USE OF TRITIUM LABELED PREPARATIONS FOR DIRECT MEASUREMENTS OF HUMIC SUBSTANCE HYDROPHOBICITY AND THEIR INTERFACIAL ADSORPTION IN TOLUENE – WATER SYSTEM

G. A. Badun, V. Yu. Pozdnyakova, M. G. Chernysheva, Z. A. Tyasto, I. V. Perminova

Lomonosov Moscow State University, Department of Chemistry, Leninskie Gory,
Moscow 119992, Russia, badun@radio.chem.msu.ru.

Key words: humic substances, tritium labeling, hydrophobicity, interfacial adsorption, partition coefficients.

I. INTRODUCTION

Humic Substances (HS) are characterized by the presence of highly oxidized aromatic core and bound to it saccharidic-peptidic periphery. The dualism of the structure results in surface activity of HS and their ability to participate in hydrophobic interactions. However, up to date there are no direct measurements of HS hydrophobicity. The most common quantitative parameter of hydrophobicity is an octanol-water partition coefficient (log K_{ow}) that reflects affinity of the compound to organic versus water phase (Leo et al. 1971). The higher log K_{ow} is, the more hydrophobic the compound is. Octanol is most commonly used as a reference organic phase, but there are some studies on a use of the different organic solvents. So, in case of tritium labeled organic compounds the most beneficial organic phase is toluene, which is used for liquid scintillation counting of the samples. Taking into consideration that the tritium path length in the condensed medium reaches few micrometers, it is obvious that tritium labeled compounds can be used not only for determination of a labeled compound in toluene, but for evaluation of its sorption on the interface as well. In our previous investigations, we have demonstrated that the scintillation method based on liquid scintillation spectroscopy of water – toluene system can be used for determination of equilibrium concentrations of labeled compounds both in organic and water phase, and on the interface (Badun et al. 2003a). In this research we used the above mentioned approach for determination of partition coefficients of humic substances and their interfacial adsorption in toluene - water system.

II. MATERIALS AND METHODS

Humic acids and fulvic acids used in this study were isolated from highland peat located in Ivanovo Region, Russia (PHA, PFA) according to standard technique using alkaline extraction with 0.1 M NaOH. A sample of brown coal humic acids (CHA) was a commercially available preparation Powhumus (Humintech, Germany).

The thermal activation method is used for introduction of tritium in the structural fragments of humic macromolecule (Badun et al. 2000, 2003b). Size exclusion chromatography (SEC) analyses on the labeled HS-preparations were conducted according to (Perminova et al. 1998) using ABIMED-HPLC-system.

Liquid scintillation spectroscopy determinations for water–toluene system were carried out in the standard plastic vials of a 1.5 cm diameter. Aliquote amount (0.8 ml) of the labeled preparation in 0.028M phosphate buffer was placed into the vial and then toluene with an addition of 2,5-diphenyloxasol (0,45 % mass) was carefully added atop of the aqueous layer. Then, the vial was put into the liquid scintillation spectrometer RackBeta 1215 (LKB Wallac, Finland) for radioactivity measurements.

Kinetics of the labeled humic preparations redistribution between the phases was estimated by the alteration of a counting rate. After reaching the equilibrium in the investigated system, the counting rate of a vial (I) was measured, and then a portion of organic phase (V_1 ml) was transferred into another vial. After that, the counting rate of the sampled portion (I_1) and of the residual solution (I_2) was measured. The "volume" constituent part of the counting rate was determined from the equation $I_V = (V/V_1)\cdot I_1$, and "surface" constituent part was calculated using two equations: $I_S = I - (V/V_1)\cdot I_1$ and $I_S = I_2 - (V/V_1 - 1)\cdot I_1$. The divergence between two values of the "surface" constituent part obtained by the different ways of determination did not exceed 5 %. As a rule, 3 ml of organic phase were used, and 2 ml of the solution were sampled after reaching the equilibrium. The quantity of a preparation transited into the organic phase and that concentrated at the organic-aqueous interface was calculated from the efficiency of tritium registration in the volume of organic phase $\varepsilon_v = 0.55 \pm 0.03$ and from the interface $\varepsilon_s = 0.5 \cdot \varepsilon_v$.

III. RESULTS AND DISCUSSIONS

The dependence of HS equilibrium concentration in the organic phase versus that in the aqueous phase is plotted in Figure 1. The corresponding relationship was linear that allowed to use the following expression for calculation of the partition coefficients: $D = c_{or}/c_w$. The corresponding data are summarized in Table 1. The partition coefficients for PFA and PHA turned out to be very close to each other and significantly less than those for CHA.

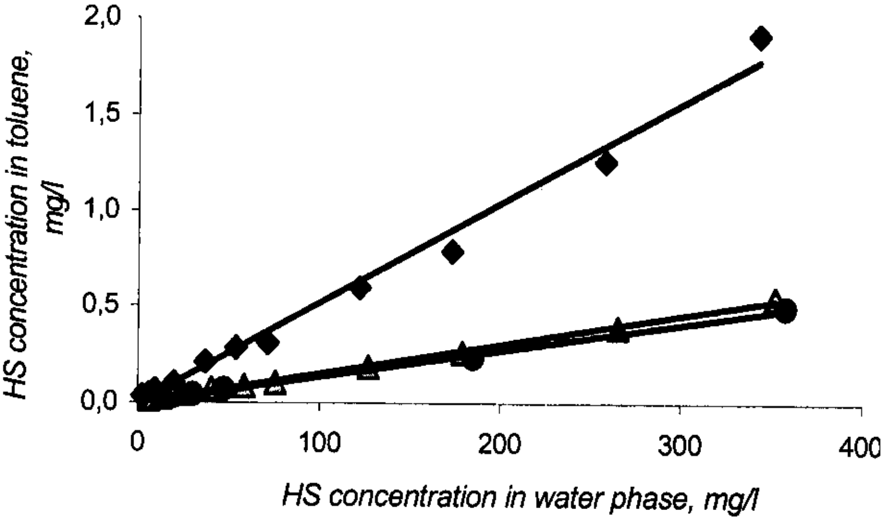

Figure 1 – Dependence of equilibrium concentration of HS in organic phase from there concentration in water phase (1 – CHA, 2 – PHA, 3 – PFA).

Table 1 – HS partition coefficients for toluene-water system.

HS sample	**Partition coefficient, $D \times 10^4$**	***log D***
^{3}H-CHA	50.0±4.0	-2.30
^{3}H-PHA	14.6±0.6	-2.84
^{3}H-PFA	14.0±1.1	-2.86

The sorption isotherm for CHA is shown on Figure 2. In the range of low concentrations of CHA (<10 mg/l), a rapid sorption of the labeled molecules at the toluene-water interface was observed. Excess quantity of CHA on the interface was increased up to higher value at concentration in water phase 60 *mg /l*. The maximum value of CHA adsorption on toluene-water interface in the studied range of HS concentration in water of 60-120 *mg/l* was 0.087±0.003 µg/cm². This value was agreed with monolayer of CHA macromolecules on toluene-water interface.

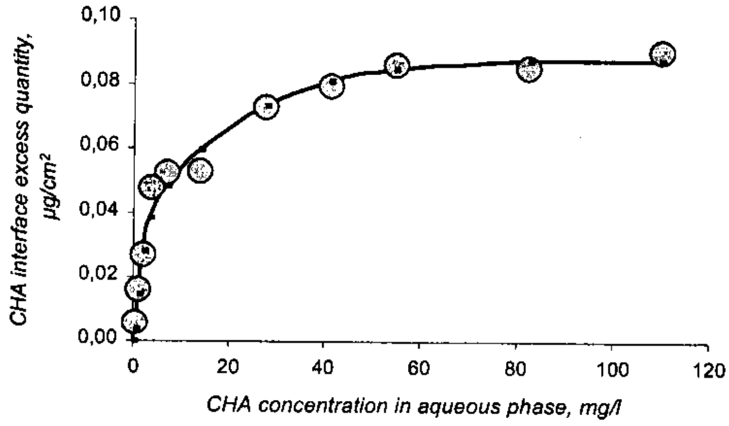

Figure 2 – Dependence of CHA excess quantity on aqueous solution-toluene interface from its equilibrium concentration in aqueous phase.

Acknowledgements

This work was financially supported by the Russian Foundation for Basic Research (grant # 03-03-32410) and by the Research Center for Environment and Health (GSF, Neuherberg, Germany) FE 75184, BA 31/139166/02/U.

References

Badun, G.A., and Fedoseev, V.M. 2000. Physicochemical basis of atomic tritium applications for investigation of solid surface. Proc. of 5th Int. Sc. Conf. "Physical and Chemical Processes on Selection of Atoms and Molecules" October. 2-6, 2000. Zvenigorod, Russia, 169-173.

Badun, G.A., Pozdnyakova, V.Yu., Chernysheva, M.G., Shchepina, N.E., Fedoseev, V.M. 2003. Determination of hydrophobity and surface activity of substances in system toluene - water with help of tritium labeled compounds. Proc. of 8th Int. Sc. Conf. "Physical and Chemical Processes on Selection of Atoms and Molecules" October 6-10, 2003. Zvenigorod, Russia, 193-198.

Badun, G.A., Pozdnyakova, V.Yu., Kudryavtsev, A.V., Perminova, I.V. 2003. Preparation of tritium-labeled humic substances using thermal activation method. Ecology and Future. Bulgarian J. of Ecological Science. 2(3-4):26-27.

Leo, A., Hansch, C. and Elkins, D. 1971. Partition coefficients and their uses. Chem. Rev. 71:525-616.

Perminova, I.V., Frimmel, F.H., Kovalevskii, D.V., Abbt-Braun, G., Kudryavtsev, A.V., Hesse, S. 1998. Depelopment of a predictive model for calculation of molecular weight of humic substances. Wat. Res. 32:872-881.