ transition.

To minimize the influence of the byproduct formation we minimized the time of pyrene irradiation – collection of a single spectra at 334 nm excitation lasted 10 seconds. We also performed test experiments that imitated the measurement procedure: fluorescence quenching curves were measured without addition of HS. All parameters of these test experiments (number of spectra

obtained, exposition times etc.) were the same as for real experiments. As a result, no deformation of fluorescence spectra was observed either for 334 nm or 360 nm excitation.

Absorption spectra of pyrene in different solvents

Absorption spectra of pyrene measured in four different solvents (hexane, methanol, ethanol and acetonitrile) are presented in Fig. S3. In order to better resolve the $S_0 \rightarrow S_1$ absorption region of pyrene, the higher concentrations were used (40 mg/L instead of 0.4 mg/L used for the $S_0 \rightarrow S_2$ absorption measurements). We note that the values of extinction coefficients for the allowed S_0 - S_2 transition and the for the forbidden $S_0 \rightarrow S_1$ transition differ more than two orders of magnitude, and that the $S_0 \rightarrow S_1$ absorption of pyrene in water was not measured because of its low solubility (135 µg/L). The polarity of the selected solvents decreases in the following sequence: acetonitrile>ethanol>methanol>hexane. It can be deduced from Fig. S3, that along with a decrease in polarity the opposite trends for the absorption cross-sections corresponding to the $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_1$ transitions are observed: while the $S_0 \rightarrow S_2$ extinction increases, the $S_0 \rightarrow S_1$ extinction declines.



Fig. S3 Absorption spectra of pyrene in the solvents of different polarity: a) $S_0 \rightarrow S_2$ absorption band, $C_{Py} = 0.4 \text{ mg/L}$ and b) $S_0 \rightarrow S_1$ absorption band, $C_{Py} = 40 \text{ mg/L}$. The arrow indicates an

increase in the solvent polarity, the black, blue, green and magenta lines correspond to hexane, ethanol, methanol and acetonitrile (in order of increasing solvent polarity), respectively.

Approximation of fluorescence quenching curves

The obtained fluorescence quenching curves were fitted to linear Sterm-Volmer equation, disregarding non-linear character of the curves, and to Eq. (6). The results of approximation for CHP are presented in Figure S4.



Fig. S4. Fluorescence quenching curves for CHP and its linear (static quenching, red solid lines) and nonlinear (non-complete pyrene quenching, green solid lines) approximations.

References

- 1 R.R. Engelbretson, R. von Wandruszka, Env. Sci. Technol., 1994, 28, 1934-1941.
- 2 M. M. D. Sierra, T.G. Rauen, L. Tormen, N.A. Debacher, E.J. Soriano-Sierra, *Water Res.*, 2005, 39, 3811-3818.
- V.A. Ganaye, K. Keiding, T.M. Vogel, M.L. Viriot, J.C. Block, *Env. Sci. Technol.*, 1997, 31, 2701-2706.